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TECHNOLOGICAL EVOLUTION AND REVOLUTION IN FOOD MICROBIOLOGY

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Burgos (Spain)

ABSTRACT BOOK

Welcome address

Dear colleagues,

It is a great pleasure to welcome you all to our city of Burgos in Spain to attend the 28th ICFMH International Conference, FoodMicro 2024. Since the International Conferences began in the last century, this is the first time it has been held in Spain. We want to express our gratitude to the executive committee of the ICFMH for choosing the city Burgos for this occasion.

Burgos is a nice city located 220 km north of Madrid, in the midway to the Atlantic seashore in Santander in northern Spain. An area that traditionally has been inhabited from more than 1 million years. Here, in the surroundings of the city, in the archeological site of Atapuerca, the remains of the so called the first European have been discovered, dating back more than 1.2 million years. The climate of this area has done the rest and in Atapuerca human fossils from most of the human phylogeny of Europe have been found, bones that you can see in the Museum of Human Evolution, which is located in the center of the city, next to the Congress Venue Fórum Evolución. Hence, Burgos is the European city of Human Evolution.

Here, in Burgos is where we developed our scientific career and research in food microbiology over the last 30 years in the Department of Biotechnology and Food Science of the University of Burgos, which was also born 30 years ago. Therefore, we think it will be a nice celebration for our university to welcome everyone to Burgos to attend FoodMicro 2024. During that time, there has also been a great evolution in food microbiology research in preparation of the big revolution that is now being produced involving new and powerful methodologies based on omics and other techniques. All this gives us a new insight of classical topics as food fermentations, probiotics or protective cultures, new technological interventions to extend the shelf-life and to improve food safety, new topics related to food environments such as primary production, food producing plants and its interaction with food products, the microbiome, etc.

For this reason, Burgos is the right place to talk and debate about this technological evolution and revolution in Food Microbiology. It is the place where everything begins with our ancient parents fighting to survive in a hard environment to evolve to the

present day. A city in the middle of Saint James' Way, where the knowledge exchange 800 years ago, brought through this way made possible the construction of the magnificent Gothic cathedral that stands in the middle of the city, which you will observe while discussing food microbiology in the Congress Venue.

Dear colleague, don't miss this opportunity to exchange ideas and do networking with other colleagues in the 28th International Conference of ICFMH. We have prepared an excellent program to facilitate this purpose including for the first time in FoodMicro Conference: Flash Communications to give more opportunities to young researchers to explain their research to the audience; Round Tables to give the audience a different way to discuss the research ideas behind the topics proposed and a congress party in the gardens of the university where you could enjoy, meet each other better and explore synergies for future research collaborations.

Welcome all of you to Spain and Burgos, enjoy our city, our culture and gastronomy and the good wines elaborated in this area.

Ah and don't forget your sunglasses!







Jordi Rovira (President), Beatriz Melero, Isabel Jaime
University of Burgos
Local Organizing Committee

Dear Colleagues and Friends,

It is my great pleasure to welcome you to the upcoming International Committee on Food Microbiology and Hygiene (ICFMH) congress in Burgos, Spain. This year, our focus is on the latest advances in new technologies in food production and microbiological analysis, and we are thrilled to bring together experts, researchers, regulators, and industry professionals from around the world to share knowledge and exchange ideas.

Revolutionary advancements in technology are transforming the field of food microbiology and hygiene, leading to unprecedented opportunities for scientific evolution. The congress will offer an exciting opportunity to delve into cutting-edge research and technological breakthroughs, exploring the ways in which innovation is driving improvements in food safety and quality. This will surely cover all aspects from the wet lab studies to in silico work as well as global changes that influence food web.

Keynote speakers and scientific sessions will cover a range of topics, including new trends in food processing, innovative approaches to food safety and quality, and novel methods for microbial analysis. We will also for the first time tackle some emerging fields such as microplastics and microbiome interactions. By sharing insights and exploring new ideas together, we hope to spark new collaborations and inspire future innovation in the field.

Beyond the scientific program, we have organized social events that will give you the opportunity to immerse yourself in the rich history and culture of Burgos, Spain. We are confident that this congress will offer an unforgettable experience for all attendees, fostering new collaborations, sharing insights, and providing opportunities to expand your knowledge.

We look forward to welcoming you to the ICFMH congress in Burgos, Spain, where we will explore the ways in which the revolution in technology is driving scientific evolution in the field of food microbiology and hygiene.

Sincerely,

Prof. Dr. Andreja Rajkovic

President, International Committee on Food Microbiology and Hygiene (ICFMH)



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ORAL COMMUNICATIONS

FERMENTED FOODS

Keynote 3.1

NUTRITIONAL AND HEALTH-PROMOTING POTENTIAL OF A NOVEL GLUTEN-FREE FERMENTED BEVERAGE OBTAINED FROM GERMINATED OAT

Natalia Aparicio-García, Cristina Martínez-Villaluenga, Elena Peñas, Juana Frías

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Fermented beverages have displayed an important role in human diet around the world in recent years. The development of dairy-alternative fermented products from innovative plant-based sources has become one of the most interesting food research topics to expand the horizons of food science. Oat is a gluten-free cereal with outstanding nutritional and bioactive properties that has been scarcely used as ingredient in fermented beverages.

Recent data obtained by our research group has demonstrated that germinated oat is an excellent source of nutrients and health-promoting compounds. Therefore, the aim of this study was to develop a novel gluten-free fermented beverage from germinated oat using a probiotic lactic-acid bacteria strain (*Lactobacillus plantarum* WCFS1) and evaluate their nutritional and bioactive potential, their anti-inflammatory activity in a macrophage inflammation model as well as their health-promoting properties in celiac adults through a nutritional intervention study.

The germinated oat fermented beverage (GOFB) contained high viable cell counts of the starter culture (8.9 Log colony-forming units, CFU) and good levels of protein (1.7 mg/mL), thiamine (676 µg/100 mL), riboflavin (28 µg/100 mL), β-glucan (79 mg/100 mL), phenolic compounds (61 mg GAE/100 mL), and antioxidant activity (164 mg TE/100 mL). GOFB showed anti-inflammatory activity, caused the reduction of cholesterol levels and improved the microbiota composition in the celiac group consuming GOFB beverage for 6 months compared to the control group. These findings support the beneficial effects derived from GOFB consumption in celiac individuals.

Keynote 3.2

PROMOTING INNOVATION OF FERMENTED FOODS (PIMENTO). COST ACTION CA20128

Marta Laranjo[1], Juana Frías[2], Effie Tsakalidou[3], Photis Papademas[4], Guy Vergères[5], Smilja Todorović[6], Marie Christine Champomier Vergès[7], Antonio Del Casale[8], Vittorio Capozzi[9], Zuzana Ciesarová[10], Kathryn Burton-Pimentel[5], Biljana Trajkovska[11], Christophe Chassard[12]

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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. The 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 European communities.

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 collaborations on FF.

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 and the "Farm to Fork" strategy by enhancing research and innovation into 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 more than 500 participants from cerca 50 countries. This regional diversity will play an important role through considering a differentiated panel of FF in diets.

The project has been ongoing since November 2021. So far, we have performed a Cartography of Fermented Foods in the diet of COST Countries, with a particular emphasis on Mediterranean countries, and developed the first European Fermented Foods Frequency Questionnaire (FFFQ) that has translated into more than 30 different European languages and was applied in 40 countries. The health benefits and risks of Fermented Foods have been investigated through 16 independent EFSA or satellite projects conducting systematic reviews of the literature. Finally, there is an ongoing survey on the bottlenecks for innovation in the field of fermented foods and starters designed for companies in the food sector. To disseminate the PIMENTO Cost Action and its achievements to the whole scientific community, PIMENTO has been presented in several international scientif conferences and a series of webinars has been organised since the beginning of 2024.

Keynote 5.2

FROM SPOILERS TO COLD FERMENTATION APPLICATIONS?

Johanna Björkroth

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Psychrotrophic lactic acid bacteria prevailing in foods comprise some latilactobacilli, *Leuconostoc* and *Lactococcus* species. They grow well at 4 °C and we have mostly studied them in association with spoilage of packaged, perishable food items. Our research has dwelled into their metabolism over many years but recently inspired from the example of kimchi fermentation we have started to consider if these organisms could be utilized in other vegetable fermentations. Kimchi fermentation can take place at various temperature but also under refrigerated temperatures leading to the growth of psychrotrophic leuconostocs. We will test that if cold fermentation can be used to control too rapid acidification and how adding enzymes to break down complex carbohydrates will work in cold fermentation. This kind of fermentation may provide useful in the case of many vegetables that should not develop too low acidity.

Our research is focusing on the use and metabolism of *Leuconostoc gasicomitatum, Leuconostoc gelidum* and *Lactococcus carnosus/paracarnosus* -group. We have been studying the interactomes of these organisms and how various factors are associated with diacetyl production through non-respiring or respiring metabolism. *L. gasicomitatum* can respire if heme is present in the medium which leads to formation of diacetyl.

In addition, survival of *L. gasicomitatum* is increased with heme dependent respiration. There also seems to be cases of VBNC cells among aerobically grown *Leuconostoc*, and we have shown that these cells can be rescued by addition of catalase. Thus, presence of oxygen shows a great difference for the growth of psychrotrophic *Leuconostoc*. In vegetable environment heme is not present but growth on pentoses may direct metabolism towards diacetyl formation. The other psychrotrophic leuconostocs than *L. gasicomitatum* do not respire. In our growth studies we have detected that acetate is growth limiting for aerobic growth of psychrotrophic *Leuconostoc* in traditional MRS medium and this should be taken

into consideration when these organisms are cultured. Currently we are testing the growth of these strains in oat-based matrix. We have inoculated these strains and are following their growth and sensory characteristics of the matrix. Our aim is to study if these organisms can be used also in the fermentation of other vegetable matrixes, such as fava bean, at low temperatures.
Some preliminary tests with other strains have shown that rapid acidification leads to non-pleasant sensory properties that might be and the low temperature can be used to protect from growth of non-desirable organisms.

Oral 3.1

DEVELOPMENT OF INNOVATIVE FERMENTED BEVERAGES AND "YOGURT"-TYPE GELS WITH IMMUNOMODULATORY PROPERTIES FROM ALMOND AND/OR CHICKPEA MILK ANALOGUES

Charles Silande[1], Nathalie Daniel[1], Andrea Hickisch[2], Marie-Bernadette Maillard[1], Séverine Chevalier[1], Gwénaële Henry[1], Laura López-Mas[3], Zein Kallas[4], Ingrid Aguilo[5], Gwénaël Jan[1], Valérie Gagnaire[1]

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Mediterranean nuts and legumes offer opportunities to diversify fermented products and to gradually move towards a more plant-based diet. Developing alternative fermented products to those derived from milk, requires selecting i) appropriate plant-based resources and ii) bacteria able to ferment them and provide adequate final sensory and probiotic properties.

Among the different nuts and legumes studied in the European LOCALNUTLEG project, almond milk analogue was chosen for preparing fermented beverages, as well as mixes of almond and chickpea milk analogues for preparing yogurt-type analogues. Different strains of lactic acid bacteria (LAB) and propionic acid bacteria (PAB) were tested. *Streptococcus thermophilus* CIRM-BIA772, *Lacticaseibacillus casei* CIRM-BIA1643, *Lactobacillus helveticus* CIRM-BIA100 and *Propionibacterium freudenreichii* CIRM-BIA141 were selected. The carbohydrates were fermented by the LAB and probiotic properties were provided by both *L. casei* and *P. freudenreichii* in beverages and by *L. helveticus* in yogurt analogues.
Sensory analyses were performed on the fermented beverages by free choice profiling technique. The fermented beverages and yogurt analogues with various almond-chickpea ratios, were deeply characterized by targeted metabolites content (organic acids, residual sugar, peptides and amino acids), and immunomodulatory properties. These latter were evaluated by changes in the production of IL-8 in intestinal human epithelial cells, after a challenge with the different fermented products, in the presence and in the absence of an inflammatory stress. The texture of the yogurt analogues was characterized by rheological measurements.
The fermented beverages were differently perceived by consumers, regarding the presence of the two LAB strains and of the LAB and PAB strains and addition or not of sucrose. All the fermented beverages had immunomodulatory properties, in contrast to the initial vegetable milk analogues. The change in the almond/chickpea ratio resulted in gels that were more consistent with increasing amount of chickpea. The almond/chickpea ratio also influenced the LAB strains growth with higher population observed in 50/50 ratio and higher immunomodulatory properties in 25/75 ratio.
These innovative fermented almond beverages and almond/chickpea yogurt analogues can be an alternative to widely consumed fermented plant-based products, with functional properties, and may help diversifying flexitarian and vegetarian consumers' diets.

Oral 3.2

HEALTHFERM: INNOVATIVE PULSE AND CEREAL-BASED FOOD FERMENTATIONS FOR HUMAN HEALTH AND SUSTAINABLE DIETS

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Fermented foods are consumed in Europe and across the globe. Especially in the past decades, fermented foods have been hailed for their nutritional and perceived health benefits. Yet little is known about the impact of fermentation on human health or how fermentation can be leveraged to enhance the use of sustainable plant-based raw materials. The EU research project HealthFerm which kicked off September 2022, is a collaboration of 23 partners from across Europe and will shed light on this forward-looking topic. Funded through the European Union's Horizon Europe Framework Programme for Research and Innovation and the Swiss government, the project has a budget of EUR 13.1 million (11.3 and 1.8 from EU and SERI, respectively) over four years. HealthFerm is coordinated by Prof. Christophe Courtin from the Laboratory of Food Chemistry and Biochemistry at KU Leuven.

The HealthFerm project targets the societal and industrial transition from traditional to sustainable plant-based fermented foods by design for a healthy everyday diet. This will be achieved by (1) disentangling the interaction between food fermentation microbiomes, grain-based foods and the human gut microbiome and health and (2) using microbial resources and fermentation technology to develop healthy pulse- and cereal-based food and diets that cater to the desires and needs of EU citizens.

Drawing from a community science approach, HealthFerm will identify micro-organisms and metabolic pathways that may result in desired nutritional and health effects. The impact of microbial fermentation on raw materials will be examined at the molecular level. Fermentation technology will be used to develop novel, healthy and nutritious liquid and (semi-) solid foods based on legumes (pea and faba bean) and cereals (wheat and oat). The impact of these foods on human health and the gut microbiome will be assessed in several intervention trials. Consumer acceptance of fermented foods, their technologies and their role in the transition towards a more sustainable healthy diet will be studied in different social contexts. Finally, extensive ecosystem building and training activities will contribute to HealthFerm's strong participatory approach.

In this presentation, an overview of the different work packages and the first results of the project will be shown.

Oral 3.3

CHARACTERIZATION OF NON-DAIRY KEFIR-LIKE BEVERAGES BY METAGENOME AND VOLATILOME ANALYSIS

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Milk kefir is a traditional dairy beverage produced through the natural fermentation of milk by a kefir 'grain' consisting of a symbiotic community of bacteria and yeast, whose regular consumption has been associated with several health benefits. In recent years, an increasing awareness of sustainability, climate change and animal welfare is leading consumers and producers to look for non-dairy alternatives of this fermented food. The aim of the present study is to provide a basis for the development of healthy and appetizing non-dairy kefir alternatives using microbiome-guided precision fermentation processes.

Two kefir grains of different geographical origins and three commercially available plant-based milk alternatives (oat, coconut and soy beverages) have been tested to obtain non-dairy kefir-like beverages. Cow's milk was used as a control substrate. The fermentation took place at 24 °C and

total mesophilic, lactic-acid bacteria and yeast counts were evaluated at times 0 and 24 h. Additionally, samples were taken after 0, 4 and 24 h of incubation for pH measurement and to analyse their microbial composition by metagenome shotgun sequencing. Volatile organic compounds were profiled by gas chromatography-mass spectrometry (GC-MS). A pH decrease was observed in all tested substrates and with both kefir grains, with the pH drop being quicker and greater in the coconut and oat beverages compared with in the control cow milk and the soy beverage. An increase of around 2 logarithmic units in the total mesophilic and lactic-acid bacteria counts was observed after 24 h-fermentations for all combinations. However, a higher variability was found in the case of the yeast counts. The metagenome analysis showed a higher impact of the kefir grain than of the substrate and incubation time on the final microbial composition of the fermented beverages. The kefir grains and T0h samples were mostly dominated by *Lactobacillus kefiranofaciens*, *Lentilactobacillus kefiri* and *Lactobacillus helveticus* while in the T24h samples *Lactococcus cremoris*, *Lactococcus lactis* and *Leuconostoc mesenteroides* were the predominant species.

These results will guide the selection of substrates and starter culture microorganism to improve non-dairy kefir fermentation processes in a targeted way.

Oral 3.4

UNDERSTANDING THE CONTRIBUTION OF OAT-ASSOCIATED ENZYMES TO FERMENTATION-INDUCED CHANGES IN OAT CONSTITUENTS

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Fermentation is regarded as a sustainable strategy to improve the nutritional quality of cereal-based foods, e.g. by phytic acid degradation, fibre solubilisation or a reduction of the glycaemic index. pH-dependent enzymes play a crucial role in the conversion of cereal constituents during fermentation. To which extent these enzymatic conversions are induced by enzymes of the inoculated microorganisms or cereal-associated enzymes (i.e those endogenously present or originating from microbes associated with the outside of the kernel) remains unclear. Oats were chosen as they have an interesting nutritional profile. In addition, oats are traditionally heat treated (kilned) to prevent rancidity development by strongly reducing the activity of oat-associated enzymes.

To assess the impact of the kilning process on the suitability of oat as a raw material for food fermentation, both kilned and non-kilned oats were included in this study. Enzymatic assays indicated that kilning strongly reduces amylase, endoxylanase, β-glucanase and phytase activities, which could affect biochemical conversions that occur during fermentation. To assess the changes induced by oat-associated enzymes, oat wholemeal suspensions were incubated at pH 4.0 and 6.4 (the pH in which a typical food fermentation ranges) for 24 hours at 30 °C in the presence of antibiotics, the latter to prevent spontaneous fermentation. In this setup, the levels of free phosphorus and water-extractable arabinoxylan increased by 33% and 18%, respectively, in the case of non-kilned oats, whereas little to no changes were found for kilned oats.

These results were compared with a fermentation experiment using a similar setup but with the addition of a *Lactiplantibacillus plantarum* starter culture. The extent and rate of acidification and microbial growth were slower for kilned compared to non-kilned oat. This probably could be explained by the low amylase activity in kilned oat. No further increase in the extent of arabinoxylan solubilisation or phytic acid degradation was observed compared to the incubated samples.

In conclusion, the results of this study (i) underpin the importance of cereal-associated enzymes during cereal-based fermentation and (ii) highlight the need for specific starter cultures with targeted enzymatic activities in oat-based fermentations.

Oral 3.5

YELLOW CAROTENOID PRODUCTION OF *LACTOBACILLI* IN THE *LACTOBACILLACEAE* FAMILY IS ASSOCIATED WITH INSECT-ADAPTED LIFESTYLE

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Carotenoids are antioxidants, anti-inflammatory and present in various fermented foods dominant by lactic acid bacteria. A yellow C30 carotenoid, 4,4'-diaponeurosporene, is produced by members of the *Lactobacillaceae* family, but the mechanism and phenotype have only been described in *Lactiplantibacillus plantarum*. The gene cluster responsible for producing the pigmented compound is identified as the *crtMN* operon. This operon has been cloned in *Bacillus subtilis* and *E. coli* for potential mass production of C30 carotenoid. A *Fructilactobacillus* sp. possesses this operon and produces yellow pigment which proves that the operon is functional. C30 carotenoid production gives ecological advantages to the organisms by providing multistress resistance including resistance to oxidative stress and low pH. Carotenoids can be degraded during fermentation and food processing, but a carotenoid-producing lactobacilli can enhance nutritional and functional values in fermented foods. So far no studies have investigated the origin, distribution and function of the *crtMN* operon in members of the *Lactobacillaceae* family.

This study aimed to confirm the presence of 4,4'-diaponeurosporene using LC-MS and characterize the *crtMN* operon in lactobacilli using phylogenetic and genomic analyses. Preliminary liquid chromatography showed one peak different between the *crtMN* positive and negative strain whose retention time close to the identified 4,4'-diaponeurosporenic acid. A BLASTp search against available genomes of the *Lactobacillaceae* family in NCBI database identified multiple genes with 30 – 60% identity.

Result indicated 9 genera in the *Lactobacillaceae* family carry the operon; of these, 54% and 39% are species of the genera *Fructilactobacillus* and *Leuconostoc*, respectively. Among the positive *Fructilactobacillus* species, all strains (100%) are carriers whereas carrier strains in *Leuconostoc* vary from 5 to 100%. Phylogenetic and GC content analyses revealed that the operon is associated with the insect-adapted lifestyle and indicated evidence of horizontal gene transfer.

This study provides insights to the ecological fitness of lactobacilli as well as the application of carotenoid-producing lactobacilli in food fermentation.

Oral 5.1

LARGE SCALE METAGENOMIC ANALYSIS OF THE FERMENTED BEVERAGE KEFIR

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Milk kefir, a fermented dairy beverage, has a long history of safe consumption. Particular milk kefir beverages and the strains therein, have also been associated with specific health benefits. A comprehensive metagenomics-based understanding of the microorganisms present within milk kefir communities can help to define the milk kefir pan-metagenome, which includes details such as how the core milk kefir microbiome and the extent non-core components can vary. Such insights are of considerable scientific and commercial value and can contribute to future efforts to create tailored kefir-based microbial communities for different applications.

To facilitate this we undertook a large-scale metagenomic analysis of milk kefir samples, generated by inoculating full fat, pasteurised cow's milk with 64 separate kefir grains sourced from 25 different countries, to study patterns of strain, species, and functional pathway variation. Ultimately, this study provides the most comprehensive insight into the microbiomes of kefir to date through the establishment of a pan-metagenome, yielding novel information into the species, including putative new species and those residual of the unfermented milk, strain variability and potential functionality that can occur in kefir microbiomes derived from kefir grains sourced from across the globe. We further apply genome-resolved metagenomic analysis to milk and water kefir metagenomes derived from the same initial microbial population of bacteria, yeast and bacteriophage to study compositional change and microbial evolution at the species and strain level.

This was facilitated through the contributions of citizen scientists who used the common source of milk or water kefir grain to carry out fermentations using different substrates and growth conditions for up to 21 weeks. We observed an initial period of rapid compositional change over weeks one (wk01) to nine (wk09) in both the milk and water kefir liquid and grain microbiomes, including in some instances changes in community subtype as represented by the dominant species present, followed by a period of greater stability for many, though not all, metagenomes for the remainder of the study. Ultimately, our combined findings provide a new insight into how microbial ecosystems change and evolve over successive fermentations.

Oral 5.2

MICROBIAL BIOMASS AS FOOD INGREDIENT: HIGH NUTRITIONAL QUALITY AND RESOURCE EFFICIENCY OR HIGH PRODUCTIVITY?

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Traditional food production systems face challenges in meeting the growing global demand for protein, leading to resource depletion and environmental degradation. The need for more sustainable food production in combination with the increased consumer awareness has currently created a momentum on the pursuit for alternative protein sources. In this context, microbial biomass has emerged as a promising sustainable protein alternative. Nevertheless, often no consideration is given to the fact that the cultivation conditions affect the composition of microbial cells, and hence their quality and nutritional value. Apart from the properties and nutritional quality of the produced microbial food (ingredient), this can also impact its sustainability.

In this work, we investigate the effect of operational reactor parameters such as nutrient availability, temperature and pH on the nutritional quality, efficiency of resource utilization and production rate of microbial biomass from pure cultures of bacteria and yeasts to be used as a food ingredient. Our analysis shows that high nutritional quality and high resource utilization efficiency cannot be combined with high productivity, thereby indicating that a careful parameter selection should be made.

Deviation from optimal growth conditions significantly affects the microbial growth rate, directly influencing biomass production rate. Beyond the production rate, resource utilization efficiency is also negatively impacted by the deviation of operational parameters from the optimal range for growth. Lower growth rates result in biomass with increased protein content and decreased nucleic acid content, while higher rates yield larger cells, potentially improving biomass harvesting efficiency. Proteome allocation also varies across different growth rates, with more ribosomal proteins at higher rates, potentially influencing the techno-functional properties of the biomass. The distinct amino acid profiles of cellular components lead to variations in product quality, suggesting that targeted growth rates could reduce the need for costly external amino acid supplementation.

In summary, our study highlights significant tradeoffs between nutritional quality, production efficiency and production rate in microbial biomass due to the operational parameter selection, which need to carefully be evaluated when designing and implementing microbial biomass production processes. This knowledge is fundamental for the development of sustainable and high-quality and economically viable microbial food ingredients.

Oral 5.3

TO GUIDE OR NOT TO GUIDE? COMPARISON BETWEEN GUIDED AND SPONTANEOUS FERMENTATION OF BOLETUS EDULIS IN LABORATORY AND CULINARY CONDITIONS: IMPACT ON MICROBIAL POPULATION AND AROMATIC COMPOUNDS

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Fermented foods are part of the culinary tradition of cultures all over the world. Fermentation of raw materials has been conducted for millennia mainly because of the extension of the shelf-life and for safety reasons, but also to provide an enhancement of organoleptic features. Due to these, the fermentation process is not only perpetuated on an industrial and more controlled scale but is also conducted in domestic conditions. Also, cooks around the world have started to discover the possibilities of using fermentation to enhance and confer new flavors to a variety, sometimes unusual, of food substrates. Despite very fascinating, the trend to produce homemade fermented foods by spontaneous fermentation, in less controlled conditions, may represent potential risks to consumers' health and lead to poor quality foods.

This work aimed at comparing spontaneous and guided fermentation of *Boletus edulis* from a microbiological and aromatic perspective. Both fermentations were conducted at a lab scale and in a restaurant located in Andalo (Trento, Italy) by a professional chef. The *Boletus edulis* samples were added with 2.5% NaCl and fermented under anaerobiosis spontaneously or by inoculating 7 Log CFU/g of *Lactiplantibacillus plantarum*. The effect on microbial diversity of the two different types of fermentation and locations was evaluated by counting lactic acid bacteria (LAB), yeast, moulds and total viable microorganisms and by monitoring the pH values for 6 days.

The results underlined a faster acidification in the guided fermented samples and a predominance of LAB during both fermentations. All the samples were then analyzed using the DNA shotgun metagenomics technique to unravel both the taxonomic composition and functional profiles of the microbial community. The results showed a variable microbial composition of the spontaneously fermented samples and a high prevalence of *L. plantarum* in the guided fermented ones. Volatile fraction was characterized for all the samples by HS-SPME/GC-MS analysis, indicating higher variability in the spontaneously fermented samples.

The results of this study proved the feasibility of *Boletus edulis* guided fermentation also in restaurant conditions, which could increase product quality's standardization and safety.

Oral 5.4

INTERSPECIES YEAST INTERACTIONS DURING WINEMAKING PROCESS: SACCHAROMYCES CEREVISIAE AND STARMERELLA BACILLARIS PERFORMANCE DURING MIXED FERMENTATIONS IN NATURAL RED GRAPE MUST

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During wine fermentation, yeast-yeast interactions among non-*Saccharomyces* and *Saccharomyces cerevisiae* species have a key role in wine quality. Numerous studies have been conducted to understand the nature of these interactions. It has been confirmed that the dominance of *S. cerevisiae* over non-*Saccharomyces*, is not solely associated with the concentration of toxic compounds, mainly ethanol, and nutrients depletion. With the use of classical microbiological tools, it was demonstrated that the physical separation of *S. cerevisiae* and *Starmerella bacillaris* during fermentation of natural red grape must leads to longer survival of the latter. There is however a lack of understanding on the causes that lead to this phenomenon. By utilizing a transcriptomics approach, it should be possible to comprehend the reasons behind these phenotypic observations. This becomes an important step to further expand the use of non-*Saccharomyces* yeasts in wine making.

A pasteurized Nebbiolo grape must was fermented under semi-anaerobic conditions at 25 °C by *S. cerevisiae* and *Starm. bacillaris* (two strains) in pure culture, mixed in flasks, and physically separated using a double-compartment bioreactor equipped with a filtering membrane. The growth kinetics, assessed by plate count, showed viable *Starm. bacillaris* cells in mixed fermentation with physical contact until the seventh day. On the other hand, physical separation of the two species delayed the disappearance of the non-*Saccharomyces* yeast until the fourteenth day; hereby, not all sugars were consumed, although a higher amount of glycerol was produced, and ethanol was reduced by about 1% (v/v). The main enological parameters analyzed by HPLC confirmed an equal exchange of metabolites in both compartments through the membrane. Moreover, in all mixed fermentations, *S. cerevisiae* didn't reach the same cell concentration as observed in purity.

Cells were collected at different time points from all fermentation conditions to make multiple comparisons through transcriptomic analysis. RNA sequencing makes it possible to evaluate changes in gene expression, highlighting over- and under-expressed metabolic pathways. Comparing data across different times and conditions allows for a more comprehensive understanding of their involvement in the observed behavior, bridging the lack of information regarding interaction mechanisms, contributing to yeast modulation and wine quality management.

Oral 5.5

NEW AND STABLE LACTIC ACID BACTERIUM-YEAST CONSORTIUM TO BE USED AS STARTER CULTURE FOR SOURDOUGH PRODUCTION

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Sourdough production consists of a fermentation of a flour-water mixture by mainly lactic acid bacteria (LAB) and yeasts. The use of starter cultures allows to steer this process to enable enhanced flavour formation and a highly reproducible and fast production of a desirable and safe sourdough. However, the use of mixed-strain starter cultures requires stable interactions among the composing strains that have to overrule the background microbiota under the appropriate production conditions.

In this study, a screening of sourdough LAB strains concerning fruity ester formation was carried out. A genotypic assay (*in silico* genome mining of ester biosynthesis genes) and a phenotypic assay [monitoring of ester production in wheat sourdough simulation media (WSSM)] were applied. The LAB strain *Companilactobacillus crustorum* LMG 23699 possessed the genes targeted, produced several esters in the presence of the necessary precursors, and was stable during Type 3 sourdough productions. Moreover, a stable consortium with a strain of the yeast *Wickerhamomyces anomalus* evolved.

This new LAB-yeast consortium successfully performed as mixed-strain starter culture for the production of Type 2 and Type 3 sourdoughs. Furthermore, co-cultivation of these strains in WSSM led to a faster and total depletion of the carbohydrates and a faster drop of the pH compared to the monocultures, providing competitiveness toward background microorganisms. In addition, the higher carbon dioxide production during co-cultivation could result in desirable bread volumes.

Finally, sourdough breads with only the LAB strain and with both the LAB and yeast strains were made and compared with breads without sourdough and involving baker's yeast or *W. anomalus* solely. These breads varied in specific volume, acidification profile, volatile organic compound composition, and flavour.

To conclude, this new and stable *Coml. crustorum*-*W. anomalus* consortium could be used to produce novel sourdoughs and flavourful breads thereof.

Flash communications

FROM WASTE TO VALUE: UNLOCKING THE POTENTIAL OF RED WINE POMACE AND MICROALGAE THROUGH TAILORED FERMENTATION

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In the search for healthy and sustainable nutrient sources, functional food ingredients that are highly bioavailable and bio-accessible are becoming increasingly appreciated. To achieve this, the integration of microalgae and wine pomace through fermentation was explored to enhance the nutritional value and bioactive properties of the resulting food ingredient. Five lactic acid bacteria (LAB) and five yeast strains were selected based on their metabolic potential and diverse origins. Over 72 hours of fermentation period, this synergistic combination addressed the issue of limited growth observed in substrates composed solely of pomace. All LAB and yeasts effectively utilized sugars and released microbial metabolites in the mixture of *Chlorella vulgaris* and wine pomace. Additionally, the incorporation of wine pomace enriched the phenolic profile of *C. vulgaris*. Employing a tailored fermentation approach, we obtained a significant enhancement in the bioavailability of phenolic compounds, particularly flavanols, flavonols and procyanidins. Furthermore, fermentation underscores the presence of potential bioactive peptides and amplifies the antioxidant activity of the mixed fermented samples. The best performing strains were chosen for evaluating the protein digestibility.
The study showed that targeted fermentation can enhance the nutritional quality and bioactivity of *C. vulgaris* and wine pomace, increasing protein digestibility. These findings highlight the potential of this approach to generate a health-promoting food ingredient and consequently its potential applications in the food industry.

MICROBIAL DYNAMICS AND PHYSICO-CHEMICAL CHANGES DURING 6-MONTH STORAGE OF FERMENTED SEA FENNEL PICKLES STABILIZED BY MILD PASTEURIZATION

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Lacto-fermentation and pickling have traditionally been applied to the processing of vegetables, primarily to inhibit the growth of spoilage and pathogenic bacteria, extend the shelf-life, and enhance the sensory appeal of the final product. Sea fennel (*Crithmum maritimum* L.), a halophyte widespread in the coastal areas of Southern and Western European countries, is considered an emerging crop, with a great potential for food innovation due to its functional and sensorial qualities.
The present study aims to explore the use of sea fennel for the manufacture of new fermented pickles. To this end, fresh sea fennel sprouts and leaves were fermented in brine using a multi-strain lactic acid bacteria starter. Fermented sea fennel was then distributed into 500-mL glass jars, each filled with 200 g of sea fennel and 300 mL of pickling liquid containing 3% (w/v) saccharose, mineral water and either white wine vinegar or apple vinegar, to achieve the following total acidity at equilibrium: 0.05%, 0.20%, 0.5%, and 0.7%. For each type of vinegar and percentage of total acidity, three technical replicates were produced, resulting in a total of 24 samples. All jars underwent a mild thermal treatment equivalent to 74°C for 3 min. Pickles were assayed immediately after pasteurization and at regular intervals during their six-month storage at room temperature for the following parameters: pH, titratable acidity, color, and viable counts of total mesophilic aerobes, lactic acid bacteria, yeasts, moulds, and *Enterobacteriaceae*.
No significant differences were observed in the physico-chemical traits of pickles during the monitoring period, regardless of the type of vinegar. For color, slight variations were noted during the 6-month storage. Regarding the enumeration of key microbial groups, at the end of the monitoring period, pickles with the lowest acidity (0.05%)

showed mean viable counts of molds and total mesophilic aerobes equal to 3.6 ± 0.1 and 4.7 ± 0.1 Log CFU g–1, respectively, whereas for 0.20%, 0.5%, and 0.7%, viable counts were below the detection limit.
The preliminary results of this research support the exploitation of lacto-fermentation combined with pickling for the manufacture of new shelf-stable preserves with sea fennel.

ADVANTAGES OF CONTROLLED FERMENTATION VS. SPONTANEOUS FERMENTATION OF A TRADITIONAL NIGERIAN FERMENTED BEVERAGE, KUNU-AYA

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Africa is known to be rich in resources and agriculture, particularly the country of Nigeria, which is the most populous country in Africa. Many traditional fermented foods originate in Africa because of the natural preservation that fermentation offers. However, spontaneous fermentation has its disadvantages, one being that the naturally occurring microorganisms lead to spoilage of fermented foods and can overgrow fermenting bacteria, resulting in a microbiologically unsafe product. One way to combat this problem is to introduce controlled fermentation by developing starter cultures that are able to inhibit the autochthonous bacteria and produce a product that has a longer shelf-life. The shelf-life aspect is very important in countries such as Nigeria due to the limited cold-chain processes.
In this collaborative project, lactic acid bacteria were isolated from various spontaneously fermented tiger-nut beverages, namely Kunu-aya, which is a popular fermented beverage in Nigeria. These isolates were phenotypically screened for their antifungal activity against 3 different molds known to cause spoilage in plant-based products. The most promising antifungal strain, *Levilactobacillus brevis*, was chosen in addition to a *Lactiplantibacillus plantarum* strain, which was a species predominantly occurring in Kunu-aya, to be used for the controlled fermentation of Kunu-aya. A co-culture consisting of both strains and a spontaneous fermented control were also included. An *in-situ* challenge test of the controlled vs spontaneous 12-hour fermented Kunu-aya was performed with *Aspergillus flavus; Fusarium langesthiae* and *Penicillium crustosum* and the growth of the mold was monitored over 10 days.
Our results showed that Kunu-aya fermented with a co-culture of *L. brevis* and *L. plantarum* delayed the growth of all three mold species for 7 days compared to visible spoilage of the spontaneously fermented control. The pH was monitored during fermentation and samples were taken at T0, 6h and 12h after fermentation for HPLC. Analyses of organic acids and sugars showed that higher acetic acid production in the Kunu-aya fermented with the co-culture could be responsible for the delay in mold growth.
This project highlights the advantages of controlled fermentation and offers insight into what all can be achieved by using functional starter cultures.

NUTRITIONALLY RELEVANT TRANSFORMATIONS OF PEA FLOURS RELATED TO LACTIC ACID FERMENTATION

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Recently, the demand for high-protein flours in the food industry has increased significantly. Legumes are the most commonly used raw material for their production, as they are also highly nutritious. However, they also contain anti-nutrients, such as phytic acid, which can reduce the nutritional value of the final product. There are several physical, chemical, and biological methods to reduce the content of phytic acid and thus increase the nutritional value of legume flour. One such approach is lactic acid fermentation, resulting in anti-nutrients degradation and formation of bioactive compounds that can either additionally increase or decrease the nutritional value of food.
In our research, we performed 24-h and 48-h spontaneous and guided fermentations (*L. plantarum* LMG 6907) in order to examine their effect on the nutritional value of pea flour fractions with different protein contents. We found large differences between spontaneous and guided fermentations in the extent of proteolysis and accumulation of lactic acid,

biogenic amines and GABA, and the phytic acid content. The extent of these transformations depended on the type and duration of fermentation as well as the initial flour composition. Major nutritional benefits were related to accumulation of GABA and partial degradation of histamine and phytic acid. The main downsides of spontaneous fermentation were low reproducibility, accumulation of nutritionally undesired biogenic amines, and excessive proteolysis.
The results of our work show un unpredictable nature of spontaneous fermentations, which can be effectively avoided by using starter cultures with known metabolic activity and thereby standardizing the fermentation process to achieve better nutritional value and safety of the final product.

LACTIC ACID BACTERIA FERMENTATIONS, A SUSTAINABLE TOOL TO IMPROVE THE TECHNOLOGICAL AND FUNCTIONAL PROPRIETIES OF NON-CONVENTIONAL PLANT-BASED SOURCES

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The growing worldwide population and decreasing food sources are important factors that are driving the interest of consumers and companies in the use of non-conventional plant-based sources. On the other hand, this kind of ingredient presents different challenges in food production, due to their scarce technological proprieties, organoleptic acceptability, and safety.
To overcome these problems, lactic acid bacteria (LAB) fermentations represent a valid, and sustainable tool to enhance the technological proprieties, flavour, and stability of the products. LAB are universally recognized as safe, fast-growing and ubiquitarian in a lot of different food matrix. In the toolbox of their metabolism, they have the ability to produce aromatic compounds for the flavour, exopolysaccharides for the texture, and antimicrobial compounds for the maintenance of safety.
This study aimed to evaluate the effect of LAB fermentation on the physicochemical properties of chickpea, sorghum flours and pea protein extract (PPE). All the strains used were wild strains belonging to the University of Parma Culture Collection (UPCC). For the two flours, three different LAB strains were selected according to their known different phenotypic characteristics and their origin: *Lactobacillus delbruekii lactis*, *Lacticaseibacillus casei*, *Leuconostoc sp.*
Results obtained using impedometric analysis, showed that all the tested strains were able to grow on both flours reaching a concentration over 108 UFC/g, showing a short lag time, rapid growth, and a good acidification capacity. PPE was found to be a difficult matrix for the LAB fermentation, due to the initial presence of an elevated number of autochthonous spore-forming bacteria, that were also able to grow during fermentation and compete with LAB. In fact, only 2 out of 25 LAB strains tested were able to grow. *Streptococcus thermophilus* and *Lactobacillus delbruekii subsp. bulgaricus* were then used for the fermentation and they showed rapid growth, reaching pH below 4.5 necessary to grantee the safety of the final product.
Even better results were observed when the two strains were used in co-culture, as they were able to further increase the viscosity of the final product and inhibit the growth of the contaminants.

FUNCTIONAL YAM-BASED ICE CREAM FERMENTED BY A INDIGENOUS PHYTASE-PRODUCING LACTIC ACID BACTERIA STRAIN

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Lactic acid bacteria strains can ferment a variety of substrates, such as cereals, roots, and tubers, and improve the food's functional characteristics. The aim was to select phytase-producer lactic acid bacteria strains able to ferment yam. Moreover, develop and characterize a yam-based fermented ice cream. For selection: 13 amylase-producing LAB strains were evaluated based on their phytase production in a modified MRS medium, positive strains were selected for the yam fermentation assay. Total allantoin and diosgenin contents were evaluated.
Afterward, the starch content (Somogyi–Nelson methodology), minerals (absorption spectrophotometry and flame photometry), total polyphenol content (TPC)

(spectrophotometer according to the Folin-Ciocalteu method), radical scavenging activity –1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3 ethylbenzo-thiazoline-6-sulfonic acid) (ABTS)–, and rheology of the unfermented/fermented yam ice cream were evaluated. Also, the LAB viable cells were determined through fluorescence microscopy and population by MRS-plating. *Leuconostoc lactis* CCMA 0415, *Lactobacillus plantarum* CCMA 0744, and *Lactobacillus fermentum* CCMA 0745 produced phytase, acidify the substrate, and grow during 24 h of fermentation.

Among the selected strains, *L. lactis* CCMA 0415 maintained a cell viability above 8 log CFU/mL and did not reduce diosgenin concentrations (around 8.0 µg/mL) after 24 h of fermentation, generating a potentially functional yam food. The allantoin concentration remained constant (around 0.5 mg/mL) in all fermentations. The fermentative performance of *Leuconostoc lactis* CCMA 0415 was evaluated during the preparation and storage of yam-based ice cream. During 90 days of storage, *Leuconostoc lactic* CCMA 0415 maintained viable (107 CFU/g). The fermentation process reduced the starch concentration from 26.82% to 22.35% and the protein concentration from 4.68% to 3.99%. However, the concentration of some minerals (K, S, Cu, Mn, Zn, and Fe) was increased. The TPC for fermented and unfermented ice creams were 51 and 54 mg, respectively. The radical scavenging activity was 18% and 10% with the DPPH method and 44% and 26% with the ABTS method for the unfermented and fermented samples, respectively.

The yam ice cream was characterized as non-Newtonian fluids exhibiting pseudoplastic behaviors. Yam is a suitable substitute for milk, making it an option to produce edible lactose-free ice cream with low fat.

SELECTION, USE, AND THE INFLUENCE OF LYSINE- AND METHIONINE-PRODUCING STARTER CULTURES IN THE NUTRITION AND PROCESSING IMPROVEMENT OF OGI

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The selection criteria of starter cultures (SCS) used for ogi production were reported. These SCS were used to improve processing technique, and lysine and methionine levels of ogi, a lactic acid fermented weaning food limiting in these two amino acids.

A pilot plant study used *Lactobacillus brevis* XO43 and *Saccharomyces cerevisiae* OY4 as SCS. Fermentation with SCS showed a constant final pH level of 3.35 in modified substrates after fermentation unlike spontaneously fermented; and significantly higher acidity (%TTA) indicative of faster fermentation. Adding sugar (2% w/w) and soybean flour (1% w/w) also increased the acid levels during fermentation. Fermentation of dehulled maize (DM) grains fortified with glucose (2%w/w) alone by SCS shows the best quality in all the parameters considered except the available niacin yield. The lysine is 24% higher than oven-dried ogi flour from the traditionally fermented (TF) and 11% higher than unprocessed whole maize grains (WMG). The methionine is 92% and 77% higher than the TF ogi and WMG, respectively. The total amino acids level of the sample was 32% more than the TF ogi flour and 55% more than WMG. Although the soluble protein level was 23% less than the unfermented WMG, it was 12% more than the DM substrate and ogi from TF.

Fermentation of corn into *ogi* led to losses in the initial quantity of niacin. SCS significantly improves nutrients such as lysine, methionine, total amino acids, soluble protein, and niacin more than spontaneous fermentation. Dehulling of grains, dewatering, and oven drying significantly reduce nutrients. Ogi fermentation process with the use of SCS and DM guarantee organoleptic qualities, and improves ogi production by eliminating wet-sieving, limiting water usage, and creating better nutritional products.

EVALUATION OF MICROBIAL DYNAMICS OF GREEN AND BLACK KOMBUCHA CONSORTIA AND IN VITRO BIOACTIVITIES DURING ONE YEAR OF BACKSLOPPING

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Kombucha is one of the most popular low-alcoholic drinks among many traditional fermented beverages. This fermented drink contains several health-promoting components as a result of the complex symbiotic relationship within its microbial community. The fermentation process involves cooperative and competitive processes inside a microbial consortium encapsulated in a cellulose biofilm, known as SCOBY (Symbiotic Culture Of Bacteria and Yeasts). The production process involves a backslopping technique, in which a small portion of a previously brewed and matured kombucha is used as starter for initiating fermentation of a new batch. Backslopping provides a means of transferring the beneficial microorganisms from mature kombucha into fresh sweetened tea, ensuring the continuity of the symbiotic culture responsible for the fermentation process.
This study delves into the dynamic interplay between microbial consortia involved in the fermentation and the correlated bioactivities of successive propagations, in order to investigate the effect of backslopping on green and black kombucha fermentation. To gain a more comprehensive insight into the kombucha microbiota, a culture-independent, high-throughput sequencing analysis of bacterial and fungal populations of SCOBY and kombucha from different propagations, was performed.
Following the analysis, it was detected that the two largest bacterial genera present in both green and black kombucha were *Komagataeibacter* and *Acetobacter*, while for yeasts we found *Brettanomyces* and *Saccharomyces*. In particular, we evidenced differences in microbial community structures between black and green tea, the latter supporting a better growth of *Saccharomyces* over *Brettanomyces* with respect to black tea. Regarding microbial variations among microbial groups during time, an increase in *Komagataeibacter* was detected with respect to *Acetobacter* in both kombuchas. In addition, only in green kombucha *Saccharomyces* population significantly increased compared to *Brettanomyces*.
The microbiological differences between black and green kombucha propagations, are mainly related to: organic acids (gluconic acid, acetic acid), and caffeine. Our findings provide valuable insights into how successive propagations impact the stability and biodiversity of the microbial ecosystem in kombucha and related biochemical properties. This study is pivotal for harnessing the full potential of kombucha as a functional beverage, representing the most accurate description of the microbial evolution of successive backslopping of black and green kombucha to date.

MICROBIOLOGICAL CONSUMER SAFETY RISK ASSESSMENT OF 3 FERMENTS USING DIFFERENT PROTEIN SOURCES IN A MARGARINE PROCESSING

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The present study has evaluated potential microbiological safety risks of the ingestion of fermented products added as ingredient during margarines processing. Microbiological hazards are related to pathogenic growth (linked to potential formation of toxin) formed during fermentation (40 °C) by microorganisms. These microbes can be carried over from pasteurised raw materials (e.g., spore formers such as *B. cereus* or *Clostridium spp.*). or manual handling (*S. aureus*). Potential pathogenic organism presence in final product due to cross-contamination or outgrowth during processing is therefore of concern. These could include *B. cereus*, *S. aureus*, *Listeria monocytogenes*, *Salmonella*, *E. coli* and moulds (mycotoxins). 03 different fermentations were performed at Upfield pilot plant under aerobic conditions.
Fermentations were made with the same culture (YFL02DA) and 3 different sources of proteins were used: faba beans, almond and butter milk powder (BMP). Raw materials (protein, glucose, citrate, water, and sunflower oil 10%) were pasteurized at 78 °C/20 seconds, homogenized, and cooled down. Thereafter, cultures (YFL02DA) were added, overnight at 40 °C to ferment and reach pH 4.5. Fermented products were pasteurized in 2 different ways: 1-Filled bottles at 78 °C/60 minutes in a water bath. 2-Water phase of margarine at 85 °C/25 seconds + emulsion at 80 °C/45 s. Fermented products were storage at 5 °C up to 6 weeks. Microbiological analyses (spoilage, pathogens and

sporeformers) were performed to investigate the safety of the ferments and margarine as finished product before and after pasteurization.
In summary, ferment products made with faba got the highest level of *B. cereus* (i.e. 5500 cfu/ml). For the other 2 ferments (almond and BMP), *B. cereus* was also present but at an acceptable safety level according to ready to eat microbiological specification of foods. All samples of margarine as finished product, obtained acceptable levels of microorganisms. Considering microbiological results from water phase before pasteurization, after pasteurization and finished product, samples prepared with BMP and Almond did not pose any food safety risks to consumers and therefore were acceptable for tasting purposes. Levels of B. cereus observed in the faba bean mix were considered not acceptable and were not used further in the study.

SELECTION OF LACTIC ACID BACTERIA AND YEASTS TO INCREASE WATER EXTRACTABLE ARABINOXYLAN CONTENT IN WHEAT BRAN, AND THEIR EFFECT IN PASTA APPLICATION

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ZHAW

Wheat bran (WB) is one of the largest sidestreams worldwide making up 150 million tons annually. Despite its promising nutritional profile, intrinsic factors, such as the presence of insoluble pentosans, impede its inclusion in food products. The process of making pentosans soluble, particularly by enzymatic conversion of water-unextractable arabinoxylan (AX) to its water-extractable form (WAX), has been shown to enhance bread volume and texture in wheat baking. One potential strategy is degradation of the ferulic ester bridges, which crosslink AX chains, by enzymes of the endoxylanase group, such as ferulate esterases (FE). There has been limited exploration to integrate WB into food products other than baked goods, e.g., pasta.
Therefore, in this study, a phenotypic screening of 154 strains of lactic acid bacteria (LAB) for FE was performed and revealed 28 consistently positive strains. Four candidate strains belonging to the species *Limosilactobacillus fermentum, Levilactobacillus brevis*, and *Lactobacillus amylovorus* were applied in model WB fermentations (100 g), where they increased WAX up to ca. 35%, measured through a colorimetric method based on phloroglucinol. Further, three *Saccharomyces cerevisiae* strains with for reported unspecific xylanase activity were tested but did not lead to WAX contents higher than the unfermented WB. LAB-yeast co-cultures lead to ca. 26% WAX increase.Nevertheless, aiming at pH reduction by LAB and therefore suppression of unwanted microorganisms, a *L. brevis-S. cerevisiae* co-culture was selected for a scaled-up fermentation (750 g) followed by integration in pasta production.
The resulting dough quality measurements of breaking resistance, dough elasticity, and firmness failed to reveal significant differences between fermented and non-fermented WB. Furthermore, hedonic tests revealed higher acceptance of pasta with fermented WB compared to the unfermented version but lower acceptance than traditional durum spaghetti. The panel described durum-WB spaghetti as nutty, earthy, and soggy, while the durum-WB fermented spaghetti had a distinctive profile characterized as banana-like, fruity, acidic, and earthy.
Consequently, the selected process for altering fiber in WB resulted in products with higher potential consumer acceptance. However, the chosen dough analysis methods lack the sensitivity to detect differences produced by this method, emphasizing the need for further investigation in this promising field.

REDUCING BIOGENIC AMINES IN FERMENTED FOODS: IDENTIFYING HELPERS AND EMPOWERING THEM FOR ENHANCED EFFICIENCY

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Biogenic amines (BAs), low-molecular-weight organic bases, result from the microbial decarboxylation of free amino acids. They are present in diverse fermented foods such as sausages, salted fish, soy sauce, cheese, and wines. Excessive intake of these compounds poses potential risks to human health. The involvement of a complex microbial community in the food fermentation process underscores the importance of identifying both the 'enemies', such as *Morganella* sp., *Klebsiella* sp., and *Hafnia* sp., that contribute to BAs production, and the 'helpers', such as lactic acid

bacteria (LAB), that reduce BAs. Regarding this issue, our recent efforts have encompassed:

i. Optimizing a StageTip-based preprocessing method for detecting BAs in fermented foods. This technique, using only 0.05 g of samples, enables precise detection, revealing significant variations in histamine and putrescine content across different locations on the same salted fish.

ii. Screening LAB 'helpers', including *Lactobacillus* sp., *Tetragenococcus* sp., and *Weissella* sp., capable of reducing BA levels in various fermented products like salted fish, sausages, and soy sauce. Further research indicates these strains act through two main pathways: inhibiting 'enemies' bacteria growth and metabolism responsible for BAs production and directly degrading pre-existing BAs.

iii. Characterizing a LAB-derived BA-degrading enzyme, multiple copper oxidase (MCO), to reduce BAs in diverse food matrices. Our study emphasizes the significant influence of the food matrix on MCO's catalytic activity, with potential competitive inhibitors present. Moreover, matrices rich in natural mediators enhance oxidative reactions. These findings propose the design of tailor-made MCOs for specific foods for high-efficiency applications.

Accurate detecting BAs distribution - identifying helpers to reduce BAs - investigating their "weapons" - constitutes the focus of our recent research. Exploring their mechanisms forms the scientific basis for industrial reduction of BAs in fermented foods. In the future, we hope more scientists will contribute to this field, enhancing the safety of fermented foods worldwide.

FERMENTATION OF CALENDULA, ECHINACEA AND MALVA OFFICINAL PLANT EXTRACTS AS A MEANS OF INCREASING THEIR SENSORY, ANTIOXIDANT, ANTIMICROBIAL AND NUTRACEUTICAL PROPERTIES

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Plants are well known sources of bioactive compounds and of indigenous microflora, that can have beneficial effects on human health. Fermentation is an ancient food technology, still today subject of study and improvement, that can enhance sensory, organoleptic, nutritional quality and shelf-life of products. Moreover, fermentation of plant-based extracts can improve antioxidant activity and bioaccessibility of natural bioactive compounds, as well as reduce antinutritional components and toxic substances in plant foods.

In this research project, three plant extracts were used for fermentation tests with microorganisms capable of developing on these substrates. Aqueous extracts of *Calendula officinalis*, *Echinacea purpurea* and *Malva sylvestris* were prepared and separately inoculated with *Lactiplantibacillus plantarum* 299V, *Pediococcus acidilactici* 12B, *Lacticaseibacillus rhamnosus* GG and *Bacillus subtilis natto*. Appropriate pasteurization treatments, inoculum levels and fermentation conditions were developed. Extracts before and after the fermentation were evaluated for the presence of new properties or potentially beneficial molecules, especially in terms of microbial count, acidity, sugars, organic acids, proteins, minerals, poliphenols, tannins, vitamins, total phenolic compounds and antioxidant activity.

In all plant extracts lactic acid bacteria grew well, increasing their populations by more than 3 log, and inducing a decrease of about two pH units. An increase in some minerals was observed in fermented extracts of all three plants in comparison to the non-fermented controls. Total phenolic compounds and antioxidant activity increased in fermented extracts of *Echinacea* and *Malva* in comparison to the non-fermented ones, while no significant differences were

detected in *Calendula* extracts. In all three fermented extracts a decrease in glucose and fructose and a consequent lactic and acetic acid production were observed.
The results obtained encourage further studies on the possibility to use such fermented extracts in the production of nutraceutical postbiotic foods.

EXTRACELLULAR VESICLES FROM LACTOCOCCUS LACTIS DELIVER VITAMIN K_2 TO HUMAN CELLS

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Fermentation can improve the nutritional quality of foods due to activities or metabolites from fermenting microbes. Lactic acid bacterium *Lactococcus lactis* is widely used for food fermentation worldwide, and is a natural producer of vitamin K_2, offering unique opportunity to introduce this vitamin to food products. Vitamin K_2 is a lipophilic/hydrophobic vitamin accumulated in the membrane of producing bacteria, and plays essential roles in human health as a carboxylation co-factor.
However, the hydrophobicity of vitamin K_2 forms poses challenges to their uptake by target cells of the human host to achieve desired biological function. In this study, *L. lactis* has been shown to secret bacterial extracellular membrane vesicles (EVs) that contain vitamin K_2. When these EVs were applied to *in vitro* grown osteosarcoma cells, the carboxylation status of an important calcium-binding bone protein named osteocalcin, increased, indicating functional delivery of bioactive vitamin K_2 by bacterial EVs. Notably, the efficiency of vitamin K2 delivery by EVs appeared higher than adding solvent-dissolved pure compounds at similar concentrations.
Therefore, this study provides proof of principle that bacterial EVs are ideal vehicles to deliver lipophilic compounds like vitamin K_2 to human cells. Research on EVs produced by bacteria that are related to dairy environments or key players in dairy/food fermentations, will promote the applications of bacterial EVs in efficient delivery of bioactive, nutritional compounds from the microbial origins to the human host, contributing to improved nutrition and conceivable health benefits. Moreover, microbial EVs and the diverse cargo components also suggest their potential roles in microbial interactions, and influences on (human) microbiota and ecology.

FOOD MICROBES IN PLASTISPHERE: MICROBIAL ECOSYSTEM ON MICROPLASTICS

Oral 4.5

MICROPLASTIC MEDIATED TRANSFER OF TETRACYCLINE RESISTANCE: UNVEILING THE ROLE OF MUSSELS IN MARINE ECOSYSTEM

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Microplastic pollution has become a major environmental concern in aquatic ecosystems supporting the dissemination of antimicrobial resistance genes. Mussels, due to high susceptibility to microplastic accumulation, are now recognized as important indicators of marine microplastic pollution.

In this study, we investigated the transferability of tetracycline resistance mediated by the conjugative transposon Tn*916* from *Enterococcus faecium* UC7251 to two *Listeria monocytogenes* strains in a complex marine environmental model. Firstly, individual biofilms of the donor and recipients strains were created on microplastic particles. These biofilm-coated microplastics were then introduced into an aquarium housing *Mytilus galloprovincialis*, and the conjugation rate was evaluated after four and seven days of exposure. The transfer of *tet*(M) primarily occurred in mussels, exhibiting conjugation rates of 10-4 T/D. Interestingly, this phenomenon was also observed in the salt water, albeit at lower conjugation rates of 10-5 T/D.

This study demonstrated that the transfer of the *tet*(M) gene from *E. faecium* to both *Listeria* strains was facilitated by the presence of microplastics combined with filtering effect of mussels.

Flash presentations

PLASTICS AS A POTENTIAL VECTOR FOR SPREAD OF ANTIMICROBIAL RESISTANCE AND PATHOGENS FROM WASTEWATER DISCHARGE TO THE MARINE ENVIRONMENT AND HUMAN FOOD VALUE CHAIN

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Antimicrobial resistance (AMR) and marine plastic pollution are significant environmental and global health concerns. However, there is limited knowledge concerning the occurrence of pathogens and antibiotic-resistant bacteria (ARB) in wastewater (WW) effluents from urban areas and in the coastal marine environments into which they are discharged. Furthermore, the potential role of plastics as a vector for transmitting pathogens, ARB, and antibiotic-resistant genes (ARG) throughout the marine environment and into the human food chain is poorly understood. The PlastiSpread project studies the role of the microbial community in biofilms associated with marine plastics, and how they are influenced by variations in urban WW discharges. Furthermore, the potential role of marine (micro)plastic litter to act as a reservoir and vector for the development and spread of AMR and pathogenic bacteria in marine environments and into the human food chain is evaluated.

By combining field- and lab-scale plastic model systems, the influence of WW effluent composition and polymer type (PE, PS and PVC), on the bacterial community composition

in plastic-associated biofilms will be investigated in relation to other materials (wood, glass). The occurrence of potential pathogens, ARBs and ARGs will also be studied. Laboratory experiments performed in Norway and Greece will utilise microplastic forms of each material (300-500 µm) and will investigate to what degree differences in antibiotic use will influence the occurrence of ARB and ARGs in local effluent WW. The field model system will contain plates (10 x 5 cm) of each selected material, which will be placed at exposed and non-exposed sites in Norway. Marine bivalve molluscs will be used as indicators for exploring the effects on marine organisms present at the same sites. Laboratory experiments will also be performed to determine the process of horizontal gene transfer of ARGs from WW to susceptible plastic-associated biofilm bacteria.

The knowledge obtained in the PlastiSpread project will increase our understanding of marine plastic litter as a vector for pathogens and ARB in the marine environment in general and further into the human food chain. Initial results from WW characterisation will be presented at the conference.

FOOD MICROBIOTA AND IMPACT ON HUMAN MICROBIOME

Keynote 1.2

DISENTANGLING THE COMPLEX RELATIONSHIP BETWEEN FOOD MICROBIOTA AND GUT MICROBIOME: AN UPDATE

Paola Mattarelli

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The relationship between food and gut microbiota is complex and multifaceted, playing a significant role in human health. Food like fermented foods can contain indigenous beneficial microbes, microbial metabolites, and other bioactives, which are harnessed to target gut microbiota and play a significant role in human health and well-being. Therefore, food microbiota can offer an affordable dietary intervention strategy being involved in the reduction of a risk factor such as cholesterol and trimethylamine oxide (TMAO) and the production of health-improving factors such as folate, gamma-aminobutyric acid (GABA), conjugated linoleic acids (CLA), exopolysaccharides (EPS), short-chain fatty acids, etc.

On the other hand, it has to be considered that probiotics exhibit significant variability in their engraftment within the gut microbial community, occurring in some subjects but not in others. Studying the interaction between food and gut microbiome is becoming increasingly crucial in this scenario. As basic research continues to unveil the effects of various novel dietary components and their microbial byproducts on the gut microbiome, the extent of their efficacy in enhancing gut health and addressing gastrointestinal diseases awaits clarification through population studies employing the microbiome, probiotics, and epidemiology approach.

Oral 1.4

BACTERIA-NEURONS INTERACTION: A NEW INTERPRETATION OF THE GUT-BRAIN AXIS

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The last twenty years have been characterized by intense efforts to investigate the role of gut microbiome in human health and wellbeing. It is now well accepted by the scientific community that the microorganisms that we have in our gastrointestinal tract (GIT) and their functional activities play a pivotal role in health and disease, and how a well-balanced GIT ecology is preventing the onset of non-communicable diseases (NCDs).

Gut microbiome can influence not only the functioning of the intestine, but also organs that are relatively far from the intestine, such as the brain, the lungs and others. For this reason, several gut-organ axes have been established and represent a new frontier of research and understanding on the importance of the human gut microbiome. In this scenario, the gut-brain axis, believed to be based mainly on chemical messages, produced in the gut the microorganisms and then transported to the targets through the circulatory system, is nowadays investigated taking into account the impact that physical contact of bacteria with neurons can produce.

In this study, we have investigated the physiological and transcriptomic responses of primary cortical rat neurons (RN) in contact with the putative probiotic *Lactiplantibacillus plantarum* 02T60C (Lpb02).

First, adherence of LpbO2 cells on neuron surfaces has been observed after 5 minutes and progressively increased along time by reaching the maximum level at 30 minutes, regardless of the multiplicity of infection (MOI) used. Temporal analysis of calcium imaging indicated an immediate calcium-mediated sensing response of the neurons to the LpbO2 presence, which remained constant until the thirtieth minute of co-culturing. Furthermore, immunological assays highlighted a greater formation of synapsis in co-cultured neurons compared to untreated ones, but no caspase-related signs of apoptosis. Accordingly, the transcriptomes of neurons co-cultured with LpbO2 and alone have been assessed after a timeframe of 30 minutes. We observed that 15 % of the 17,743 transcribed genes were differentially expressed (DEGs) in co-cultures compared to untreated neurons. On the basis of DEGs, pathways related to tissue regeneration, anti-inflammation activities and response to axon injury were significantly enriched in co-cultures.

Oral 1.5

A METHYL ESTERASE FROM *BIFIDOBACTERIUM LONGUM* SUBSP. *LONGUM* RESHAPES THE PREBIOTIC PROPERTIES OF APPLE PECTIN BY TRIGGERING DIFFERENTIAL FECAL MICROBIOME MODULATORY CAPACITY

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Pectin structures have received increasing attention as emergent prebiotics due to their capacity to promote beneficial intestinal bacteria. Yet the collective activity of gut bacterial communities to cooperatively metabolize structural variants of this substrate, remains largely unknown.
Herein, a pectin methylesterase, named BpeM, and whose encoding gene was almost exclusively present in bifidobacterial strains belonging to *Bifidobacterium longum* subsp. *longum* species was characterized. The purified enzyme was able to remove methyl groups from highly methoxylated apple pectin, and the mathematical modelling of its activity enabled to tightly control the reaction conditions to achieve predefined final degrees of methyl-esterification (DM) in the resultant pectin. Demethylated pectin generated by BpeM, exhibited differential fermentation patterns by gut microbial communities in *in vitro* fecal cultures promoting a stronger increase of bacterial genera associated with beneficial effects, including *Lactobacillus*, *Bifidobacterium* and *Collinsella*.
Our findings demonstrate that controlled pectin demethylation by the action of a *B. longum* esterase selectively modifies its prebiotic fermentation pattern, producing substrates that promote targeted bacterial groups more efficiently. This opens new possibilities to exploit biotechnological applications of enzymes from gut commensals to programme prebiotic properties.

Flash communications

FUNCTIONAL SWISS-TYPE CHEESES PROMOTE BENEFICIAL EFFECTS ON MICE HEALTH AND GUT MICROBIOME DURING INFLAMMATORY BOWEL DISEASE

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Backgrounds and aims

Inflammatory Bowel Diseases (IBD), including Ulcerative Colitis (UC), coincide with alterations of the gut microbiota. Immunomodulatory probiotic bacteria may induce or prolong remission in UC patients. Fermented foods, including cheeses, constitute major sources of bacteria. New *in vivo* evidences

revealed anti-inflammatory effects in selected strains of the dairy bacterium *Propionibacterium freudenreichii*, used as a cheese ripening starter. We thus hypothesized that consumption of a functional cheese, fermented by such a selected strain, may exert a positive effect on IBD.

Methods

We developed an experimental single-strain cheese solely fermented *P. freudenreichii* CIRMBIA-129. We moreover produced, in industrial conditions, an Emmental cheese using the same strain, in combination with *Lactobacillus delbrueckii* CNRZ327 and *Streptococcus thermophilus* LMD-9, as starters. We tested both cheeses, in healthy conditions and as preventive food in the context of Dextran Sodium Sulphate (DSS)-induced colitis in mice. We monitored the gut microbiota of mice using shotgun metagenomic sequencing. We assessed the taxonomic and functional profiles through sequence alignment to NCBI taxonomy and KEGG pathways databases.

Results

The experimental cheese and the Emmental cheese, both fermented by *P. freudenreichii*, reduced severity of subsequent DSS-induced colitis, weight loss, disease activity index and histological score. Both reduced small bowel IgA secretion, restored occludin gene expression and prevented induction of TNFα, IFNγ and IL-17. None of the cheeses disturbed the microbial community ecology in healthy conditions. Emmental intake increased symbionts as *Romboutsia* and *Akkermansia muciniphila*. Metabolic pathway analysis suggests that *A. muciniphila* may produce bioactive metabolites as acetate and cooperate with other commensal species to produce indole and gamma-aminobutyric acid. Some of the impaired microbiome metabolic functions, in colitis mice, were restored by single strain cheese, while Emmental promoted an increase in *Ligilactobacillus murinus*. This taxon presented several genes with immunomodulatory activity potential.

Conclusions

Combinations of starter bacteria immunomodulatory strains lead to anti-inflammatory cheeses, as revealed in an animal model of colitis. Such cheeses may restore beneficial bacteria in the gut microbiota. Further studies are undertaken to investigate their role in regulating the gut-brain axis. This opens new perspectives for personalised nutrition in the context of IBD.

PROBIOTIC LACTIC ACID BACTERIA ASSOCIATED WITH FERMENTED MILLET-BASED MILK BEVERAGE 'BRUKINA' AND THEIR EFFECTS ON THE GUT MICROBIOME

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Brukina is an indigenous spontaneously fermented millet-based milk beverage widely consumed in Ghana. To prepare, milled millet is steam cooked and mixed with fermented cow milk and sold as ready to eat food. This study sought to determine the microbial profile of *brukina,* potential probiotics associated with the product and the impact of consumption on the gut microbiome.

FDA approved *brukina* were obtained from supermarkets in Accra, Ghana and Lactic acid bacteria load was determined. 16S rRNA amplicons from cultured LAB strains and direct DNA from *Brukina* were sequenced. Acid and bile tolerance, adhesion capacity, antimicrobial activity and antibiotic susceptibility profile of the isolated lactic acid bacteria were determined. Human participants and animal models were fed with *brukina* samples for four weeks. Fecal samples were collected at different time points and 16SrRNA amplicons of microbial DNA sequenced. Microbial abundance was analyzed using Shannon indices.

LAB load was 104 to 106 CFU/mL and were identified as *Lactobacillus fermentum, Lactobacillus delbrueckii, Lactobacillus johnsonii, Lactobacillus prophage,* and *Lactobacillus taiwanensis* with antimicrobial activity against four enteric pathogens (*Escherichia coli, K. pneumoniae, Salmonella typhi, and Staphylococcus aureus*). There was good adhesion to Caco-2 cells, appreciable tolerance to acid and bile coupled with low resistance to antibiotics. Direct DNA extraction from brukina showed the presence of *Limosilactobacillus fermentum*, *Enterobacter hormaechei*, *Alishewanella agri, Neobacillus fumarioli, Bacillus safensis* and *Faecalibaculum rodentia.* There was an increased

abundance of *Lactobacillus delbrueckii* and *Lactobacillus fermentum* in the gastrointestinal tracts of human participants and rat models after four weeks of feeding.

Brukina is a promising African functional food due to its significant LAB content with probiotic potential. The abundance of lactic acid bacteria in the GIT tract after regular consumption of *Brukina*, helps in the competitive elimination of enteric pathogens. However, the presence of diverse microbes in the product underscores the complexity of the microbial community in the fermented millet-based milk beverage, hence making a case for the use of starter cultures.

SUPPLEMENTATION WITH BENEFICIAL MEDITERRANEAN DIET INGREDIENTS ENHANCES GUT MICROBIOME COMPOSITION AND METABOLIC POTENTIAL

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The Mediterranean Diet (MD) is a plant-based dietary pattern rich in polyphenols, which exert beneficial effects in the human body, such as anti-inflammatory and antioxidant activities. However, evidence suggests that these beneficial outcomes might be partly attributed to the human gut metabolism, which can potentially be modulated by the MD ingredients and compounds. To elucidate the dynamics occurring in the human microbiome following adherence to the MD, we conducted a randomized, placebo-controlled trial.

In detail, a superfood simulating the composition of the Mediterranean Diet or a placebo was administered to 122 overweight subjects for 8 weeks. The gut microbiome was sampled and analyzed by shotgun sequencing at baseline and at the end of the treatment. Overall, the biodiversity of the gut microbiome significantly increased in the treatment group at the end of the experiment. Also, we found significantly higher abundance of several potentially beneficial taxa from the genera *Faecalibacterium*, *Roseburia* and *Bifidobacterium*.

Furthermore, we explored the functional potential of these communities, highlighting a shift towards a health-promoting metabolism. In fact, the abundance of genes linked with butyrate production and conversion of glucosinolates to isothiocyanates significantly increased during the experiment in the treatment group. Similarly, the overall abundance of Glycosyl Hydrolase (GH) CAZymes increased during the treatment, suggesting an enhanced potential for carbohydrate (including fibers) degradation by the gut microbiome.

Overall, our results highlight the potential of the MD-based superfood to modulate the gut microbiome by selecting beneficial taxa with health-enhancing metabolic traits.

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FOODBORNE PATHOGENS

Keynote 6.1

SOURCE ATTRIBUTION OF *LISTERIA MONOCYTOGENES* TO DIVERSE ECOLOGICAL NICHES IN A ONE HEALTH APPROACH

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In a comparison of different production chains within the EU, food processing establishments were classified as "*Listeria* free or colonized" based on available self-monitoring data. Subsequent sampling revealed a clear picture - all FPEs were sporadic, hot spot or heavily contaminated with *L. monocytogenes*. This example illustrates the problem we face in monitoring: There is an obligation to provide data on *L. monocytogenes* contamination in FPEs, raw material characteristics, batch and shelf life of ready-to-eat foods as part of self-monitoring, but these data, if available, provide only a snapshot of the safety of the food produced. It is noticeable that mainly hypervirulent and outbreak-related genotypes are detected in the environment and also in farm animals.

The presence of *L. monocytogenes* is most frequently observed in the agricultural environment, in feces, in the contamination of lying surfaces, i.e. the skin, and thus upon entry into the slaughterhouse and milk processing rooms. In particular, clonal complexes (CC) of genetic lineage I (CC1, CC2, CC4, CC6, CC191, CC59) and lineage II (CC7, CC11, CC14, CC37, CC204) are regularly isolated from excreting animals, fecal samples and the farm environment. We recently assisted a company with remediation after an outbreak and had to ask ourselves the question: When is a food facility back under control? Multiple *L. monocytogenes* genotypes can be introduced through raw materials within a manufacturing process. The question is how to remove them and whether hypervirulent genotypes (e.g. CC1, CC2, CC4, CC6) also have persistence properties. Therefore, the food industry and regulatory authorities need a detailed analysis of the hypervirulent and outbreak-associated genotypes circulating in the environment and in primary production in order to develop improved biosecurity measures. The mapping of *L. monocytogenes* genotypes to the source of entry contributes significantly to risk assessment and improvement of food safety.

The workflow for *L. monocytogenes* genome analysis is as follows. Step 1 is to analyze the Illumina Miseq data using SeqSphere software to determine the clonal types (wgMLST). A second step is to combine the Miseq data with long read sequencing data (Nanopore) to identify mobile elements (virulence hotspots, bacteriophages, plasmids) associated with hypervirulence and niche adaptation. Data gaps related to the entry of *L. monocytogenes* into the hazard stage (e.g. slaughterhouse) will be identified and strategies to reduce the risk of *L. monocytogenes* contamination in the first steps of production will be elaborated.

Keynote 6.2

INTERVENTIONS FOR THE CONTROL OF NT-*SALMONELLA* SPP. AND *CAMPYLOBACTER* SPP. IN CHICKEN MEAT: CONCLUSIONS OF JOINT FOOD AND AGRICULTURE ORGANIZATION (FAO)/WORLD HEALTH ORGANIZATION (WHO) EXPERT MEETINGS AND INITIATIVES FOR THE COMPREHENSIVE STRATEGIES

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Contaminated food is a leading cause of diarrheal infections. According to the report from WHO, Non-typhoidal (NT)-*Salmonella enterica* from food sources results in approximately 79 million cases worldwide each year. Additionally, *Campylobacter* spp. is responsible for 96 million cases per year responsible of significant burden.

Worldwide poultry will supply 41% of meat protein in 2032 and the increasing demand and consumption of poultry meat may lead to the increase of foodborne diseases. Over 20% and 30% of the world's foodborne Salmonellosis and Campylobacteriosis are caused by broiler meat handling, preparation and eating. Though various strategies have been implemented to reduce these pathogens in poultry, challenges persist until today, which prompts the Codex Committee on Food Hygiene to consider updating guidelines to control these pathogens in chicken meat. FAO and WHO convened two Joint Expert Meetings on Microbial Risk Assessment (JEMRA) in September 2022 and February 2023 to evaluate recent data and evidence, providing scientific advice on control measures in the chicken meat production chain for NT-*Salmonella* spp. (*Salmonella*) and *Campylobacter* spp. (*Campylobacter*).

In the JEMRA meetings, the Experts Committee reviewed recent literature related to chicken meat production from the primary production to the post-processing stage. They concluded that several interventions could be useful for the control of both pathogens in chicken meat. Good hygiene practices are essential all over the food chain. In the primary production stage, biosecurity measures are highlighted with measures such as continuous control of incoming flocks. During the processing stage, irradiation can be effective to control both pathogens. Some chemicals such as chlorine and acid water additives show reduction of the pathogens, but their effectiveness is variable. Regarding *Campylobacter*, immersion and air chilling are useful in reducing concentration and prevalence in the carcass, as were scalding, marination and freezing processes. For *Salmonella*, the use of high-pressure processing can be effective. In the post-processing stage, to encourage a positive food safety culture through human behavior and consumer education is needed to avoid cross-contamination at home.

Though no stand-alone control measure is sufficiently effective in reducing either the prevalence or concentration with *Salmonella* and *Campylobacter* in chicken meat, the emphasis should be put on control strategies based on multiple intervention steps to control these pathogens. To guide on the development of the intervention strategies for the future, there is the need to pursue more accurate quantitative microbiological risk assessments, with the advent of next generation technologies including machine learning, omics, and tools for traceability in the chicken meat production chain.

Strategies to reduce both pathogens in chicken meat have been successful in some countries, but control has stalled, showing the need to change the approaches. Based on the outcomes and recommendations of JEMRA meetings, WHO in collaboration with FAO and WOAH is developing quantitative risk assessments and surveillance tools in promoting and supporting comprehensive strategies to *Salmonella* and *Campylobacter* risk reduction in chicken meat.

Keynote 8.1

MAPPING AREAS AT RISK OF FOODBORNE PATHOGEN CONTAMINATION IN FRUIT AND VEGETABLE PRODUCTION

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Due to the nature of their production horticultural crops are at risk of microbiological contamination from a range of sources including soil, irrigation water, bird droppings, wash water, processing equipment, staff etc. Washing cannot remove all contamination from fresh produce so it is therefore imperative that all reasonable steps are taken to minimise contamination risks during production, processing and transport.

This is particularly important given that these commodities are often eaten without cooking. Growers need to be aware of where risks may arise on farm so that targeted interventions can be put in place to ensure produce safety, and reduce the possibility of cross contamination of produce with foodborne pathogens.

A number of case studies will be presented which will outline the areas in horticultural production at greatest risk of contamination, the factors which can influence this, and mitigation measures which can be employed to reduce contamination. The application of sequencing technologies to further understand contamination patterns will also be presented.

Keynote 8.2

JOINT FOOD AND AGRICULTURE ORGANIZATION (FAO)/WORLD HEALTH ORGANIZATION (WHO) EXPERT MEETING ON VIRUSES IN FOODS: FOOD ATTRIBUTION, ANALYTICAL METHODS, AND INDICATORS

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The Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) on microbiological risk assessment of viruses in foods was convened in September 2023 in response to the request by the Codex Committee on Food Hygiene (CCFH). The Expert Committee: 1) reviewed the literature and available surveillance databases, which ranked foodborne viruses according to frequency and severity; 2) ranked the relevant food commodities of highest public health concern; 3) discussed methods for virus testing performed for outbreak investigation and product testing as part of surveillance and monitoring strategies; and 4) reviewed current and potential indicators for viral contamination.

Human norovirus was identified as the leading cause of viral foodborne illness, followed by hepatitis A and hepatitis E viruses. Hepatitis A virus and hepatitis E virus were ranked equally but higher compared to norovirus in terms of clinical severity. When considering both frequency and severity, the ranking for these viruses are: 1) Norovirus; 2) hepatitis A virus and hepatitis E virus ranked in order; 3) rotavirus, sapovirus, enterovirus, astrovirus, and enteric adenovirus ranked in order.

Despite the methodological advancements, there remain challenges in their use, most notably ensuring accurate interpretation; application to other viruses and/or matrices; integration of sequencing technologies; and implementation in low resource countries. A variety of indicators for viral contamination have been investigated, including bacteria, bacteriophages, and plant and animal viruses. Additional research is needed to determine if there is an appropriate viral indicator for use in other commodities associated with foodborne virus contamination.

Oral 6.1

COMPARATIVE GENOMICS OF *ARCOBACTERACEAE* SPECIES AND INSIGHT ON *ARCOBACTER BUTZLERI* TRANSCRIPTOME DURING INFECTION OF A GUT MUCUS PRODUCER HUMAN CELL LINE

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The *Arcobacteraceae* bacterial family includes species isolated from several matrices and hosts. The division of *Arcobacteraceae* in several genera was proposed recently. *Arcobacter butzleri* represents the species most associated with food and waterborne outbreaks.

Aims of this work were the obtainment of genomics and transcriptomics information related to *A. butzleri* strains and linked to virulence potential. The study was consequently centered on *Arcobacteraceae* type strains to conduct pangenome assessments.

The *A. butzleri* virulence potential was evaluated on gut mucus producer (MP; Caco-2/HT29-MTX-E12) and not mucus producers cell models (NMP; Caco-2; Caco/HT29) infected with 32 strains isolated from humans and animals. Colonization and invasion capacities were evaluated jointly to whole genome sequencing (Illumina) and data analysis. Subsequent RNA-seq analysis on MP models after 30 and 90 minutes of infection were performed, including chemical analysis (HPLC) aimed at validating the gene expression of genes of particular interest. Finally, the study focused on the pangenome of 20 *Arcobacteraceae* was aimed to link their genomic traits with phenotypical characteristics and isolation sources.

A. butzleri has shown the ability to colonize host cells but demonstrated higher colonization in MP. Among the virulence genes, the *tonB* operon (iron transport and virulence) was detected in all *A. butzleri* strains. *A. butzleri* has demonstrated similarities between strains isolated from the same intestinal tract of pig. Transcriptome analysis demonstrated the

overexpression of *tonB* operon in strain LMG11119 (higher colonization). Furthermore, an overexpression of genes involved in acetate metabolism was observed, among them *actP-yjcH*, whose functionality was confirmed by a decrease in acetate levels during host-bacterium contact. The *Arcobacteraceae* comparative genomics demonstrated a genome size reduction in animal-related species suggesting an evolutionary adaptation to the host with variation in gene classes percentages and with correlation to specific orthogroups (e.g. TonB-related genes). The pangenome partitions do not support the division of *Arcobacteraceae* family into different genera revealing partitions with numbers comparable to other Gram-negative bacterial genera.

The obtained results provided insights into the virulence genes of *A. butzleri*, enhancing understanding of its virulence mechanisms and associated pathways. Pangenome analysis of *Arcobacteraceae* suggested adaptive capabilities in animal-related species that showed smaller genomes.

Oral 6.2

NOVEL APPROACH FOR THE CONTROL OF CAMPYLOBACTERIOSIS USING *CAMPYLOBACTER*-SPECIFIC PHAGES

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Campylobacter, in particular *Campylobacter jejuni* and *Campylobacter coli* species, are the main causative agent of one of the most prevalent foodborne diseases worldwide, campylobacteriosis. This zoonotic pathogen has been included by the World Health Organisation in its list of priority bacteria resistant and multi-resistant to available antibiotics, in order to urgently seek solutions for its control. *Campylobacter*-specific bacteriophages, campylophages, are a promising solution to address human health, animal health and food safety issues.

With the aim of developing a new biocontrol tool against this pathogen, more than 300 campylophages were isolated from different environmental and food samples, especially from chicken skin. After a host range study, the most promising campylophages were selected and their main stability, specificity and safety properties characterised. Finally, the efficacy of single phages and phage-cocktails was evaluated both *in vivo,* in the animal model *Galleria mellonella,* and in chicken skin.

Morphological analysis by transmission electron microscopy revealed that the phages belong to the family *Myoviridae*, with an icosahedral head and a long contractile tail. They are stable under different pH and temperature conditions of application, and designed cocktails infect more than 70% of the tested *Campylobacter* strains.

Studies in *G. mellonella* suggested that the combined use of antibiotic and bacteriophages enhances treatment efficacy compared to their individual use, resulting in lower mortality (16.2%), than the application of antibiotic alone (41.7%) after *C. jejuni* infection.

Regarding bacteriophages efficacy on food, more than 1.5 Log (CFU/g) reduction in *C. jejuni* load was found when artificially contaminated chicken skin samples were treated and stored under refrigerated conditions.

These results suggest that the application of specific bacteriophages targeting *Campylobacter* have great potential as a food safety strategy for the prevention and control of this pathogen throughout the entire agri-food change, from the farm-to-table.

Oral 6.3

MOLECULAR MECHANISMS MEDIATING THE SURVIVAL AND GROWTH OF *SALMONELLA ENTERICA* SUBSP. *ENTERICA* ON DICED ONIONS

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Salmonella enterica subsp. *enterica* is one of the most important foodborne pathogens worldwide. In recent years, many salmonellosis outbreaks were caused by the consumption of contaminated fresh produce. Recent outbreaks in the years 2023, 2021 and 2020 were linked to the consumption of raw onions. An in-depth understanding of the key mechanisms that mediate pathogen fitness on

specific, relevant food matrices is a crucial starting point to implementing highly targeted preventive measures.
The aim of this study was to identify the genetic determinants underlying the survival and even growth of *S. enterica* on ready-to-eat onions. For this, a barcoded transposon insertion sequencing library of 200,000 mutants in *S.* Typhimurium 14028s was screened on diced onions. The onions were stored at 8°C during a time period of five days. Sampling was carried out daily starting one hour after inoculation. Sequencing libraries for Illumina sequencing were prepared using PCR. The data for the input and output libraries was statistically analyzed using DESeq2 and subjected to KEGG and GO enrichment analyses. Genes inducing a significant ($p < 0.01$) fitness effect on onions (> 1.0 or < -1.0 Log_2 fold change between timepoints) were selected for subsequent competition assays comparing specific single gene deletion mutants and the wild type.
First results showed 26 genes with a significant fitness effect for resilience of *S.* Typhimurium on onions at 8 °C. These genes are involved in different pathways, for example in carbohydrate- and nucleotide biosynthesis, as well as in the processing of genetic information. Results of a similar screening on alfalfa sprouts identified five genes with a significant fitness effect. The identified genes differ between the two food matrices, suggesting that *S. enterica* might require a different subset of metabolic pathways for the two food matrices analysed in this project. In terms of food safety, this knowledge may enable highly targeted interventions for individual food matrices, but also common genetic determinants could be applied for broader targeted interventions.

Oral 6.4

QUASI-METAGENOMIC APPROACH FOR THE DETECTION AND CHARACTERIZATION OF *L. MONOCYTOGENES* ISOLATES OBTAINED FROM THE FRESH PRODUCE INDUSTRY

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Listeria monocytogenes, the causative agent of listeriosis, is a significant foodborne pathogen that continues to cause worldwide food outbreaks. It is widely distributed in the environment, capable of growth at refrigeration temperatures, and can persist in food production and processing facilities for extended periods, ranging from months to years. Due to the associated health risks, monitoring and surveillance of its occurrence in food processing plants are crucial. Currently, to assess the risk level and monitor the presence of *L. monocytogenes*, whole-genome sequencing (WGS) of isolates obtained from a selective medium is required. This enables characterization through the identification of virulence factors, resistance genes, subtypes, etc. However, despite its effectiveness and precision, this technique typically takes around 8-11 days to complete owing to its necessary do an enrichment broth (1-2 days), culture in a selective media (3-5 days) and an *in silico* typing through WGS (4 days).
This study aims to develop a methodology to reduce the time needed for the detection and characterization of *L. monocytogenes* strains that can be found in the environment of the fresh produce industry. The objective is to implement a WGS approach through metagenomics, referred to as quasi-metagenomics, which involves direct sequencing of microbial enrichments. To achieve this, it is necessary to enrich the sample in a culture broth, in order to obtain higher DNA yield and, consequently, an adequate sequencing depth. In this way, the genome can be assembled from the metagenomic data. A protocol has been developed consisting of sample collection, incubation in enrichment broth, DNA extraction and Nanopore long reads sequencing, leading to the detection and characterisation of *Listeria* using bioinformatics tools.
This study is funded by the State Research Agency (AEI) of the Spanish Ministry of Science, Innovation and Universities

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Oral 6.5

UNRAVELLING THE MYSTERIES OF YERSINIOSIS IN NEW ZEALAND

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Yersiniosis is a gastrointestinal infectious caused mainly by the bacteria *Yersinia enterocolitica* (YE). In New Zealand (NZ), there were 1,294 notified cases in 2022, which at a rate of 25.3 cases per 100,000 population is at least 10-fold higher than other industrialised countries.

We report a case-control study involving notified human cases of yersinosis cases within two NZ regions, as well as whole genome sequencing (WGS) analysis of YE obtained from clinical cases and from source testing.

Of the 291 notified yersiniosis cases reported within the study period, 247 cases were interviewed and consented to participating in the case-control study completing telephone questionnaires of potential exposures and disease burden. Pathogenic YE bioserotype 2/3, O:9, corresponding to sequence type (ST) 12 (McNally scheme) predominates in NZ, with 46% of consenting cases confirmed with this type. Pathogenic YE bioserotype 2/3, O:5, 27, and 4, O:3 (corresponding to ST14 and ST18, respectively) were also observed in 5% and 3% of consenting cases, respectively. Exposure to pork was identified as a significant risk factor for pathogenic YE and source testing (n = 499) confirmed the presence of pathogenic YE in 15% of retail raw meat, primarily raw pork. The use of WGS analysis confirmed multiple genetically very closely related clusters of pathogenic YE, predominately from raw pork with clinical cases from both NZ regions.

The pathogenicity of YE Biotype (BT) 1A remains controversial internationally but this BT is notifiable in NZ and was observed within 27% of consenting cases. A diverse range of STs were observed within the YE BT 1A isolates obtained from both clinical and food samples. Risk factors associated with YE BT 1A included the consumption of cooked seafood, sushi, tofu and some vegetable types.

This study demonstrates the power of combining the analysis of specific risk factors and WGS to indicate that raw pork is a significant risk factor for yersinosis attributed to pathogenic YE bioserotypes but not for YE BT 1A. This study has generated up-to-date evidence on potential sources of YE in NZ and will help devise control strategies to improve the yersinosis public health outcomes.

Oral 8.1

WHOLE-GENOME SEQUENCING OF FOODBORNE *BACILLUS CEREUS* ISOLATES INDICATES NO CLEAR ASSOCIATION BETWEEN THEIR GENOMIC DIVERSITY AND FOOD MATRIX TYPES

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Bacillus cereus sensu lato (s.l.) is a group of closely-related bacterial species frequently involved in foodborne intoxications. It is ubiquitous in nature and also reported in various types of foodstuffs such as starch-based foods, dairy, ready-to-eat foods and vegetables. Its virulence gene repertoire is usually assessed using (q)PCR targeting the commonly studied enterotoxin and cereulide biosynthesis genes. Hence, whole-genome sequencing (WGS) could be of added value as it enables a full characterization of the virulence gene repertoire in one analysis. Additionally, these WGS data can be employed for other applications such as strain typing.

This study reports the genotypic characterization of 170 *B. cereus s.l.* isolated from 13 food types and soil over a period of more than two decades. The prevalence of virulence genes was assessed by short-read WGS. Additionally, MLST, cgMLST, SNP and *panC* typing were also performed.

A high proportion of the isolates contained the genetic information for the production of the three main potent enterotoxins (*nheABC*, *hblCDA* and *cytK2*) and cereulide, as well as other potential virulence genes coding for hemolysins, metalloproteases and phospholipases. The WGS characterization displayed a high diversity illustrated by the a high number of distinct MLST sequence types (n = 86), including 29 (34%) not previously described. All seven *panC* types were detected. cgMLST and subsequent SNP-based phylogeny on the four most prevalent sequence types (ST8, ST15, ST24 and ST26) confirmed this high diversity. Interestingly, these phylogenetic characterizations indicated no overall association between food matrix and isolate, although clusters of closely-related isolates with shared epidemiological metadata were detected. Furthermore, some isolates were found to be genetically closely related, while originating from distinct food matrices and/or isolated several years apart.

Oral 8.2

FUNCTIONAL ANALYSIS OF SIGH IN *BACILLUS CEREUS* ATCC14579 AND REGULON PREDICTION IN THE *B. CEREUS* GROUP

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B. cereus is a common human pathogen responsible for frequent outbreaks of foodborne diseases manifested by diarrheal or emetic syndromes. The ability to form highly resistant endospores allows survival of mild processing conditions. Subsequent germination and outgrowth of spores may cause food spoilage and food-borne intoxication.
Here we report on an assessment of the role of *Bacillus cereus* ATCC14579 SigH (Spo0H), an alternative transition-phase associated RNA polymerase sigma factor, in stationary phase survival and initiation of sporulation. Comparative phenotypic analysis of *B. cereus* ATCC14579 and its *sigH* deletion mutant revealed a role in a wide range of relevant phenotypes, in addition to a confirmation of its role in sporulation. Phenotypic analysis showed a dramatic loss of growth with over 200 di- and tri-peptides and a 3 log reduction in stationary phase survival under non-sporulating conditions.
In addition, a severe reduction of liquid-air interphase biofilm formation and altered swimming and swarming behavior, including an altered chemotaxis response, was observed for the SigH mutant. *SigH* expression was assessed during growth in non-sporulation and sporulation medium, and comparative transcriptome analysis was performed at relevant time-points during growth. In-silico analysis was combined with whole genome ORF based micro-array experiments allowing the identification of a *B. cereus* SigH promoter consensus. SigH regulons were obtained to interlink genotype and phenotype and compare regulon functionality for a selection of *B cereus* group members showing large overlap and motif conservation.
The involvement of SigH in the newly described phenotypes and the large number of transcriptional regulators and genes coding for chaperones under SigH control, point to the crucial role for this alternative sigma factor in transition phase performance and cellular differentiation in *B. cereus* ATCC14579 and other members of the *B. cereus* group.
In conclusion, this data highlights the importance of SigH in this food pathogen and points to possible roles in transmission in food processing environments and clinical settings.

Oral 8.3

THYMIDINE AUXOTROPHY OF *CLOSTRIDIUM BOTULINUM* GROUP II STRAIN BELUGA TRIGGERED BY LABORATORY DOMESTICATION

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Repeated cultivation of (micro)-organisms under controlled laboratory conditions can lead to the accumulation of genetic alterations and physiological changes as an adaptation to the laboratory environment. These events have the potential to alter the original phenotype, resulting in what is commonly referred to as laboratory domestication, observed not only in bacteria but also in higher organisms. While this is a desirable outcome during microbial strain optimization for industrial processes, it can have significant and deleterious implications for food safety research, where domesticated bacterial foodborne pathogen model strains may not

represent the original phenotype or its full virulence, growth, or resistance potential.

In the context of routine cultivation under optimized laboratory conditions, changes in domesticated bacterial strains may go unnoticed, potentially impacting the outcomes of food safety challenge tests. In our study of multiple spore stocks of *Clostridium botulinum* Group II strain Beluga, widely employed in food safety research, we identified a thymidine auxotrophy under nutrient-poor growth conditions in the majority of our tested spore stocks. Using whole-genome sequencing (WGS), we identified a mutation in *thyA*, a gene encoding thymidylate synthase crucial for *de novo* synthesis of dTMP required for DNA synthesis. Notably, this mutation was absent in a stock that grew without thymidine supplementation, revealing that the mutation may have emerged and gone undetected in the spore stocks for nearly 30 years.

Genetic alterations undetected during routine cultivation may yet have significant impact on food safety studies. For example, efficient dTMP synthesis by ThyA has been linked to virulence in bacterial pathogens and is considered a critical factor for growth and fitness. Inadvertently utilizing strains with an impaired *thyA* in food safety research could lead to an underestimation of bacterial growth, impacting the outcomes of challenge studies and the development of predictive models. To mitigate the risk of undesired laboratory domestication, we strongly recommend maintaining low passage numbers, avoiding single-colony passaging, and conducting frequent WGS to identify newly emerged mutations that may compromise the reliability of food safety study outcomes.

Oral 8.4

DISTRIBUTION OF VIRULENCE GENES AMONG CAMPYLOBACTER SPP. ISOLATES FROM SPAIN

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Campylobacter spp. is the most common bacterial cause of human gastroenteritis in the world. However, its pathogenesis remains unclear as the relevance of its putative virulence factors is commonly poorly understood. In this study, we further characterized the repertoire of virulence-associated genes in *Campylobacter* for improved understanding of the dynamics of the pathogen.

We selected 135 *Campylobacter jejuni*, 7 *Campylobacter coli* and 3 *Campylobacter lari* from different steps of the poultry supply chain in Spain (86 isolates), such as farm (11 isolates), slaughterhouse (32 isolates), poultry processing plant (14 isolates) and retail (29 isolates), and from human clinical samples of the Spanish region of Burgos (59 isolates). The isolates were sequenced, and the raw reads were filtered and further assembled using CamPype (https://github.com/JoseBarbero/CamPype).

An in-house database of 333 virulence genes was blasted twice against the draft and annotated genomes, followed by manually correction of overlapping hits based on Bidirectional Best Hit and gene synteny. For those virulence-associated genes affected by phase-variation (PV), the ON/OFF status was also analyzed using Phasomelt. Despite 191 virulence genes were identified in all *Campylobacter* isolates and only the *ggt* gene (colonization) and seven genes of the Type IV Secretion System (T4SS) were absent, the isolates showed a heterogeneous virulence profile.

High occurring variability was observed in those genes involved in the capsule biosynthesis and transport, O-linked flagellar glycosylation and lipooligosaccharide biosynthesis loci, with a great impact of PV that led to 72 different virulence genes patterns among isolates. Between sampling locations, isolates from retail significantly possessed a reduced number of virulence and PV genes on average compared to isolates from any other stage ($p < 0.05$) that could evolve to be less virulent. In contrast, isolates from farm, slaughterhouse, poultry processing plant and hospital seem to be equally pathogenic regarding their virulence gene content.

Furthermore, the highest occurrence of virulence genes (287) was found in *C. jejuni*, that would confer this species increased virulence and ultimately would explain its stronger predominance. Therefore, understanding the virulence potential of *Campylobacter* spp. through Whole-Genome Sequencing is critical to define how the bacteria undergo evolution and adapt to hosts.

Oral 8.5

A PROOF OF CONCEPT: INDUCTION AND TRANSDUCTION OF STX PHAGES DURING SEED GERMINATION

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Shiga toxin-producing *E. coli* (STEC) encodes Shiga toxin (Stx) on prophage and produces toxin and phages in response to environmental stress. Sprouts are associated with foodborne outbreaks of STEC, which are often linked to the contamination of dry seeds. Drying results in cell oxidation, which may induce Stx prophages of STEC survived on dry seeds. Conditions for sprouting are ideal for the growth of bacteria and dissemination of *stx* via phage transduction.

Thus, the study aimed to prove the concept of induction and transduction of Stx phage during seed storage and sprouting. The challenge study was conducted by drying of mung bean inoculated with phage donor *E. coli* O104:H4Δ*stx2::gfp::ampr* (8 log CFU/g), followed by inoculation of generic *E. coli* (7 log CFU/g) during sprouting for 4 d. *E. coli* O104:H4Δ*stx2::gfp::ampr* was constructed previously to quantify the *stx* expression by the fluorescence of GFP. *E. coli* O104:H4Δ*stx2::gfp::ampr* expressed GFP after drying and rehydration. About 1 in 104 CFU/g of *E. coli* acquired ampicillin resistance, considered as putative lysogens. 71% of 103 putative lysogens carried *gfp* by PCR detection, confirming phage transduction.

The study also determined the host range of the bacteriophage from *E. coli* O104:H4 Δ*stx2::gfp::ampr* to assess the infectivity of the Stx phage. Forty-five strains of non-STEC were incubated with Stx phage, and 5 strains acquired *stx*, including generic *E. coli*, *E. coli* O103:H2, ETEC, and *S. sonnei*. The expression level of the same phage differed among lysogens. Overall, transduction of Stx phage mediates the spread of *stx* and magnifies the number of STEC by transduction during sprouting, thus increasing the probability of severe disease outcomes after ingestion.

Flash communications

DETECTION OF ZOONOTIC HEPATITIS E VIRUS (HEV) IN GAME MEAT IN POLAND

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Hepatitis E virus (HEV) is recognised as a zoonotic pathogen transmitted via food. Its presence in food mainly relates to animal meat. The aim of this study was the aassessment of the occurrence of HEV in game meat intended for human consumption.

In total, 766 muscle samples representing meat of culinary importance such as neck, butt, ribs, and loin were collected from 271 carcasses of the following animal species: wild boar (n = 165), deer (n = 30), roe deer (n = 70) and fallow deer (n = 6). HEV extraction from meat and isolation of viral RNA were performed using TRIzol (TRI Reagent®) and a NucliSens kit (BioMérieux). For a quantitative detection of HEV RNA in meat samples, a real-time duplex RT-qPCR was employed followed by a subtype identification of detected HEV strains. Moreover, the presence of anti-HEV antibodies in meat juice samples was assessed using an ID Screen® Hepatitis E Indirect Multi-species ELISA (IDvet).

HEV RNA was solely detected in 0.75% (5/660) of wild boar ribs at the concentration ranging from 1.0 × 103 to 1.5 × 104 G.C./g of muscle tissue. HEV was not found in any meat sample of deer, roe deer and fallow deer. The sequence analysis of the ORF2 genome fragment of detected wild boar HEV strains showed their phylogenetic relationships to other HEV-3 strains previously found in the population of European wild boars. The anti-HEV IgG were found in 12.12% (20/165) of wild boar meat juice and none of the tested meat juice sample of wild ruminants contained virus-specific antibodies. Wild boar meat can be a source of HEV infection for humans, but its sporadic detection in meat suggests that a risk of foodborne virus transmission seems to be low. Furthermore, findings from this study indicate that wild ruminants do not play an important role in the epidemiology of HEV infections in Poland.

GENOME ENGINEERING APPROACH TO EVALUATE THE PATHOGENICITY POTENTIAL OF STEC O174 STRAIN ISOLATED FROM DAIRY ENVIRONMENT

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In the last years, the number of outbreaks linked to the contamination of dairy products made with raw milk by Shiga toxin-producing *Escherichia coli* (STEC) are increasing. Despite many efforts to study the pathogenicity of STEC, the genome plasticity typical of E. coli makes the unambiguous identification of virulence factors very difficult.

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*. Moreover, instead of the more known Locus of Enterocyte effacement (LEE) pathogenicity island, this strain carried the Locus of Adhesion and Aggregation pathogenicity island (LAA-PAI), reach of additional virulence factors. With the aim to investigate the virulence potential, we applied the lambda red recombinase-based genome engineering approach to create new strains with deletions in one or both shigatoxins and a region of LAA PAI harbouring some virulence factors. The lethality of these mutated strains was assessed in *Galleria mellonella* larvae, a well-known *in vivo* model to study host–microbe interactions. The analysis revealed a significant decrease of mortality for both single and double mutants. Indeed, while the lethal doses (LD50) of the parental strain was 6.0 CFU/10 µl, the values for the single mutants were 81.7 CFU/10 µl and 50.5 CFU/10 µl. As expected, the double mutant showed a LD50 of 100-fold higher respect to the wild type, but it was interesting to notice that these deletions only reduced the pathogenicity. The further deletion of portions of LAA PAI led to obtain a LD50 of 105 CFU/10 µl, revealing that virulence factors present in this region could be considered as key pathogenicity factors, together with *stx* genes, to assess the pathogenicity of food contaminating STEC strains. Larvae injected with negative controls showed no mortality.

Overall, our results demonstrated that the virulence factors harboured by LAA PAI were responsible for a part of pathogenicity of a STEC strain, suggesting that they can be included as virulence markers of LEE-negative STEC strains.

DEFINITION OF PATHOGENIC STRAINS OF SHIGATOXIN-PRODUCING *ESCHERICHIA COLI* (STEC)

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Shiga toxin-producing *Escherichia coli* (STEC) are pathogenic *E. coli* strains that can cause serious diseases such as haemorrhagic colitis and haemolytic uremic syndrome (HUS). In its opinion of 18 May 2017, the French Agency for Food, Environmental and Occupational Health and Safety (Anses) defined the pathogenic STEC strains according to genetic criteria (presence of *stx1* and/or *stx2*, *eae* or (*aaiC* and *aggR*) genes) and serotyping. The list of high risk strains included those belonging to the following serotypes: O157:H7, O26:H11, O145:H28, O103:H2, O111:H8, O104:H4, O80:H2.

In 2020, the Efsa BIOHAZ Panel concluded that all STEC strains are pathogenic and capable of causing serious disease. The French Directorate-General for Food requested Anses to review its definition in the light of the Efsa opinion and recent French and European epidemiological data.

Based on the analysis of French epidemiological and microbiological data (2017-2021), Anses proposes a new classification of STEC strains into four groups according to their virulence potential. This classification takes into account the association of strains with severe forms of infection (mainly HUS, followed by bloody diarrhoea (BD)). The criterion used to classify strains is the positive predictive value (PPV) for HUS, then the PPV for bloody diarrhoea in case of equivalence; French and international epidemiological data show that strains with the *stx2a* and/or *stx2d* subtypes are more virulent, particularly in the presence of the eae adhesion gene (group I). *eae*-negative strains with the *stx2a* and/or *stx2d* variants (group II) also have a high potential to

cause HUS, particularly in adults (22% of HUS cases in adults compared with 1% in children under 15). STEC strains with other subtypes of the *stx* gene (groups III and IV) are less frequently associated with HUS cases and are mainly found in cases of bloody diarrhoea and diarrhoea.
Serogroup is no longer used to classify strains. However, serogroup information is still useful in human and food epidemiosurveillance. This classification can be used to define management measures (surveillance, management of contaminated batches) corresponding to the level of protection previously defined by risk managers.

UNEQUAL TRANSCRIPTION OF SHIGA TOXIN SUBUNIT GENES IN ENTEROHEMORRHAGIC *ESCHERICHIA COLI*

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Shiga toxins (Stx) produced by enterohemorrhagic *Escherichia coli* (EHEC) are AB_5 protein toxins consisting of a single enzymatically active A-subunit and a pentamer of non-covalently linked B-subunits. The respective genes encoding the Stx2 subunits, $stxA_2$ and $stxB_2$, are located in the late-regulated phage region within the genome of lambdoid prophages, downstream of the antiterminator gene Q and upstream of the genes encoding the phage lysis cassette.
The aim of this study was to quantitatively analyze the relation of the transcription of $stxA_2$ and $stxB_2$ in five EHEC wildtype strains. Due to the operon structure of the *stx* genes, we hypothesized either a 1:1 transcription ratio, or due to the AB_5 structure of the toxin, a 1:5 ratio.
To perform quantitative transcriptional analysis, total RNA was isolated and purified from *E. coli* O157:H7 strain HUSEC003, *E. coli* O157:H- strain HUSEC004, *E. coli* O103:H- strains HUSEC008, *E. coli* O26:H11 strain HUSEC018 and *E. coli* O104:H4 strain LB226692. Subsequently, qRT-PCR was performed to determine the transcript levels for *stxA2*, *stxB2*, and the housekeeping gene *rrsB,* which was used as an internal control.
Surprisingly, $stxA_2$ was expressed approximately ~ 1.3-2.5 times stronger than $stxB_2$ in all investigated EHEC wildtype strains. The analyzed genes were not expressed in the expected ratios, indicating that free A-subunits might circulate in the bacterial environment which do not find a B-pentamer for holotoxin formation. Further analysis on translational and protein level should help to clarify this phenomenon.

GROWTH OF *LISTERIA MONOCYTOGENES* AND THE SPOILAGE MICROORGANISMS ON MYCOPROTEIN DURING CHILLED STORAGE IN MODIFIED ATMOSPHERE

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In this study we investigated the safety and spoilage related potential challenges for the mycoprotein as a raw material. *Listeria monocytogenes* can grow at chilled conditions and can be established in the processing environment, therefore a challenge test was performed to estimate the growth rate of this microorganism on mycoprotein.
L. monocytogenes growth on mycoprotein was followed at 4 and 7°C, with vacuum and 30% CO_2 (70% N_2) packaging. Next, the natural microbiota able to cause spoilage during storage up to 16 days was studied by the whole population sequencing (16S RNA gene) as well as by classical plating methods.
Results: 30% CO_2 reduced the growth rate of *L. monocytogenes* significantly compared to the vacuum. The estimated growth rates µmax (1/h) using the logistic growth model were 0.068 at Vacuum (7 °C), 0.051 at 30% CO_2 (7 °C) and 0.026 at Vacuum 4 °C. At 4 °C vacuum 2 log increase was observed in 7.5 days.
On day 0, the total microbial counts were non-detectable (< 1 or < 2 Log CUF/g), except for two colonies of sporeformers. CO_2 slightly inhibited the spoilage microbiota compared to vacuum packaging. Spoilage microbiota has reached high levels in some samples already on day 8 at 7°C. Total counts on day 8 varied largely and was between 2.6 and 10 Log CFU/g, of which the majority were psychrotrophic. Species such as *Pseudomonas lundensis, Paenibacillus borealis* and *Lactobacillus paracasei* have been isolated from spoiled samples. Lactic acid bacteria occurrence was also heterogenous, and were detected after 8 days on 1 out of 4 samples at the level of 5.4 Log CFU/g. Whole population sequencing

showed domination of *Pseudomonas* and *Paenibacillus* spp. after storage.

Conclusions

Lower temperature and CO_2, compared to the vacuum, inhibited the growth of *L. monocytogenes*. The reported growth rates can be used to estimate safe shelf life scenarios for the chilled mycoprotein. The heterogenous growth of spoilage microbiota between the samples may be the result of very sporadic distribution of low levels of initial contamination.

STUDY OF THE MICROBIAL DYNAMICS OF *HERMETIA ILLUCENS* ALONG THE REARING AND PROCESSING STEPS: AN INSIGHT FOR THE FUTURE SAFE INTRODUCTION FOR HUMAN FOOD

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Hermetia Illucens (Black Soldier Flye, BSF) is a key strategy to face the increase of the population with sustainable protein alternatives and become a promising insect species for introduction in human food. However, this specie is not yet approved as a Novel Food and its safety remains of concern. Insects naturally contains diverse microbial communities, including bacteria, yeasts, and fungi, which play a key role in their development, digestion and health but can also include foodborne pathogens.

In this context, culture-independent and culture-dependent microbiological analyses were conducted to characterize the microbiome of the black soldier fly (BSF) and monitor potentially pathogenic microorganisms throughout the whole manufacturing chain, from eggs at rearing stages up to powders produced. The samples analyzed included eggs, substrates, larvae and frass at days 0, 7 and 16 and physical chemical parameters were measured (pH, temperature). The study employed 16S metabarcoding and bioinformatic analysis to investigate microbiota composition during insect farming (substrate, frass, eggs), combined with culture-dependent methods which focused on identifying food safety and spoilage-associated microorganisms.

Bacterial diversity dynamics were studied during two insect powder manufacturing processes. Bacterial loads ranged from 5 to 9 log CFU/g across the different sample types. Predominant taxa were Gammaproteobacteria (30%), Bacteroidia (30%), Firmicutes (18%), and Actinobacteria (15%). *Campylobacteria* constituted a smaller proportion (2%). No *Cronobacter* spp, *Listeria monocytogenes*, or *Salmonella* spp were detected in the samples. Presumptive *Clostridium perfringens* were found in one sample (4.28 log CFU/g). Thermal processes induced significant changes in the bacterial dynamics but did not provide insect powders free of microorganisms, compromising the food safety of this product.

The characterization of the microbiota of edible insects using culture-dependent and culture-independent analyses allowed for better identification and monitoring of potentially pathogenic microorganisms throughout the insect farming and insect powders. The results obtained provides a first complete overview of the evolution of the microbial community in a real farming industry, a key step to ensure the safety and quality of insect-based products, necessary for their integration into our food systems. It paves the way towards understanding the microbial safety of BSF matrix for better implementation of management strategies.

SLAUGHTER LINE SPEED AND CHILLING STAGE: ACCESSIBLE LEVERS FOR FOOD BUSINESS OPERATORS TO REDUCE THE CONCENTRATION OF *CAMPYLOBACTER* ON BROILER CARCASSES

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Campylobacter, the most common European bacterial foodborne pathogen, is mainly associated with poultry meat. To reduce carcass contamination and the risk of campylobacteriosis, measures can be implemented at slaughterhouse level.

Based on two industrial situations with contrasting *Campylobacter* contamination levels on final products, we aimed to explore the contribution of environmental contamination sources and slaughtering methods to final product contamination.

Two French slaughterhouses, SA and SB, were selected for their contrasting prevalence of *Campylobacter* results not complying with Regulation (EU) 2017/1495. SA showed a lower non-compliance rate (16%, versus 59% in SB), despite similar processes. From October to December 2022, nine batches were sampled over three days at each slaughterhouse. *Campylobacter* concentration was determined in caecal contents (C.CC), air in hanging ($C.Air_{hanging}$), plucking ($C.Air_{plucking}$) and evisceration ($C.Air_{evisceration}$) rooms, neck skins before and after chilling stage ($C.NS_{after\text{-}chilling}$). Partial Least Squares regression (PLSR) was conducted to explore the relationship between the response associated with the contamination of the final product, $C.NS_{after\text{-}chilling}$, and various variables, such as contamination of environmental sources and process parameters, like slaughter line speed (SLS) and chilling ($\Delta C.NS_{chilling}$).

$C.NS_{after\text{-}chilling}$ was greater in SB (2.59 ± 1.17 log_{10}CFU/g) compared to SA (1.85 ± 1.14 log_{10}CFU/g), mirroring the mean SLS differences (10,429 versus 8,150 broilers/hour). The PLSR model was shown to explain 87.4% and 84.7% of the variance of $C.NS_{after\text{-}chilling}$ and the explanatory variables, respectively. Variables significantly contributing the most to explaining the variance of $C.NS_{after\text{-}chilling}$ (represented by their *t* values) were *Campylobacter* contamination of air during evisceration, $C.Air_{evisceration}$ (6.16), process parameter SLS (5.10), and caecal contamination C.CC (3.64). Following were $C.Air_{plucking}$ (2.89) and $C.Air_{hanging}$ (2.82) and $\Delta C.NS_{chilling}$ (1.87) which also contributed significantly. Unlike the other contributing variables, $\Delta C.NS_{chilling}$ affected significantly negatively $C.NS_{after\text{-}chilling,}$ producting a 0.85-log_{10}CFU/g reduction in SA.

This study emphasized the importance of air contamination during evisceration and caeca contamination as environmental sources, as well as the impact of SLS and the chilling process on *Campylobacter* contamination levels in neck skin samples after chilling. Variations in process parameters might explain the varying contamination levels. Optimizing chilling processes and reducing SLS could constitute accessible levers to mitigate *Campylobacter* contamination.

KINETIC AND PROTEOMIC STUDIES IN UHT AND RAW MILK REVEAL DISTINCT PATTERNS AMONG HYPERVIRULENT AND HYPOVIRULENT *LISTERIA MONOCYTOGENES* CLONAL COMPLEXES

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Listeria monocytogenes clonal complexes (CC) show an uneven distribution among different food products. Hypervirulent CC like CC1, CC4, and CC6 are strongly associated with dairy products. Whether the overrepresentation of these hypervirulent isolates in dairy products is the consequence of a growth advantage in this type of food, is currently unknown. We investigate the role of temperature (37 and 4 °C) on the growth kinetics of five *L. monocytogenes* isolates (CC1, CC4, CC6, CC9, and CC121) in ultra-high temperature (UHT) milk and raw milk.

Regarding refrigeration temperatures, hypovirulent CC9 and CC121 isolates exhibit better growth parameters compared to the hypervirulent CC1, CC4, and CC6 isolates in UHT milk. Moreover, in raw milk at 4 °C, the CC9 isolate displayed a better growth rate and reached higher maximal final concentrations than the rest. Conversely, when incubated at 37 °C, the mammalian host temperature, hypervirulent isolates (CC1, CC4, and CC6) grew faster in UHT milk than their hypovirulent counterparts (CC9 and CC121).

The proteomic analysis performed of CC1 and CC9 (representative of hyper- and hypovirulent isolates, respectively) in UHT milk showed that they respond to milk cues differently with CC-specific traits. Regarding membrane and cytosol proteins, CC1 and CC9 isolates grown in UHT milk upregulated 22 and 13 proteins, respectively,

when compared to the same bacteria grown in BHI. Ten proteins related to metabolism (e.g. LysA or different phosphotransferase systems), and stress response were upregulated in both isolates during growth in UHT milk.
Concerning cell surface proteins associated with the peptidoglycan, CC1 and CC9 isolates grown in milk upregulated 8 and 4 proteins, respectively, when compared to the same bacteria grown in BHI. Of these, only a predicted class I SAM-dependent methyltransferase was associated with growth in milk in both isolates.
These findings improve risk assessment and provide insights into the strong association of hypervirulent *L. monocytogenes* isolates to dairy products, discarding a growth advantage of these hypervirulent isolates in milk when stored at refrigeration temperatures, and showing for the first time through a proteomic approach that there is a *Listeria* CC-specific and a *Listeria* CC-common response to the milk environment.

EVALUATION OF YEAST SURFACE DISPLAY AND SECRETION OF CRISPR-CAS9 INTEGRATED ENDOLYSIN PLY511 AGAINST *LISTERIA MONOCYTOGENES*

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Endolysins, bacteriophage-derived enzymes, have emerged as promising antimicrobial agents due to their ability to specifically target and degrade bacterial cell walls. In this study, we investigate the efficacy of *Saccharomyces cerevisiae* to deliver the endolysin Ply511 against *Listeria monocytogenes* as a proof of concept towards an engineered probiotic.
Listeria monocytogenes is a Gram-positive pathogen causative of human infections, resulting in febrile gastroenteritis, perinatal infection, and central nervous system infections. These infections are frequently acquired through the ingestion of contaminated food.
In our study, we employed CRISPR-Cas9 to genetically modify *Saccharomyces cerevisiae* to display the listeria endolysin Ply511 on its surface or to secrete it. Flow cytometer analyses confirmed the expression of the genetic construction integrated in the recombinant yeast. Also, the enzymatic peptidoglycan-degrading activity of the engineered yeast was confirmed by using heat-killed *L. monocytogenes* cells.
In preliminary killing assays, the recombinant yeast displaying or secreting the endolysin did not show CFU reduction of *L.monocytogenes* in comparison with the wild-type. The secretion of the endolysin showed similar results. These preliminary findings suggest that further optimization is needed at this stage for both the yeast display and secretion of endolysin Ply511 to enhance their effectiveness within an engineered probiotic context, ensuring the achievement of effective bacterial killing.

ANTIBIOTIC RESISTANCE OF *VIBRIO PARAHAEMOLYTICUS* STRAINS ISOLATED FROM SEAFOOD PRODUCTS

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Introduction

Vibrio parahaemolyticus, a Gram-negative bacterium naturally occurring in the marine environment, is experiencing an increased prevalence attributed to global warming. *V. parahaemolyticus* has been linked to numerous gastroenteritis outbreaks associated with the consumption of raw or undercooked seafood. Concurrently, the surge in antimicrobial resistance poses a significant threat, with bacteria capable of exchanging genetic elements, including resistance genes. In this context, *V. parahaemolyticus* can play a role as a transmission vector and/or reservoir of these genes.

Purpose

This study aims to elucidate the presence of phenotypic resistance and associated resistance genes within a cohort of 875 *V. parahaemolyticus* strains isolated from seafood products intended for consumption.

Methods

To achieve our objective, we conducted antibiotic susceptibility tests using the disk diffusion method according to CLSI protocols. Resistance to 16 antibiotics, belonging to antibiotic families classified as critical by the WHO, was assessed in 875 strains isolated from seafood destined for the market. Subsequently, we performed whole genome sequencing (WGS), incorporating both short and long reads, on 48 strains. Following this, the genomes were analyzed by comparing them to various antimicrobial resistance databases (NCBI-amr, Resfinder, Bacmet, and Card), as well as mobile genetic elements databases (Plasmid finder and MOB-suite).

Results

The study revealed that 191 *V. parahaemolyticus* strains exhibited phenotypic resistance to one or more antibiotics, with 42 categorized as phenotypically multidrug resistant. Predominantly, resistance was observed against tetracycline, co-trimoxazole, chloramphenicol, and streptomycin. WGS analysis facilitated the correlation of phenotypic resistance with specific resistance genes, such as *blaNDM-1*, *blaCTX-M15*, *blaOXA-10*, or *tetA*. However, some strains displayed phenotypic resistance without associated resistance genes.

Significance

This study unveils crucial insights into antimicrobial resistance dynamics in *V. parahaemolyticus* within seafood, emphasizing the urgent need for surveillance and intervention to address the rising prevalence of resistant strains and safeguard public health and the seafood industry.

MICROBIAL VOLATOLOMICS BY SPME-GC-HRMS FOR PHENOTYPING *LISTERIA MONOCYTOGENES* RESPONSE TO ACID STRESS

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Foodborne pathogens can undergo preventive treatments involving lethal stresses during processing, such as cleaning and disinfection procedures. However, sometimes these pathogens can survive. That results in increased robustness (resistance) when, further down in the food chain, they encounter stronger lethal stresses. Therefore, studying how pathogens respond to these stresses, is crucial for controlling microbial safety through the food chain.

Hence, the aim of the present work was to assess the relevance of microbial volatolomics (*i.e.* analysis of volatile organic compounds, VOCs, emitted by microorganisms) in characterizing the response of foodborne pathogen when exposed to an acid stress. Taking *Listeria monocytogenes* (*L. monocytogenes*) as a model pathogen, we implemented a two-step strategy.

The first step aimed to determine the most informative bacterial extract for further volatolomics. Based on case-control experiments (cultures on synthetic growth medium with/without *L. monocytogenes*), volatolomics was performed by headspace solid phase microextraction (HS-SPME) hyphenated with gas chromatography and high-resolution mass spectrometry (GC-Q Exactive-HRMS Orbitrap®) on three types of samples: (i) the pellet collected after centrifugation of the whole culture which concentrates bacterial cells; (ii) the supernatant collected after centrifugation of the whole culture, which includes what the bacteria released into the growth medium; (iii) the whole culture, which contains global information diluted by the growth medium, but avoids handling of the bacteria samples required by the centrifugation step. The non-targeted analysis of the volatolome of the 3 types of samples using Compounds Discoverer® software, shows that pellet, on the one hand, and whole culture and supernatant, on the other

hand, provide different and complementary information on the presence of *L. monocytogenes* in the culture medium.
Based on the results of the first step, the response of *L. monocytogenes* to acid stress was secondly studied through volatolomics analysis of pellet and whole culture samples, collected after bacterial culture. The examination of volatolomics results reveals distinct markers of different acid stress levels applied to *L. monocytogenes*. The discussion will focus on the origin of these markers with regard to the literature and on the perspectives of this volatolomics study.

SALMONELLA ENTERITIDIS CONTAMINATED FRESH PRODUCE. A LYTIC PHAGE TO THE RESCUE

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The application of bacteriophages presents an effective and sustainable strategy to curb the spread of foodborne pathogens. Serovars of *Salmonella enterica* subsp. *enterica* are widely recognized pathogens frequently associated with outbreaks from consuming contaminated ready-to-eat (RTE) fruits and vegetables. *Salmonella enteritidis* (SE) is a serovar known to cause salmonellosis in Sub-Saharan Africa. Therefore, we aimed to isolate and characterize lytic bacteriophages targeting this particular serovar.
The lytic *Salmonella* phage SCF was isolated using *Salmonella enteritidis* (ATCC 13076) as the bacterial host and chicken feces as the phage source. Morphological examination using transmission electron microscopy demonstrated that phage SCF possesses an icosahedral capsid and a flexible, elongated tail, matching the morphology of phages within the class *Caudoviricetes*. The whole genome sequence analysis of phage SCF revealed a genome size of 108.56 kb, a G+C% of 39.02, and enabled the assignment of phage SCF to the genus *Tequintavirus*. The genome of phage SCF most closely matched *Salmonella* phage PS3-1 (Genbank OP594723). Critical functional and structural phage genes encoding endolysin, tail fibers, holin, and capsid proteins were identified within the phage's genome, while virulence or lysogenic genes were absent.
The phage demonstrated lytic activity against *Salmonella* Typhimurium but not against *E. coli, S. marcescens, E. cloacae, P. aeruginosa,* or *K. pneumonia*. It exhibited a burst size of 72 virus particles per bacterial host cell with a latency period of approximately 20 minutes. Phage SCF was stable within a temperature range of 8 - 50°C and a pH range of 4 to 8. Using the crystal violet assay confirmed that phage SCF inhibited biofilm formation by *Salmonella enteritidis* up to 70% and substantially reduced pre-existing 72-hour biofilms formed on stainless steel surfaces. Moreover, phage SCF effectively controlled SE contamination on tomato surfaces.
Treatment of SE-contaminated tomatoes with phage SCF for 2 hours at room temperature reduced viable SE counts by approximately 5 logs at a multiplicity of infection (MOI) of 0.1. These characteristics render phage SCF a promising biocontrol candidate against *Salmonella enteritidis* in food and food processing environments.

WHOLE-GENOME SEQUENCING FOR THE CHARACTERIZATION OF *LISTERIA MONOCYTOGENES* ISOLATES FROM ENVIRONMENTAL SAMPLING OF THREE FRESH-CUT PRODUCE PROCESSING PLANTS

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Listeria monocytogenes is a relevant human pathogen linked to the consumption of different types of food, such as ready-to-eat (RTE) fresh produce. *L. monocytogenes* can withstand and survive cold temperatures encountered during refrigeration, representing an increasing concern in fresh produce processing plants, where temperature and moisture conditions are beneficial for its survival and growth. The establishment of good manufacturing practices and management programs to reduce contamination of *L. monocytogenes* in the processing facilities is critical to avoid cross-contamination to the final product.
The aim of this study was to determine the diversity and persistence of *L. monocytogenes* isolates from three different processing plants, including cut vegetables, cut fruits and prepared salads, obtained after cleaning and disinfection activities. Systematic environmental sampling was performed three times throughout the year with a periodicity of 3-4 months to detect seasonal variability. A total of 275

environmental sites were monitored, where 94 corresponded to cut vegetables, 85 to cut fruits and 96 to prepared salads. A total of 47 isolates of *L. monocytogenes* were typed by multilocus sequencing typing (MLST) and core-genome MLST (cg-MLST).

The results revealed two clusters: Lineage II, serotype 1/2a;3a, and Lineage I, serotype 4b;4d;4e. According to the genetic characterization of the isolates, two different sequence types were found, ST155 and ST6, indicating a low diversity among all samples. Genome analysis of the isolates showed they harboured virulence genes, as well as biocides and antibiotic resistance genes, indicating the relevance of the isolates to public health.

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CHARACTERIZATION OF THE VIABLE BUT NOT CULTIVABLE (VBNC) CELLS IN LONG-TERM CULTURES OF *LISTERIA MONOCYTOGENS*

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Listeria monocytogenes (*Lm*) is a pathogen involved in the development of listeriosis, a foodborne disease with high mortality rates. Its ubiquitous nature gives the bacterium the ability to survive and grow in food-related environments, where cross-contamination easily occurs. Several studies demonstrated that *Lm* has the potential to escape adverse environments by entering dormancy states as persistence and viable but nonculturable (VBNC), which lead to the chronic presence of the species into food-processing sites.

In this project, long-term cultures subjected to nutrient deprivation were used as a strategy to evaluate the production of VBNC cells by three *Lm* strains in five different replicates. Cultures were incubated at two different temperatures, one corresponds to the optimal temperature of growth (30 °C), while the other is the typical refrigeration level (4 °C) and monitored for a period of 360 days. At selected time points, aliquots were withdrawn from every culture for testing. Cells were enumerated by traditional plating methods and by quantitative PCR (qPCR) complemented with propidium monoazide (PMAxx) dye to detect the portion of population composed by persister and VBNC cells. The transcriptome of the populations at the different time points was analysed to detect any significant difference in the gene expression between viable and dormant cells. A chemical-based sequencing method for genomic methylome analysis, NT-seq, was also performed to determine if epigenetic modifications play a role in the formation of persister and VBNC cells.

The results of this study demonstrated the presence of a high number of VBNC cells (up to 106 VBNC cell/mL) starting from 15 days of incubation for cultures at 4 °C and from 30 days for cultures at 30 °C, with variability among strains for numbers and phenotypical characteristics.

IMPACT OF CLIMATE CHANGE ON FOOD SAFETY AND SPOILAGE

Keynote 2.2

WATER SCARCITY: RECLAIMING AND REUSING IN FRESH PRODUCE PRODUCTION

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Water scarcity stands as a primary constraint affecting the sustainability of food systems. Freshwater plays a pivotal role in food production systems, from cultivating crops to raising animals, and is equally crucial during food processing. Here, water serves not only as a critical ingredient but also for cleaning and disinfecting processing areas, and in operations such as food washing or blanching. International organizations endeavor to raise awareness about the challenges posed by water scarcity, emphasizing the need for improved water management efficiency and research into alternative water resources.

Reclaimed and reconditioned water stand out as alternative water sources that have been extensively explored. Reclaimed water refers to urban wastewater treated to meet specific water quality standards, intended for a variety of uses. In 2020, the European Commission introduced new European Legislation for water reuse in agricultural irrigation (EC, 2020), which comes into effect in June 2023. While this legislation marks significant progress in reducing biological and chemical risks associated with reclaimed water use for crop irrigation, further research is necessary to identify potential risks related to other significant hazards. On the other hand, establishing minimum water use requirements in food production often proves challenging. According to the Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA), water used throughout food production chains should be "fit-for-purpose" to ensure the production of safe food. This implies that water quality can vary at different points and for different purposes within the food processing chain but must always align with its intended use. In the case of fresh fruits and vegetables (FFV), determining the required water quality for specific purposes at various production stages relies on an assessment of health risks associated with specific contaminants or product pathways. Based on current international guidelines, to mitigate microbial risks in FFV at the point of consumption, water's microbiological quality should improve along the production chain, from farm to consumer.

Developing a risk assessment for health hazards stemming from specific contaminants necessitates a wealth of data, yet many potential hazards lack sufficient data. This is particularly evident concerning the spread of antimicrobial-resistant bacteria (ARB) and antimicrobial resistance genes (ARG) in FFV through irrigation with reclaimed water and the industry's use of recycled processing water. Current scientific evidence in this area remains limited, underscoring the need for further research to bridge this data gap. Three ongoing research projects: 1) The State Research Agency (AEI) of the Spanish Ministry of Science and Innovation (TED2021-131427B-C2), the AGROALNEXT program (MCIN and NextGenerationEU funds, PRTR-C17.I1) and a recently launched tender by EFSA (WateResist: OC/EFSA/BIOHAW/2023/01)—aim to generate valuable data to better understand the potential risks associated with these hazards. WateResist's primary objective is to gain insights into the occurrence and variety of ARB and antimicrobial resistance determinants (ARDs), encompassing ARGs, plasmids, and other mobile genetic elements carrying ARGs. This research focuses on reclaimed wastewater used during pre-harvest stages, as well as recycled processing water in post-harvest handling and processing operations for FFV, to assess the role of this water in the spread of ARB and ARDs across various European regions. Conversely, the overarching objective of the coordinated RECWATER and the Agroalnext research project is to evaluate and validate efficient water disinfection methods for safe water reclamation in irrigation and water reconditioning for FFV production and processing, aiming for sustainability.

Oral 2.4

POTENTIAL HEALTH RISK OF MICROCYSTIN ACCUMULATION IN GREEN-HOUSE GROWN CROPS

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Cyanobacteria produce Microcystin congeners during prolific growth in freshwater systems, also known as cyanobacterial blooms. Using bloom-contaminated water for irrigation can result in the accumulation of these hepatotoxins in crops, resulting in potential human health risks. While lab-based studies already provided valuable data, they do not always take into account the variation in plant growth during larger-scale production of crops.

Therefore, larger greenhouse experiments were set up with different crops (carrots, strawberries and lettuce) to better reflect large-scale scenarios. The crops were exposed for 4 to 5 weeks to 100 µg/L of MC-LR through the irrigation water. A combined spray- and drip-irrigation approach was used for lettuce, whereas spray- or drip-irrigation were used separately to provide water for carrots and strawberries. After harvest, MC-LR was quantified in both plant and irrigation water samples using a validated UHPLC-MS/MS method. The irrigation water was collected at 8 time points during the experiments. Eventually, Observed MC-RL values in the crops were combined with the available food consumption data to provide a deterministic health risk assessment.

During the experiments, MC-LR concentrations in the irrigation water were shown to be stable. Furthermore, MC-LR concentrations were reduced when comparing the outer and inner leaves of the lettuce. Moreover, only a few carrot plants showed detectable levels of MC-LR in either the taproot or leafy greens. However, MC-LR accumulated in all strawberry plants when spray irrigation was used, with the higher concentrations observed in the roots, followed by the greens and strawberries, sequentially. Strawberry plants grown with drip irrigation only accumulated MC-LR in the roots and the greens. This does imply the transport of the MC-LR from the roots to the other plant parts. Multiple exposure scenarios were defined and showed a potential health risk for different age categories when contaminated, spray-irrigated strawberries are consumed.

Accumulation of MC-LR was observed In this study, crop and irrigation method dependent accumulation of MC-RL was shown. An initial deterministic risk assessment also showed potential consumer health risks. However, further research should be conducted including an assessment of more microcystin congeners in different crops.

Oral 2.5

ASSESSING THE MICROBIOLOGICAL QUALITY OF ROOF-HARVESTED RAINWATER AS AN ALTERNATIVE WATER SOURCE FOR PRODUCE IRRIGATION

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Irrigation water is increasingly being relied upon within horticultural production, to increase product yield and quality. Seasonal droughts and alterations in weather patterns caused by climate change have become a serious threat to water availability; hence, assessing the potential of alternative water sources such as rainwater harvesting has gained considerable prominence in recent years. Despite the advantages of harvested rainwater, it is at risk of microbial contaminations arising from a range of sources including faecal matter from birds, rodents, insects and animals on roofs which can be washed into rainwater collection and storage tanks. Contaminated irrigation water is widely recognised as an important source of contamination for fresh produce and therefore it is imperative to ensure that safe irrigation water sources are identified.

The objective of this study was to investigate the microbial quality of roof-harvested rainwater from different roof

surfaces sampled from selected rural and urban horticultural growing sites on the east coast of Ireland. Over a six-week period (August-September 2023), 42 rainwater samples, collected from 7 different sites were assessed for the presence of indicator bacteria: total viable counts (TVC), Escherichia coli and enterococci using standard culture based methods. Results indicated that 100%, 90% and 90% of the rainwater samples were positive for TVC, E. coli and enterococci respectively. The mean concentrations were 5.3 ± 1.1 $\log_{10}$ CFU/mL, 1.3 ± 0.7 $\log_{10}$ CFU/mL and 1.7 ± 0.7 $\log_{10}$ CFU/100 mL, respectively.

Variations in the concentrations of these bacteria across the sampling weeks were observed for all sites, indicating that roof surface, weather patterns, and proximity of rainwater tanks to trees, housing bed nests may be factors in contamination levels. Metagenomic based analysis of the rainwater samples provided more in-depth information on the dominant microbial communities from each roof type and sampling site; information which can be utilised in the assessment of treatment options.

The detection of bacteria in the harvested rainwater supports the need for effective treatment before any potable or irrigation use as it may pose a risk to consumers if used untreated.

Flash communications

ROLE OF COAT LAYERS IN HEAT RESISTANCE AND GERMINATION OF *B. SUBTILIS* SPORES PRODUCED AT HIGH SALINITY CONDITIONS

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Bacterial spores are highly heat-resistant, able to survive pasteurization treatments and germinate during food storage leading to spoilage and foodborne diseases. Heat treatment at temperatures above 100 °C is the most effective method to inactivate spores. However, processing conditions need to be carefully optimized to minimize the loss of nutritional and sensorial quality of foods, this requires a deep knowledge of the sources of variability in spore behaviour. For instance, sporulation conditions influence germination and resistance properties of spores. While the impact of sporulation temperature has been widely studied, there is limited data on other environmental factors like water activity (a_w). As global warming and desertification are progressively increasing the osmotic stress suffered by soil bacteria, which constitutes an important source of spore contamination of foods, there is a need to assess the behaviour of spores produced in high salinity environments. Thus, in this study, we compared heat inactivation and germination kinetics of B. subtilis spores produced at high salinity (S_{salt}) and optimal conditions ($S_{control}$). Heat resistance of S_{salt} spores was approximately 1.9-fold higher than that of $S_{control}$ spores. On the other hand, heat and non-heat-activated S_{salt} spores displayed decreased germination efficiency to L-alanine, L-valine and AGFK mixture (L-arginine, D-glucose, D-fructose and potassium) than $S_{control}$ populations, but not in rich growth medium. Furthermore, germination induced by Ca2+-dipicolinic acid was slower in S_{salt} spores than in $S_{control}$ spores, while the former population germinated faster to dodecylamine. To further investigate if variations in resistance and germination between both populations was due to modifications in the coat layers, we studied the behaviour of mutant spores defective in coat morphogenesis sporulated under optimal and high salinity conditions. Germination kinetics of ΔcotY and ΔcotE mutants produced at high salinity was similar to that of mutant populations sporulated at optimal conditions. Furthermore, heat resistance of S_{salt} ΔcotY spores was equal to that of $S_{control}$ parental spores.

Thus, high salinity environments could constitute a source of problematic spores, characterized by increased heat resistance and heterogeneous nutrient germination response. These changes are likely due to modifications in the crust and the outer coat layer.

REVEALING THE IMPACT OF ENVIRONMENTAL DYNAMICS UNDER CLIMATE STRESS CONDITIONS ON MICROBIAL RESPONSES

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This study investigates the intricate relationships between the dairy sector, microbial dynamics, and climate variations, emphasising the impacts of increasing atmospheric carbon dioxide and temperature levels. It includes multivariate analysis, gas solubility in dairy waste, and Escherichia coli growth and gene expression under stress conditions. Specifically, the multivariate analysis uses historical datasets from Spanish farms from 2014 to 2019 to understand how climatic variables influenced raw bovine milk quality.

Solar radiation and global carbon dioxide levels emerged as crucial factors influencing milk quality parameters based on the selectivity ratio. A novel spectrophotometric method also estimated pH and dissolved carbon dioxide in various solutions, providing insights into gas dissolution under different environmental conditions. Accurately monitored dissolved carbon dioxide conditions were then related to the microbial growth rate of Escherichia coli, while their impact on gene expression was assessed at specific conditions.

The study uncovered significant correlations between climatic factors and milk quality, revealing negative impacts on raw bovine milk fat, protein, and dry lean percentages due to temperature and solar radiation. Positive associations were found between average temperature and somatic cells, while total bacterial count negatively correlated with atmospheric carbon dioxide levels.

The study also highlighted variations in dissolved carbon dioxide concentrations across different solutions under changing temperatures. Growth assessments of Escherichia coli underscored critical temperature and carbon dioxide levels. Specifically, at 42 °C, a rise of the dissolved carbon dioxide by 80% resulted in a 0.81 1/h decrease in the specific growth rate. However, this increase raised the specific growth rate by 0.72 1/h at a lower temperature of 27 °C. Genetic and metabolic responses of Escherichia coli to environmental stress revealed up-regulated pathways related to flagellar assembly and aromatic hydrocarbon catabolism. In conclusion, this study provides insights into the complex relationships among climate change, dairy waste, and microbial dynamics, offering a foundation for adaptive farm management practices within the dairy industry.

The results highlight the need for sustained efforts to ensure the sustainability and safety of the dairy sector amidst evolving climatic changes.

EVALUATION OF CLIMATE- AND ECOSYSTEM-INDUCED STRESSES ON THE DYNAMIC OF *ARCOBACTER* SPP. PRESENT IN MANILA CLAMS COLLECTED IN THE VENICE LAGOON

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While the occurrence of zoonotic pathogen Arcobacter spp. in marine ecosystems is well recognized worldwide, the effect of climate and ecosystem induced stress on its dynamic remains unclear. Besides, filter-feeder bivalves accumulate microbiota in edible tissues issuing human health. In previous studies, Arcobacter was identified as one of the predominant taxa in several sites of the Venice lagoon with higher relative abundance in the polluted areas.

This study aimed to investigate the temporal and ecological dynamics of the Arcobacter community in Manila clams on the Venice lagoon. Sampling was performed during summer and winter (2018 -2020), in a breeding site (Chioggia) and a chemical-polluted site (Porto Marghera). A combination of applying two methods of culturing and whole genome sequencing resulted in the identification of eighteen isolates, including eight A. butzleri, eight A. cryaerophilus, one A. aquimarinus, and one A. mytili. These strains shared 12 virulence, 34 motility, and 9 chemotaxis genes with a high presence of 83-100% and a low presence of 11% for 4 urease genes. Among these genes, 10 virulence-associate and 4 chemotaxis genes have been already identified in Campylobacter genera. Regarding antibiotic resistance genes, all strains showed resistance to Nalidixic acid,

belonging to the class of quinolone antibiotics. Regarding metabolic detoxification pathways, 17 genes were detected that highly encoded biodegradation and metabolism of xenobiotic compounds and benefit bacteria to be able to resist chemical pollutants. No linear correlation was detected between the distribution of genes and collection season/site. Additionally, quantitative PCR was applied to DNA extracted from clam homogenates to track the abundance of Arcobacter spp. The load of bacteria (9 × 104 cfu.mL−1) in summer samples of Porto Marghera was found significantly 10 times more than in summer samples of Chioggia. Considering the similar distribution of detoxification pathways genes between the isolates, it could be hypothesized that the higher abundance of Arcobacter in summer samples of Porto Marghera could be due to other environmental factors arising with higher temperature. Namely, higher concentrations of some pollutants-derived compounds arising in summer could enhance the bacteria proliferation. Our survey revealed the presence of pathogenic Arcobacter in clams and highlighted the combined effects of pollutants and climate change on marine health.

MICROBIAL FOOD ECOLOGY

Keynote 1.1

MICROBIAL FOOD ECOLOGY: FROM PROCESSING PLANTS TO FOOD

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A wide diversity of microorganisms in varying numbers and behaviors co-exist in the plant (and plant-based food) microbiome. As food microbiologists we mainly have a background in food processing, food preservation and food safety, and usually look at food-associated microorganisms in a targeted approach, focusing on specific spoilage organisms or human pathogens. We often handle microorganisms in a negative manner, as agents associated with food deterioration and foodborne illness and refer to them as unwanted contamination due to unhygienic handling. Some microorganisms are also associated with beneficial impacts in food production, such as during fermentation or when used as protective (probiotic) cultures.

The presence of enteric pathogens such as *Salmonella*, of *Listeria monocytogenes* as wide-spread environmental contaminant and of spore-forming microorganisms such as *Bacillus cereus* in plant-based foods can compromise both safety and quality of the final product. In food control, primarily, our focus is on the genomic features and behavior of these microorganisms of interest themselves but also the interactions within complex microbial communities and plant- or food (environment)-microbiome interactions and how this can be exploited deserves attention. Some examples and lessons learned will be discussed:

1. The difficulty in defining a (bacterial) pathogenic species within a microbial community has multiple implications. Microbial hazard identification and hazard characterization is in need of a multi-method approach. This is exemplified in the present debate on the safety assessment of *Bacillus thuringiensis* as a biological control agent in plant primary production.
2. As our understanding of the factors shaping microbial assembly, plant-microbe interactions and microbial function in plant systems is enhanced, exploiting the plant-microbiome to suppress the establishment and growth of human pathogens in fresh produce might become a realistic prospect. This will be exemplified in research characterizing and investigating indigenous microbiota from fresh-cut romaine lettuce to control *Listeria monocytogenes* growth.
3. While plant-based alternative products may share similarities with animal-based products in terms of taste and physical attributes, it is crucial to acknowledge that their chemical composition and microbial contamination profile are not identical and pose new challenges in food processing. There are still gaps in knowledge regarding microbiological risks in the production process and the final product. This will be exemplified with some case studies e.g. some foodborne illness outbreaks were associated with cheese alternatives.

Oral 1.1

SYNERGISTIC INTERACTIONS IN MULTISPECIES BIOFILM COMBINATIONS OF BACTERIAL ISOLATES RECOVERED FROM DIVERSE FOOD PROCESSING INDUSTRIES

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Most biofilms within the food industry are formed by multiple bacterial species which co-exist on surfaces as a result of interspecies interactions. These ecological interactions often make these communities tolerant against antimicrobials. Our previous work led to the identification of a large number (327) of highly diverse bacterial species on food contact surfaces of

the dairy, meat, and egg industries after routine cleaning and disinfection (C&D) regimes.
The aim of this work was to characterize the biofilm-forming potential of these isolates and to develop industry-specific four-species interaction models that represent persist bacteria on food contact surfaces that likely become tolerant against C&D.
Biofilm-forming ability of 92 bacterial strains belonging to 26 genera and 42 species was assessed and synergistic interactions in biofilm formation were investigated by coculturing species in all possible four-species combinations. Biofilm quantification was based using crystal violet staining and scanning electron microscopy was used to observe the biofilms development.
Out of the total 455 four-species biofilm combinations, greater biofilm mass production, compared to the sum of biofilm masses of individual species in monoculture, was observed in 34 combinations. Around half of the combinations showed synergy in biofilm mass > 1.5-fold and most of the combinations belonged to dairy strains. The highest synergy (3.13-fold) was shown by a combination of dairy strains comprising *Stenotrophomonas rhizophila*, *Bacillus licheniformis*, *Microbacterium lacticum*, and *Calidifontibacter indicus*. The observed synergy in mixed biofilms turned out to be strain-specific rather than species-dependent.
All biofilm combinations showing remarkable synergy appeared to have certain common species in all combinations which shows there are keystone industry-specific bacterial species which stimulate synergy or antagonism and this may have implication for biofilm control in the concerned food industries.

Oral 1.2

BACTERIAL COMMUNITY DYNAMICS AND THEIR FRUCTOPHILIC PROPERTIES DURING FERMENTATION OF TRAMINETTE GRAPE

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Traminette is an important hybrid grape and its use in wine-making has increased in midwestern and eastern United States regions. High-throughput DNA metabarcoding sequencing of the DNA extracted from Traminette grape, must, during yeast pitching, alcoholic fermentation and final wine identified an abundance of the OTU corresponding to the genera such as *Faecalibacterium*, *Peptoclostridium*, *Lactobacillus*, *Roseburia*, *Bacillus* and *Veillonella* as the dominant bacteria. It also identified *Clostridium*, *Listeria*, and *Enterococcus* that may be of safety concern in wine.
Across the different wine production stages, yeast nutrients played a role in bacteria dynamics, Venn analysis of the relative abundance indicated 53 common unique genera were shared in the Traminette R tank while 45 genera were shared in Traminette L. The roles of wild fructophilic lactic acid bacteria (FLAB) in fructose metabolism to form lactic acid and mannitol were determined as well as their possible malolactic fermentation. Cultured FLAB strains were identified using sequencing of almost complete 16S rRNA gene as *Leuconostoc citreum*, *Lactococcus lactis* subsp *Hordniae* and *Lactiplantibacillus plantarum*. Although fructophilic *Lp. plantarum* F4 demonstrated similar fructose metabolism when compared with the reference strains of *Lp. plantarum* and the *Fructobacillus fructosus* FB3a strains, randomly amplified polymorphic DNA (RAPD) gel fingerprints reveals the differences in the genome of the strain. *Lp. plantarum* F4 that did not express mannitol dehydrogenase but decarboxylases malolactic acid through the expression of mle gene in an artificial wine medium.
Data from the HPLC analysis indicated no residual fructose and L-malic acid at pH 3.2 and 12% ethanol when used for fermentation. This study supported the abundance of genera that belong to Firmicutes during Traminette grape fermentation and suggested the use of fructophilic *Lp. plantarum* F4 during wine secondary fermentation to reduce

residual fructose and to conversion of L-malic acid to lactic acid.

Oral 1.3

DISINFECTION IN THE SALMON INDUSTRY: IMPACT ON BACTERIAL COMMUNITIES AND EFFICACY TOWARDS FOODBORNE BACTERIA AND BIOFILMS

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Salmon aquaculture is the fastest growing food production system in the world. Deficiencies in the quality or safety of salmon can have global repercussions. Controlling food safety aspects during production is therefore essential. Here, we investigate the state of hygiene in a salmon processing plant using next generation sequencing and classical culture-dependent methods to characterize the surface microbiota before and after cleaning and disinfection (C&D) at ten sampling points. To assess the efficacy of commercial disinfectants, we performed susceptibility assays using advanced robotic high-throughput technologies and included both planktonic cells and biofilms formed by foodborne bacteria which may affect food safety and spoilage.
Quantification of total aerobic bacteria from surface samples taken in the salmon processing environment revealed effective reductions in the bacterial loads by C&D corresponding to an average reduction of 1-2 log CFU/cm2. These changes affected both the composition and the diversity of the surface microbiota. Before C&D, the highest relative abundance in the core microbiota was assigned to *Acinetobacter, Mycoplasmataceae, Pseudomonas* and *Enterobacteriaceae* in descending order. After C&D, we observed a sharp increase in the relative abundance of *Pseudomonas* and a decrease of *Enterobacteriaceae*. At most of the sampling points, the alpha diversity also declined after C&D. However, variations were found between conveyors, processing machines and drains. Moreover, the minimum inhibitory concentration (MIC) was determined for seven commercial disinfectants with different types of active substances, including peracetic acid, sodium hypochlorite and ammonium chloride. The assay included 128 *Pseudomonas* isolates, 46 *Aeromonas* isolates and 59 Enterobacterales isolates sampled from the salmon processing plant. Generally, most disinfectants were effective using the concentration recommended by the producer. Furthermore, biofilm assays were performed using BacTiter-Glo to detect and quantify viable cells before and after 60 single-species biofilms were exposed to different concentrations of eight disinfectants. These results will be presented in more detail at the conference.
Our study contributes to answer fundamental questions related to the efficacy of commercial disinfectants and their impact on the surface microbiota in salmon processing environments. This knowledge is essential since bacteria inhabiting these environments can strongly influence food safety and quality.

Flash communications

THE SWEET-COLD SPOT: A MULTI-OMICS STUDY ON THE IMPACT OF TEMPERATURE AND SUGARS ON EXOPOLYSACCHARIDES (EPS) PRODUCTION BY SAUSAGE SPOILAGE *LEUCONOSTOC MESENTEROIDES*

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Exopolysaccharides (EPS) synthesis by *Leuconostoc mesenteroides* in meat products can impart a slimy texture, altering their appearance and shelf-life and consumer acceptability. We studied the influence of sugars and temperature on EPS/slime production by *L. mesenteroides* isolates from industrial meat sausages.
Four isolates were studied: KS273, KS279 (High EPS producers, HEPs), KS276 and KS277 (Low EPS producers, LEPs). The impact of temperature (8-37 °C), carbon source

(glucose, sucrose, fructose) and sugar concentration (0-5%) in M17 medium on EPS production was studied through Response Surface Methodology, comparative genomics and transcriptomics.

EPS production was optimal for all the isolates in 5% sucrose at 8 °C. The highest EPS production in glucose was ~2.5-fold lower than in sucrose. In contrast to previous studies, low temperature (8 °C) was a key triggering factor for EPS production by the four strains compared to higher temperatures. A pangenomic analysis detected the presence of four dextransucrases and a complex levan biosynthetic cluster in all four strains. However, the LEPs carry one ORF encoding a levansucrase (levS) in the cluster, whereas HEPs carry an additional ORF encoding a different levansucrase (sacB_1), being the only difference regarding EPS-related genes between HEPs and LEPs. The transcriptomic analysis indicated that the expression of levansucrase genes in HEPs is ~10-fold higher at 8 °C than at 25 °C. A comparative analysis between HEPs and LEPs showed that transcription levels (TPMs) of dextransucrase and levansucrase genes are ~10-fold and ~100-fold higher in HEPs, respectively.

These results indicate that carbon source type, concentration and temperature are relevant modulators of the EPS production and, despite having similar genetic machinery for EPS biosynthesis, their expression levels are remarkably different in HEPs and LEPs. This suggests there are differential regulatory factors playing a key role, leading to the phenotypical differences.

MICROBIOLOGICAL SAFETY IN ALTERNATIVE NON-CONVENTIONAL PLANT-BASED PROTEIN EXTRACT

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The IPSUS project (https://susfood-db-era.net/main/IPSUS) explores opportunities to extract upcycled plant proteins from food sidestreams left unused, during production or processing stages, and to find novel food applications for these potential innovative ingredients. Despite there is literature available on the food safety of raw materials, no information was found regarding the microbiological safety of the extracted proteins, thus, a deeper understanding of the food safety aspects of plant protein sources is needed.

In recent years there has been a growing interest in using different plant-based protein extracts as ingredients in food to enhance the nutritional value, but also to improve the techno-functional properties. Among all the strategies currently in use to improve the potentiality of the extracted proteins, fermentation by lactic acid bacteria (LAB) is a sustainable and promising tool.

Although the growth of spoilage bacteria and pathogens has been studied in traditional meat systems, plant-based provides a relatively different nutrition environment, pH, and inner structure for microorganisms to adhere and proliferate, which may influence the survival and growth of both spoilage and pathogenic bacteria.

Moreover, even if most of these bacteria could be inactivated by the heat produced during the extrusion and other technological and extraction processes, some endospore-forming bacteria, such as *Clostridium* spp. or *Bacillus spp.*, may survive the heat treatments.

With this in mind, the present study aimed to screen the microbial contaminants present in 12 plant-based protein extracts, isolate spore-forming bacteria, identify them, and test the capacity of different wild lactic acid bacteria to ferment those protein extracts. The results underline the differences among spore-forming populations of different protein extracts, both in terms of concentration of bacteria but also in terms of different species present in the matrices. The different microbial species and concentrations in the plant-based proteins analyzed, were found to be potentially the cause of the observed different fermentation abilities of the LAB used. In fact, some contaminants were able to grow during fermentation thus, negatively affecting the growth of LAB, which, in some cases, were no longer able to complete the fermentation process.

ANTIBIOFILM EFFICACY OF BACTERIAL METABOLITES ISOLATED FROM MEAT PROCESSING EQUIPMENT

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Some locations in meat processing facilities such as hard to reach spots and floor drains may be conducive to biofilm formation. Commonly used sanitizers are largely ineffective for inactivating bacteria within biofilms. Our previous work has demonstrated that some bacterial strains isolated from meat processing equipment have antagonistic effects against *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC) when grown in dual-species cultures.

The objective of this work was to determine whether metabolites produced by these meat processing surface bacteria (MPB) were involved in the observed antagonistic effect. Four MPB strains *Aeromonas* sp., *Serratia* sp., *Hafnia* sp., and *Janthinobacterium* sp. were tested against five pathogenic strains: *E. coli* O103:H2, *E. coli* O121:H19, *E. coli* O111:H8, *E. coli* O145:H28, and *Salmonella* Typhimurium. Cell-free supernatant was concentrated through freeze-drying and this concentrate was referred to as cell free extract (CFE). CFE was mixed in equal volume with a pathogen strain suspended in Lennox broth without salt (LB-NS) in 96 well microtiter plates containing detached pegs, with a cell concentration of 105 cells/mL.

The plates were incubated at 15 °C for up to 6 days and bacterial counts on pegs were enumerated on day 3 and day 6. Significant reductions were observed for all MPB CFE, with *Aeromonas*-CFE resulting in no recovery of cells from biofilms of *E. coli* O103, *E. coli* O121 and a > 5 log reduction in biofilm cell counts for *Salmonella* after 6 days incubation. *Hafnia*-CFE also demonstrated very high antibiofilm activity, by lowering the cell counts in biofilms ranging from 3.8 to 6.8 log units, compared to their respective controls.

Whole genome sequence analysis found that genomes of all four MPB strains have predicted biosynthetic gene clusters: *Serratia* (9), *Janthinobacterium* (6), *Hafnia* (4) and *Aeromonas* (3). Heat treatment of MPB CFE by boiling for 10 min did not affect the antibiofilm activity of *Aeromonas*-CFE or *Hafnia*-CFE. Genes involved in regulation of curli and a subunit component of curli were highly down-regulated in both biofilm and planktonic co-cultured cells.

These findings suggest that MPB CFE can be very effective for controlling biofilms.

INSIGHTS INTO DEVELOPING PLANT-BASED ANALOGUES: DAIRY *VS.* SOY CURDS

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The increasing demand for plant-based alternatives requires a thorough understanding of microbial growth and adaptation on diverse substrates. In this context, our study investigated the adaptive evolution of six fungal strains growing on soy and dairy curds. It included two *Penicillium camemberti* cheese starters, two wild *Penicillium biforme* strains isolated from cheese rinds, and their two respective experimentally evolved strains in cheese agar for eight weeks, obtained from previous studies.

We analyzed the fungal growth patterns, global gene expression, and volatile organic compound production, as well as patterns of community assembly on soy and cheese matrices. Surprisingly, the commercial strains of *P. camemberti* exhibited superior growth on soy ($p < 0.001$), reaching approximately 6.5 CFU/mL in each week of growth, while in the cheese curd the growth was consistently one logarithmic unit lower.

To better understand this growth discrepancy, we analyzed the nutritional composition which revealed the presence of glucose and iron in the soy curds, potentially favoring *Penicillium* growth. Furthermore, RNASeq was employed to provide insights into the mechanisms involved in the growth patterns on different substrates. As *P. camemberti* is known for its role in cheese flavor formation, we also investigated the volatile compounds generated in both substrates.

Additionally, an *in vitro* community assembly assay was performed to compare the dynamics of fungal and bacterial communities grown on soy and cheese curds. For that,

we inoculated the matrices with microorganisms typical in bloomy cheese rinds (*P. camemberti*, *Geotrichum candidum*, *Brevibacterium aurantiacum*, *Brachybacterium alimentarium*, and *Hafnia alvei*) and harvested them at intervals of three, seven and ten days. A consistent community structure was observed after the seventh day of incubation in both matrices, emphasizing the adaptability of the microbial consortium to the plant substrate.

These findings highlight the surprising adaptability of dairy cheese strains to plant-based substrates, opening avenues for innovative plant-based product development in the food industry.

MICROBIAL RESISTANCE

Keynote 10.1

QUANTIFYING ANTIBIOTIC RESISTANCE DYNAMICS IN THE FOOD CHAIN: A ONE HEALTH APPROACH FROM FOOD TO THE HUMAN GUT

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Antibiotic resistance (AR) has become a prime public health concern worldwide. According to the latest projections, by 2050, up to 10 million deaths could occur annually. Antimicrobials have been long used in the animal husbandry and food industry, and their overuse and spread over the environment along with the incredible adaptative capacity of bacteria have led to an increased resistance to several antibiotics. An example of this is Ampicillin, a semi-synthetic beta-lactamase antibiotic that, due to its extensive use, has become a micropollutant commonly found in water, sludge, food, flora, and fauna.

As a consequence, bacteria belonging to the *Enterobacteriaceae* family, including relevant foodborne pathogens such as *Salmonella* spp. and several other clinical and food-related bacteria have shown increased resistance to the antibiotic. The routes through which AR propagates are multiple and complex, and their dynamics involve different mechanisms, from the selection of resistant populations due to different selection forces, such as exposure to antibiotics, metabolic cost, or environmental stressors, together to cross-protection and horizontal transfer of AR genes (AGRs). Although these phenomena can occur in different contexts (e.g., sewage, soil, human gut…), food environments remain a relevant resistance dynamic theater, and more importantly, a resistance modulator, as food processing factors and conditions can influence resistance patterns, reducing or amplifying certain resistant populations or allowing ARGs acquisition by foodborne pathogens.

A recent work, developed in our laboratory, on Spanish fermented foods, identified the ripening duration as a relevant factor in the phenotypic resistance patterns of Lactic Acid Bacteria (LAB) populations. These commensal microbial communities can act as AR reservoirs along the food chain and transfer ARGs to foodborne pathogens. To better understand the impact, and identify AR drivers and risk factors, microbial interactions within resistance-driven environments should be analyzed from different biological and environmental angles. Quantifying how AR microbial communities respond to environmental stresses or compete with food microbiota can help us anticipate the attendant AR risk. Transconjugation of ARGs has been identified as another important via through which ARs can propagate along different food-related environments, using microorganisms themselves as ARGs transmission routes. In this sense, new research has also proven the capacity of LAB species, isolated from foods, to transfer ARGs to foodborne pathogens such as *Listeria monocytogenes*.

This information is key to determining the connection between AR microorganisms in foods and observed foodborne disease cases. Data integration from different sources and disciplines is also essential in the AR context due to the diversity of the phenomena, and their complexity along the food chain. In this respect, Microbial Quantitative Risk Assessment (QMRA) methodology is proposed as an *in-silico* bench to unravel the entangled AR transmission mechanisms and determine their importance in the final risk.

This quantitative and integrative approach should combine predictions on microbial response to food environments, trans-conjugation pathways for ARGs, and microbial interactions in the human gut, incorporating the AR component into Dose-Response models. The associated risk dynamics may not be mitigated or prevented with a traditional approach or managed as a separate factor, regardless of other health problems but rather it requires considering a global perspective and multisector strategy, based on the One Health concept, putting together information from different disciplines.

Oral 10.1

EXPLORATION OF GENETIC DETERMINANTS OF DRY-HEAT RESISTANCE IN *SALMONELLA*

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Background

Salmonella is one of the leading causes of foodborne infectious diseases, posing a serious threat to human health. Many strains of *Salmonella* are able to survive long periods of drying and are extremely resistant to heat treatment, contributing to many outbreaks of foodborne disease worldwide during the past few decades. Therefore, exploration of genetic determinants and further understanding of the related mechanisms of dry-heat resistance in *Salmonella* is very important for the controlling of *Salmonella* in dry food.

Methodology

Twelve *Salmonella* strains were cultured in Tryptone Soy Broth (TSB) for 18 h. After overnight drying in 24-well plates, the cells were treated at 60 °C and 80 °C for 1 h with a final a_w at 0.1-0.2, respectively. Cell counts of each sample were determined consequently. To screen the most dry-heat resistant strains, whole-genome sequencing was conducted using the Illumina MiSeq system. Subsequently, selected genes were individually knocked out, and the impact of the target gene deletions was assessed by comparing the survival rates of the wild-type strains and the mutants after dry-heat treatment.

Results

Among the 12 evaluated strains, 6 strains were very sensitive to dry-heat, with the cell counts under the detection limit after the treatment, while the strain *Salmonella enterica* Infantis CICC21649 exhibited a highest resistance, with a different lethality of even 5 log(cfu/mL) comparing to the most sensitive ones. Total 138 genes were found to exhibit differential distribution between the resistant and sensitive strains. The effects of the functions regulated by the genes *gtrB* and *dam* were then investigated by gene knockout, and the biggest effect of the genes deficiency on the dry-heat resistance was observed in the mutant *Salmonella enterica* Infantis *ΔgtrB::Cm*, with a 1.55 log(cfu/mL) difference of the lethality compared to the wild type strain.

Conclusions

This study successfully screened dry-heat resistant strains of *Salmonella*, identified differentially protein-encoding genes, and further determined the contribution of them in enhancing the dry-heat resistance in *Salmonella*. Mechanisms related to the putative functions of the targeted genes therefore provides important clues for promoting the control of *Salmonella* in food.

Oral 10.2

RESISTOME CHARACTERIZATION OF RECLAIMED WATER USE AS IRRIGATION WATER OF LEAFY GREENS AND ITS LINK WITH HUMAN HEALTH RISK

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The European Commission promotes an integrated water management approach, in which reclaimed water from urban wastewater treatment plants (WWTPs) represents an alternative water source to alleviate the demand for irrigation water which is considered a priority innovation. However, there are concerns linked to the safety of using reclaimed water as irrigation water, not only due to the potential presence of pathogenic microorganisms and chemical contaminants but also to the presence of antimicrobial resistant (AMR) agents, including antimicrobial resistant bacteria (ARB) and genes (ARGs). To determine if there might be any potential risk linked to the use of reclaimed water as irrigation water for leafy greens, information about the performance of WWTPs and the characterization of the effluents is needed.

The objective of this study was to characterize the resistome of different WWTPs. For this, the presence of ARGs in influent and effluent from four WWTPs in the Region of Murcia (Spain) was analyzed by filtration and Nanopore sequencing over time.

The results showed a clear decrease in the number of ARGs between influent and effluent in the different WWTPs. However, differences were observed among the performance of the different treatments used in the WWTPs, being the membrane bioreactor systems (MBR) one of the most effective treatments. It was found that the types of ARGs showing the highest relative abundance were against macrolides, beta-lactams and aminoglycosides.

The results obtained in this study are key to reducing the risk of AMR agents and to continue implementing techniques and treatments that ensure greater food and health safety.

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Oral 10.3

BIOFILM FORMATION BY *BROCHOTHRIX THERMOSPHACTA*. IMPACT OF FOOD INDUSTRIAL SURFACES ON THE BIOFILM FEATURES AND STRUCTURE

Antoine Gaillac[1], Romain Briandet[2], Elodie Delahaye[1], Julien Deschamps[2], Evelyne Vigneau[3], Philippe Courcoux[3], Laurence Dubreil[4], Claire Gourin[1], Hervé Prévost[1], Emmanuel Jaffrès[1]

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Brochothrix thermosphacta is considered as a major food bacterial spoiler and was identified in multiple food industrial surfaces, notably as biofilm. Biofilm formation is directly correlated to bacterial persistence in food industrial environments, resulting in food contamination. An understanding of *B. thermosphacta* biofilm formation is needed to better control its presence in the food environment and prevent food contamination.

B. thermosphacta biofilms (i.e. 30 strains) were assessed by Crystal Violet staining method, Biofilm Ring Test, specific *rpoC* qPCR and Confocal Laser Scanning Microscopy (CLSM). Different food environment materials such as Polycarbonate (PC), Polystyrene (PS) and Stainless steel (SS) were used in a CDC biofilm reactor to evaluate their impact on *B. thermosphacta* biofilm. The CLSM images were analyzed with BiofilmQ toolbox.

From the 30 strains studied, 7 were classified as high biofilm producers and 27 as low biofilm producers. The structure of those high biofilm producers is flat with a higher number of cells, a higher biofilm surface area, biofilm thickness and a higher biofilm volume. A high biofilm producer strain showed the same biofilm formation on PC, PS and SS, however a highly structured biofilm on PC and PS compared to flat biofilm on SS was observed. This architecture seems to be correlated to material topography, roughness and hydrophobicity. The biofilm outer surface area and the substrate area covered by the biofilm were higher on SS. Conversely, biofilm density and biofilm volume were higher on PC and PS. High biofilm producer, associated with rough surface materials could explain the presence of *B. thermosphacta* on food industrial surfaces. A transcriptomic analysis allowed to identify the differentialy expressed genes in the biofilm state of *B. thermosphacta* and those specifically involved in the biofilm formation, such as the operon “iol” genes, responsible for the uptake of myo-inositol, which were all upregulated in the mature biofilm state.

To our knowledge, this study is the first describing the diversity of *B. thermosphacta* biofilm formation and structure with a set of complementary biofilm assays on an abiotic surface, with also a transcriptional and functional characterization.

Flash communications

MICROBIOLOGICAL AND GENETIC ANALYSIS OF *LISTERIA MONOCYTOGENES* IN BIOFILM AND PLANKTONIC STATES: IMPACT OF TRANSFER TO COOKED HAM, SUBSEQUENT STORAGE AND SIMULATED *IN VITRO* GASTROINTESTINAL DIGESTION

Salud María Serrano-Heredia, Javier Sánchez-Martín, Olga María Bonilla-Luque, José Luis López Carmona, Antonio Valero Díaz, Elena Carrasco Jiménez

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Listeria monocytogenes represents a significant risk for public health. According to the last European Union One Health 2022 Zoonoses Report, it was considered the fifth most commonly reported zoonosis in humans in the European Union. *L. monocytogenes* exhibits a high survival capacity at refrigeration temperatures and different stress conditions, being able to form biofilms that could be transferred to ready-to-eat foods during processing.

The objective of this study was to assess the survival ability and virulence of *L. monocytogenes* in planktonic and biofilm states during sequential cross-contamination events to cooked ham, followed by storage and an *in vitro* gastrointestinal digestion. For this, biofilms of three *L. monocytogenes* strains (L1C5, L196 and L138) previously isolated from meat industries, were developed on stainless steel coupons with an incubation period of 5 days at 25 °C. Afterwards, they were transferred by contact to cooked ham slices for 90 seconds.

In parallel, for the sake of comparison, part of the ham batch was contaminated with 10 µL of *Listeria* cells in the planktonic state with the same initial concentration used as for biofilm formation (104 CFU/mL). Then, from the two contamination pathways, slices were subjected to *in vitro* digestion either immediately or after a 7-day storage at 7 °C under vacuum packaging. A control batch with *Listeria* cells directly submitted to digestion was assayed. ANOVA test for *Listeria* counts was carried out using R version 4.3.2.

Finally, different virulence genes of *L. monocytogenes* (*16S, plcA, plcB, prfA, argA, inlA, inlB, inlC, inlJ, Hly, flaA, sigB and Lmo 2672*) were evaluated at all digestion steps by using qPCR. Results showed that *L. monocytogenes* was present in all the scenarios studied after the digestion process (reaching a pH value of 3), with a significant reduction in the population from the initial concentration.

For biofilm cells transferred to cooked ham, there were significant differences after digestion, both immediately or after storage ($p < 0.05$), as well as for planktonic cells ($p < 0.001$). By comparison with the control, it was found out that food matrix contributed as a protector of *L. monocytogenes* cells.

THE PERSISTENCE PHENOMENON OF FOOD-ASSOCIATED *LISTERIA MONOCYTOGENES*. A CASE OF OPPORTUNISM OR ADAPTATION?

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Foodborne pathogens, such as *Listeria monocytogenes*, can survive in food processing facilities over long time periods, with the potential to contaminate food products. This so-called persistence poses a critical food safety risk, with far-reaching economic and public health implications. Despite numerous investigations into this phenomenon, factors contributing to *L. monocytogenes* persistence have yet to be thoroughly elucidated.

In this study, we take a two-tiered approach toward investigating patterns underlying *L. monocytogenes* persistence. First, we use a meta-analysis approach, followed by a broad genome analysis of *L. monocytogenes* isolates collected from food processing facilities across Austria. The meta-analysis revealed no clear pattern of persistence across the *L. monocytogenes* clonal complexes, nor a clear pattern of gene presence/absence that might explain adaptations to food processing environments. These results, based on a limited number of genes, hint that we may be overlooking some critical factors in studies of *L. monocytogenes* persistence. In a follow-up study, we identified putatively persistent strains across food producers in Austria using whole genome SNP analysis. We then

broadly investigate gene content, focusing not only on stress resistance and virulence genes, but also on genes associated with growth and general survival.

Finally, we suggest key factors that should be considered in future studies of *L. monocytogenes* persistence in the context of food processing environments, including enhanced metadata collection, and in-depth characterization of microbial interactions. Taken together, we advocate for an expanded eco-evolutionary approach toward deciphering persistent *L. monocytogenes*, which will facilitate improved monitoring and elimination strategies in the future.

SURVEYING *SALMONELLA* ON IMPORTED CHICKEN CARCASSES: OCCURRENCE, ANTIMICROBIAL RESISTANCE, AND WHOLE-GENOME SEQUENCING ANALYSIS IN THE UNITED ARAB EMIRATES

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There exists a dearth of knowledge regarding Non-typhoidal *Salmonella* (NTS) prevalence and genomic features within the United Arab Emirates (UAE), a key market in global chicken meat consumption. In this study, *Salmonella* was detected in 16 out of 254 (6.30%) samples of imported frozen chicken carcasses. Among the recovered serotypes, *S.* Minnesota; ST 548 (10/16) emerged as the most prevalent, trailed by *S.* Heidelberg; ST 15 (4/16), and then *S.* Kentucky; ST 198 and ST 152 (2/16). Antimicrobial resistance was most prevalent against tetracycline (93.7%), followed by Ampicillin (68.7%), and extended-spectrum cephalosporins (ceftriaxone and cefoxitin) (56.2%), with 68.7% (11/16) of the isolates classified as multiple drug resistances (MDR).

Among the ten out of the eleven MDR *Salmonella* isolates, both IncC and Col(pHAD28) plasmid incompatibility types were concurrently featured. Whole-genome sequencing (WGS) analysis revealed that 10 of the 16 isolates carried the plasmidic AmpC cephalosporinases (pAmpC) gene, *blaCMY-2*. The range of virulence genes varied from 149 to 165 genes, with an average of 168 genes per isolate. With the exception of one isolate, all other isolates were found to possess genes associated with the type III secretion system (TTSS) encoded by the *Salmonella* pathogenicity island-1 (SPI-1).

These findings contribute to our global understanding of *Salmonella* epidemiology, with a specific focus on the Middle East. The insights gained from this study hold potential significance in shaping import risk analyses aimed at mitigating *Salmonella* exposure risks through globally traded chicken carcasses. The research underscores the value of WGS as a crucial tool for substantiating evidence-based food safety hazard assessment.

EVOLUTION AND PERSISTENCE OF LONG-TERM CULTURES OF *PSEUDOMONAS FLUORESCENS* STRAINS UNDER STRESSFUL CONDITIONS OF STARVATION AND REFRIGERATION TO SIMULATE THE FOOD PRODUCTION ENVIRONMENT

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The ability to persist has been extensively demonstrated in several species of microorganisms. Most bacteria are able to survive for very long periods (years) in the form of Viable But Not Culturable (VBNC) or persister. These states are always present in a certain percentage in bacterial populations and occurred as a result of stress-induced and stochastic physiological changes. The two states differ in their ability to resume growth upon stress removal, returning them to a metabolically active state. In fact, the VBNC state requires a form of awakening before regaining the ability to grow in culture. The VBNC and persister states represent hidden sources of contamination and infection in food and health services.

The current study aimed to investigate the formation of persisters and VBNCs in three strains of the *Pseudomonas fluorescence* subgroup (CECT378, L3-4, and UK1). Strains were incubated in five replicates at two temperatures, 22 and 4 áµ'C, under nutrient starvation, and characterized

phenotypically and genotypically at defined intervals. After six months, the long-term cultures at both temperatures showed high stress tolerance while containing >107 cfu.mL-1 viable cells and maintaining their capabilities, especially UK1, in biofilm formation and motility. Persisters and VBNC cell fractions were evaluated using antibiotic treatment and PMAxx-qPCR, respectively. Results of six months at both temperatures showed low values of VBNC for all the tree strains, from 0- 1.1 log, versus high variability of persister cells between the strains (0-7.95 log), which CECT378 showed the highest counts while UK1 had the lowest value.

To evaluate the dynamics of the genetic adaptation during the long-term cultures 600 single colonies of the three strains were collected at the beginning, middle, and end of the experiment for whole genome sequencing, as specific mutations seemed to contribute to stress adaptation. Additionally, significant high counts of total cells presented in strain CECT378, led us to apply an RNA single-cell sequencing, using the PETRI-seq (prokaryotic expression profiling by tagging RNA in situ and sequencing) technique to define the differences in the transcriptomes of the cell subpopulations.

NEW METHODS IN FOOD MICROBIOLOGY

Keynote 4.1

EXPLORING THE CONTINUUM FROM 'A FEW' CELLS TO COMPOSITE MICROBIAL COMMUNITIES FOR SAFER FOOD SUPPLY CHAIN

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With the advent of omics, the advancements in the technology of analytical equipment and the increased computational capacity, including artificial intelligence, in food microbiology, the frontiers between single cells, physiology, and the collective outcome of microbial communities in foods start to become '*blurred*'. Understanding stress responses and dynamics of single cells serves as a bridge between the stochastic behavior of small populations, which commonly relates to food safety issues, and the deterministic behavior of large populations, commonly relevant to food spoilage and fermentation.

Increasing our knowledge and understanding of the dynamics of single cells and microbial communities enables a systematic manipulation of the classical "*multiple hurdle*" technology to maximize shelf-life and safety of foods. Predictive microbiology is a mature discipline that offers the opportunity to quantitatively express the impact of multiple hurdles on microbial behavior. This is imperative, particularly, due to the increasing popularity of novel preservation methods, which tend to be mild and natural, i.e., with minimal impact on sensorial and nutritional properties of foods and minimum use of chemical preservatives. In the same context, new product formulations, e.g., plant-based dairy or meat alternatives pose new market and public health challenges. Proactive, science-based assessments about the perishability and safety of these foods are critical to facilitate global trade, without compromising public health, especially in view of global warming derived from the climatic change.

Moving away from intense lethality processes (thermal processing), or traditional chemical preservatives, requires that their unquestionable effectiveness is counteracted by a combination of alternative process or agents of lower intensity, yet at least equivalent efficacy. This is where predictive modelling tools can be of great assistance. Furthermore, quantitative expression of microbial responses with predictive modelling is valuable for estimating the public health burden of '*what if*' scenaria along the food supply chain, such as new product formulations, variability in distribution and retail conditions, consumer handling, etc.

The presentation aims to offer a critical overview of how the combined use of omics, predictive modelling and non-invasive methodologies may help in understanding microbial behavior and minimize food losses, while safeguarding public health. Thus, improving food security too. In the first part, food safety Issues will be addressed and particularly, the methods employed in understanding single cell physiology, the dormancy-injury-viability continuum of pathogens exposed to sub-lethal stresses, and how this may relate to population dynamics. In the second part, which is relevant to food spoilage, harnessing of succession of bacterial communities and the metabolic fingerprint of foods, as shaped up by microbial growth during storage, reference will be made to non-invasive techniques that could be used to assess food spoilage in non-destructive, real-time manner.

Keynote 4.2

REVOLUTIONISING FOOD SAFETY: HARNESSING SEQUENCING TECHNOLOGIES AND BIOINFORMATICS FOR ADVANCED MICROBIOLOGY IN THE REALM OF MICROBIAL DARK MATTER

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Sequencing technologies and the application of bioinformatic approaches are reshaping microbiology and its applications, encompassing aspects of food quality and precision food safety. The use of Next-Generation Sequencing (NGS) technologies and protocols allows a deep understanding of the genomes of microorganisms in pure cultures through whole-genome sequencing. Importantly, metagenomics has facilitated extensive comprehension of the microbiota and microbiome of food. Microbial communities along food chains have increasingly been studied to describe the genetic diversity, functionality, and succession of spoilage microflora, foodborne pathogens, and functional microorganisms used in food production by fermentation.

In this talk, several concepts and approaches will be introduced, including strategies to reveal the causes of unsuccessful microorganism culturing. We will journey through the discoveries of microbial community diversities in cheese, discuss the importance of validating computational approaches for species identification, and examine a pilot study where water kefir was used as a model to evaluate the performance of different sequencing approaches. A common aspect of all these studies is the challenges modern microbiology faces: integrating multiple strategies, repeatability of technologies, and sustainability of databases. The predictive methods to be presented have allowed for detailed descriptions of complex microbial communities. Several isolates were retrieved through culture-dependent techniques and in situ culturing approaches, previously identified as high-quality metagenome-assembled genomes (MAGs) of prominent probiotic species such as *Gluconobacter*, *Liquorilactobacillus*, *Lactiplantibacillus*, *Lentilactobacillus* and *Lacticaseibacillus*. Nanopore technology applications were used to describe the samples through full-length 16S rRNA gene sequencing and shotgun metagenomic approaches, coupled with software-controlled enrichment (Nanopore Adaptive Sampling, NAS), of the species isolated with culturing. These next-generation tools are set to radically change risk assessment approaches and methods, better pinpointing the origins of contamination events and describing potential unknown pathogens as well as beneficial microorganisms that are components of microbial dark matter.

Traditional methods for detecting microbial threats often fall short in terms of timeliness and specificity, suggesting the need for more advanced approaches. In particular, a novel strategy will be presented that combines genomic identification through the use of 16S rRNA gene diversity integrated with bioinformatics pipelines for accurate taxonomical identification, compared to the more established MALDI-TOF assay. The synergy between NGS and bioinformatics not only speeds up microbial detection but also enhances the accuracy and depth of microbial risk assessments. This integration offers a holistic approach, empowering food microbiologists and food industry stakeholders with data-driven insights for making informed safety decisions.

Oral 4.1

DEVELOPMENT OF A RAPID AND RELIABLE METHOD FOR THE IDENTIFICATION OF *BACILLUS* SPP. SPECIES IN PLANT-BASED PRODUCTS BY *TUF* GENE SEQUENCING WITH MINION

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As already demonstrated in different studies, plant-based products may run the risk of contamination by thermoduric spore-forming *Bacillus* spp., able to produce harmful toxins and surfactins. Since modern production systems are such intensive and rapid, having methodologies for the detection of foodborne pathogens able to cope with them is still a milestone.

This study addresses the development of a rapid high-throughput targeted sequencing approach using Oxford

Nanopore technologies (ONT), enabling the simultaneous detection and identification of *Bacillus* species relevant to the safety of plant-based food.

Firstly, we developed a DNA extraction method, in which a food matrix pre-processing step by enzymatic digestion treatments and a beat beating mechanical lysis were performed. The method was validated with a Droplet Digital PCR probe assay, inoculating solutions of purified spores (*Bacillus cereus, Bacillus thuringiensis, Bacillus licheniformis, Bacillus subtilis* and *Bacillus atrophaeus)* in soy beverages and meat analogues products, in a final concentration ranging from 1 × 101 to 1 × 105 spores mL−1. The *tuf* gene was amplified using modified primers tufGPF/tufGPR and libraries prepared with the Native Barcoding kit. The sequencing was conducted on a MinION device, by applying the Super accurate model of Dorado basecaller. Taxonomic classification was performed with BLASTn tool against a custom database, including *tuf* gene sequences extracted from all the *Bacillus* genomes deposited in NCBI database.

Digital PCR analysis indicated that the use of a sample pre-processing procedure in combination with mechanical bead-beating cell lysis resulted in efficient extraction of *Bacillus* spores DNA from spiked samples, with a relative detection limit of 101 spores mL−1 for both matrix categories. Thus, the sequencing pipeline resulted in a successful identification of four out of the five species tested, not allowing to discriminate between highly related *B. cereus* and *B. thuringiensis* strains. By using ONT, *Bacillus* strains could be detected at 102 spores mL−1 of sample, with actual proportions of each microorganism reflecting expected relative abundances.

In conclusion, the developed workflow revealed to be a rapid, reliable and robust method for the detection and identification of highly related *Bacillus spp.* species, potentially contributing to the improvement of food safety assessment and food control applications.

Oral 4.2

TRACING THE ORIGIN AND AUTHENTICITY OF SPANISH PROTECTED DESIGNATION OF ORIGIN HONEY USING METAGENOMICS AND MACHINE LEARNING

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Honey products are widely consumed due to their health-promoting properties and there is a general interest in tracing their geographical origin. In this regard, metagenomics sequencing and machine learning (ML) have been proposed as novel technologies to guarantee traceability and authenticity of foods. To our knowledge, no previous attempts to identify microbial biomarkers of honey products with a protected designation of origin (PDO) within the European Union have been developed.

The aim of this study was to develop a novel metagenomics approach to identify microbial biomarkers of Spanish honeys (n = 78) from three different PDOs (Granada, Tenerife and Villuercas-Ibores). 54 out of the 78 samples met all the sequencing quality criteria. ML algorithms were used to differentiate honey metagenomes from each PDO (Granada, n = 14; Tenerife, n = 25; and Villuercas-Ibores, n = 15), as well as PDOs honeys from non-PDOs (n = 9) honey products. For this purpose, the following ML models were computed: i) partial least squares (PLS), ii) random forest (RF), iii) boosted logistic regression (LOGITBOOST), iv) artificial neural networks (NNET).

According to the results obtained, non-PDO honeys and Granada PDO showed higher beta diversity values than Tenerife and Villuercas-Ibores PDOs. ML classification of honey products allowed the identification of different microbial biomarkers of the geographical origin of honeys: *Lactobacillus kunkeei*, *Parasaccharibacter apium* and *Lactobacillus helsingborgensis* for PDO honeys and *Paenibacillus larvae*, *Lactobacillus apinorum* and *Klebsiella pneumoniae* for non-PDO honeys. In addition, potential microbial biomarkers of some honey varieties

including *L. kunkeei* for Albaida and Retama del Teide varieties, and *P. apium* for Tajinaste variety, were identified. ML models were validated on an independent set of samples leading to high accuracy rates (above 90%).
This work demonstrates the potential of ML to differentiate different types of honey using ML based on their metagenomes, leading to higher performance metrics compared to conventional approaches. In addition, ML models discriminate both the geographical origin and variety of products corresponding to different PDOs and non-PDO products within Spain. Results here presented may contribute to develop enhanced traceability systems and could be applied to a wide range of foods.

Oral 4.3

APPLICATION OF THE IR BIOTYPER FOR RAPID IDENTIFICATION AND DISCRIMINATION OF *LISTERIA MONOCYTOGENES* STRAINS IN PERSISTENCE AND OUTBREAK INVESTIGATIONS

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Typing of *Listeria monocytogenes* (*Lm*), an important foodborne pathogen, traditionally relies on time-consuming and costly DNA- and sera-serotyping-based techniques. Fast identification and classification of this pathogen could significantly improve food safety and infection control, mitigating its impacts. The IR Biotyper, utilising Fourier-transform infrared spectroscopy (FTIRS), offers a novel approach capable of generating highly specific spectra, enabling strain typing within 3 h.
In this study, we assessed the IR Biotyper's efficacy in recognizing *Lm* persistence or outbreak-associated clones. Sixty-two strains underwent typing via whole genome sequencing (WGS), PCR, sera serotyping, and FTIRS. FTIRS analysis focused on the 1300-800 cm-1 region, targeting cell envelope carbohydrates. Strains grown on various agars (Blood, ALOA, PALCAM, Rapid'Lmono, Brain Heart Infusion) at 37 °C for 24 h or 48 h were analyzed. The congruence of FTIRS clustering was compared to WGS, PCR typing, and serotyping using the Adjusted Rand index and Adjusted Wallace coefficient.
FTIRS achieved 100% accuracy in distinguishing *Lm* strains, clustering them by lineage and broadly into serotypes (1/2, 3, 7, and 4). Further analysis improved the classifier's ability to differentiate the more pathogenic 4b isolates from other serotype 4 variants (i.e., 4a, 4c, 4d, and 4e). The classification resolution surpassed that of PCR typing, generally aligning with WGS. Despite challenges in separating 1/2 strains into serotypes 1/2a and 1/2c, FTIRS successfully clustered epidemiologically linked isolates from outbreaks or persistent strain contamination scenarios, even differentiating strains grown on varied media. The highest clustering coherence occurred when strains grown on the same agar were compared, indicating the media's impact on bacterial metabolic profiles. The incubation period (24 h vs. 48 h) and temperature minimally affected results, primarily influencing classification confidence, given that the classifier was created with strains grown for 24 h. Adherence to standard sample preparation protocols was deemed crucial for accurate FTIRS-based characterization.
Overall, the IR Biotyper demonstrated rapid and accurate discrimination between *Lm* strains at lineage, serotype, and clonal levels, showcasing its potential for real-time pathogen monitoring and source-tracking to enhance public health and food safety. It is proposed as an early screening method, followed by more in-depth analysis with WGS.

Oral 4.4

HOW CAN DNA-BASED OMICS EFFECTIVELY IMPROVE MICROBIOLOGICAL QUALITY CONTROL? AN EXPLORATIVE STUDY APPLIED TO INFANT FOOD PRODUCTION

Cristian Botta[1], Dimitra Tsoureki[1], Anupam Gautam[2,5], Sascha Patz[3], Katerina Pissaridi[4], Ilario Ferrocino[1], Irene Franciosa[1], Luca Cocolin[1], Daniel Huson[1], Kalliopi Rantsiou[1]

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Community profiling genomics, metagenomics and pangenomics are nowadays extensively used in medical

research to obtain a holistic view of the patient's condition and to highlight biomarkers associated with human pathologies and diseases. Although they represent well-established techniques, in food science and technology their use is still marginal and their potentialities are far from being fully understood and thus effectively exploited by industries in microbiological control. It is therefore pivotal to explore their applicability on site, starting with food processes in which safety risks lead to the highest impact on certain population, such as the production of infant food.

Therefore, a comprehensive monitoring of microbiota and microbiome has been conducted during the production of a cereal based powder for fourteen months, sampling the environment, raw materials, intermediate and final products. Total DNA of each sample was subjected to 16S rRNA amplicon-based and shotgun sequencing. In addition to these untargeted approaches, targeted detection by enrichment was performed for five microorganisms: *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus*, *Salmonella* spp. and *Listeria monocytogenes*. The presence of alive cells was confirmed by qPCR and isolation, which was followed by pangenome analyses.

Resident environmental microbiota and microbiome changed over time, being affected by different incoming raw materials of each production run, in a frame of powder foodstuffs and high airborne contaminations. Both targeted and untargeted approaches showed a high prevalence of *Bacillus cereus*, mainly in raw materials. Furthermore, the modelling of the production processes through network analysis allowed the determination of potential contamination pathways for all five microorganisms researched, which appeared to be invisible to direct linear observations. Interestingly, the hindering effect of not-bacterial DNA was less evident in community profiling data than metagenomic data, which however provided a better taxonomic resolution of minor taxa in samples with high DNA quality. Finally, the isolate-dependent nature of pangenomic data was pivotal for the discrimination between resident and transient biotypes within the processing plant.

Regardless of the process-specific outcomes, this *in situ* investigation underlines the imperative need to find thoughtful methods that simplify the complexity of omics output to allow better interpretation and application in food industries.

Flash communications

FOURIER-TRANSFORM INFRARED SPECTROSCOPY TO INVESTIGATE AN OUTBREAK OF CEREULIDE-PRODUCING *BACILLUS CEREUS SENSU LATO*

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In September 2022, 59 people got sick after eating from a catered buffet. Elevated numbers of *Bacillus cereus sensu lato* and a high concentration of cereulide, the emetic toxin produced by *B. cereus s.l.*, were both detected in a rice dish. Cereulide and emetic strains were also detected in the feces collected from affected patients.

In order to explore the diversity of *B. cereus* strains associated with the outbreak, a polyphasic approach combining PCR (*ces* gene cluster) and Fourier-transform infrared (FT-IR) spectroscopy was used to type 50 *B. cereus s.l.* isolates obtained from the rice dish (using ISO7932) and from the five stool samples (using heat treatment before isolation according to ISO7932). In parallel, Whole-genome sequencing (WGS) was used for a full characterization of isolates and further comparison with FT-IR. Core-genome multi-locus sequence typing (cgMLST) was compared to FT-IR to assess whether FT-IR could be used as a clustering tool in *B. cereus s.l.* outbreak investigation. Single nucleotide polymorphism (SNP) typing was used to assess clonality in the *ces* positive isolates.

Multiple sequence types were detected in the isolates, indicating a high diversity of *B. cereus s.l.* in the rice and feces samples. The PCR detection of *ces* in emetic isolates was confirmed by the WGS data. The *ces* positive isolates – those with the potency to produce cereulide – were typed as ST144 and ST26, and were found in the rice and fecal samples. cgMLST and SNP analyses suggest that the recovered ST144 isolates are copies of the same strain and that, most likely, some of the patients were infected by this strain through consuming the contaminated rice dish. Interestingly, different clusters of food- and patient-borne isolates were observed on FT-IR and are comparable to those obtained by (cg)MLST.

A NOVEL MULTIVARIATE APPROACH TO IDENTITY OPTIMAL STARTER CULTURES FOR THE PRODUCTION OF FERMENTED BEVERAGES

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Food fermentation has been practiced since ancient times for food preservation during harsh seasons and to obtain different beverages. The microbial action during the fermentation process also enhances the sensory and nutritional quality of the fermented product. In the past, fermentation was carried out on naturally occurring microorganisms but, nowadays, the market-increasing request for standardized and safe fermented products led to the selection and the use of starter cultures and the control of fermentation protocols on an industrial scale.

In light of this, we here describe innovative volatolomic multivariate statistical approaches to drive the selection indigenous yeast strains as candidate for optimal starter cultures. Indeed, the volatolomic analysis of small molecular metabolites in fermented foods is crucial to elucidate their quality properties and is a key tool to select yeast and bacterial strains with better fermentation performances depending on the matrix used, both in terms of quality and safety.

The proposed methods can be combined leading to an accurate selection of analytes to build reliable statistical models for the discrimination and selection of more performant strains.

In particular, a Permutation Test approach is applied to a chemical data set to identify the molecules that characterize the volatolomic profile in a statistically significant way, thus allowing a sharper characterization of the similarities and dissimilarities between the different formulations tested.

Partial Least Square Correlation, a non-asymmetric variation of PLSR, is used to study the correlation, within and between, two different datasets, thus allowing, as example, the correlation between volatolomic and sensory data.

This novel approach, recently applied to the identification of starter strains for the production of craft beers and sparkling wines, provides a systematic and data-driven method for the selection and characterization of yeast strains based on their volatolomic profiles, with a link to sensory attributes. The comprehensive strategy that integrates chemical analysis with statistical modelling to better understand and optimize fermentation processes in the production of specific beverages will be discussed.

ESTABLISHMENT OF NOVEL ROUTINE-METHODS FOR SELECTIVE DIFFERENTIATION AND QUANTIFICATION OF MULTI-STRAINED PROBIOTIC MILK POWDER FOR INFANT FORMULA

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Probiotic products have become more and more established in the food industry, where several studies have demonstrated the various applications in different food products and their beneficial health impacts. The focus of this project is probiotic bacteria in milk powder for infant formula, containing the strains *Streptococcus thermophilus (S. thermophilus), Lactobacillus casei (L. casei), Bifidobacterium animalis* subsp. *lactis* (BB12) and *Lactobacillus rhamnosus (L. rhamnosus).* This composition is similar to the beneficial bacterial colonization of breast milk and ensures an immunological balance in the infant's intestinal tract.

For quality control measurements of multi-strained probiotics, the problem of separation and quantification often arises due to the similarities of strains. However, the bacteria under study have some relevant differences in their growth characteristics, which could potentially resolve the issue. Their individual cultivation can be achieved by using different incubation strategies, like different media, temperatures, incubation times, or aerobic and anaerobic conditions. The milk powder samples were therefore cultivated on Plate-Count (PC), Man-Rogasa-Sharpe (MRS), M17, TOS+Mupirocin (TOS+MuP) and MRS+Cysteinhydrochlorid (MRS+), in combination with different temperatures, varying from 30 °C, 37 °C, 42 °C and 45 °C, and also in aerobic/anaerobic conditions. For identification of the bacteria MALDI-TOF was used.

Overall, the results have shown that it was possible to quantify and differentiate the strains under study with the above-mentioned parameters, when in single and

in co-culture. *S. thermophilus* grew selectively with PC under anaerobic conditions at 45 °C. BB12 was selectively cultivated under anaerobic conditions on MRS+ at 37 °C. For *L. rhamnosus* selective growth was obtained with M17 under aerobic conditions at 37 °C. Only *L. casei* was not possible to grow selectively and can only be quantified by subtraction of the cell count of *L. rhamnosus*. Additionally, the cell counts demonstrated that from the initial cell concentration the recovery rate was > 98%.

For the purpose of quality control measurements, the validations of these methods indicate that it is possible to identify each individual bacteria of a multi-species probiotic milk powder. Furthermore, to do justice to the aspect of food safety and to ensure positive intestinal health effects in infants, these methods showed reliable recovery rates for each strain.

INTER-LABORATORY VALIDATION TRIAL ON A MULTIPLEX REAL TIME PCR METHOD ALLOWING THE IDENTIFICATION OF 30 MAJOR MULTI *LOCUS* SEQUENCE TYPING CLONAL COMPLEXES OF *LISTERIA MONOCYTOGENES* CIRCULATING IN FOOD IN EUROPE

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Listeria monocytogenes (*Lm*) is a bacterium that causes a food-borne illness, the listeriosis. The rapid screening of the strains and the reliability of the analysis between laboratories are crucial to conduct investigation, differentiate strains representing the most significant health risks, understand the entry and the transfer of *Lm* in the food chain.

A new method based on twelve multiplex real time PCR was developed in 2021 by the European Union Reference Laboratory (EURL) for *Lm* [1]. It is an alternative for the characterization of 30 *Lm* multi locus sequence typing (MLST) clonal complex (CC) obtained conventionally by whole genome sequencing (WGS). The CCs are a universal language for strain typing and provide information on strain virulence.

The method assessment is on-going through a European Inter Laboratory Validation Trial (ILVT) involving 15 laboratories in 12 countries.

The ILVT was adapted from ISO standard 16140 part 6. The panel included 98 blinded pure strains. Four *Listeria spp.* non-*Lm* strains and four *Lm* strains different from the 30 CCs targeted, were used as exclusivity panel. Ninety strains (three per CC), were used as inclusivity panel. Results were considered as acceptable when identical to those obtained by the reference method (WGS based MLST). Acceptability limits were set to two failed results per participant.

The method validation is confirmed if at least ten laboratories obtain acceptable results. The ILVT results will be available in May 2024.

This study is presented on behalf of the ILVT study group.

ONE HEALTH

Keynote 10.2

ANTIMICROBIAL RESISTANCE AND GLOBAL HEALTH: ANIMALS, FOOD AND HUMANS, A "ONE HEALTH" CONCERN

David Rodríguez Lázaro

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The emergence antimicrobial multiresistant bacteria is one of the most relevant problems in public health and one of the most relevant challenges we face as a society. This has been recognized by the World Health Organization, the United Nations and the European Union. The World Health Organization estimated that in 2014 more than 700,000 deaths were due to infections by bacteria with antimicrobial resistance, declaring that the spread of antibiotic resistance is already a "terrible" problem. However, expectations are not at all promising, and if nothing is done about it, it is estimated that by the year 2050, deaths from this type of microorganism will be the leading cause of mortality in the world, surpassing deaths from cancer, with figures that will exceed 10 million deaths annually and will represent an economic cost of 100 trillion dollars. In 2015 in Germany, the G7 considered this threat as a global problem of an importance equivalent to global warming and, in September 2016, a monographic session on antimicrobial resistance was held at the United Nations General Assembly. This was the fourth time that the United Nations General Assembly has addressed a health-related.

In parallel, the impact of food pathogenic microorganisms on Public Health systems is very considerable. In addition to these serious connotations for Public Health, food safety is one of the main priorities for the Food Industry. This scenario represents a great challenge, in which the implementation of food safety assurance plans plays a main role, and the application of microbiological controls within these is a premise to minimize the impact of microbial agents and the risk of consumer. This problem has increased in recent decades as a consequence of the globalization of the food market, the increase in the transportation of people and goods, and the profound changes in eating habits and economic crises.

These two concerns, antimicrobial multiresistant bacteria and food safey are closely linked, and the food production chain is one of the different dispersion routes for this type of microorganisms. Therefore, we find ourselves in a delicate balance between a changing world with new emerging dangers, many possibly borne by food, and the maintenance of high-quality official surveillance systems that guarantee the safety of what we eat. It is the responsibility of society and particularly its leaders to maintain this privileged level of control and security in which we currently find ourselves, and consequently a One Health initiative is necessary, which coordinates human, animal and environmental health to address these major public health problems.

Oral 10.4

ANTIMICROBIAL RESISTANCE AND MICROBIAL TRANSMISSION IN BEEF PRODUCTION

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The food industry's environmental and socio-economic impact raises critical concerns regarding food waste and antimicrobial resistance (AMR). Microbial-induced spoilage significantly contributes to food loss, and while it is well-established that antimicrobial use is one of the main drivers

of AMR spread, there is a notable lack of direct research on AMR spread within food systems.

In this research we used a deep shotgun metagenome sequencing approach to obtain a functional profile of the microbial community found in an Austrian beef processing facility. Taxonomic profiling of the microbiota from the different samples by targeting potential pathogens and/or spoilage microbes showed *Actinobacter* spp., *Psychrobacter* spp., *Pseudomonas fragi* and *Brochotrix thermosphacta* to be present in the facility and that their abundance increased throughout the three time points collected within the same working shift. The analysis of the genomic content showed a high prevalence of AMR genes, mainly associated with enterobacteria and conferring resistance to aminoglycosides (42.1% of the total AMR genes; mainly *aph(6)-Id* and *aph(3")-Ib*, beta-lactams (31.6%; mainly different *blaOXA*), tetracyclines (10.5%) and amphenicols (7.0%).

The results of this research provide better understanding of contamination dissemination dynamics within beef processing facilities, providing insights into AMR prevalence, metabolic potential of facility microbiota and strategies to enhance hygiene standards and meat quality, thereby mitigating food waste. Our samples were selected ad hoc after a thorough inspection of the meat processing facility in order to cover a wide variety of surfaces (including conveyor belts, membrane skinners, etc.) towards the identification of potential AMR, virulence, spoilage, and pathogenicity hotspots. Beyond cataloging microbial presence and spread, this study underscores the latent hazards posed by these microbes, regardless of their pathogenic attributes.

Our findings emphasize the urgency of tailored interventions to ensure food safety and public health in these critical settings.

Oral 10.5

FOOD SAFETY NETWORK FOR THE DEVELOPMENT, HARMONIZATION AND ADOPTION OF METHODOLOGIES BASED ON ONE HEALTH ORIENTED TO THE ASSESSMENT OF NEW MICROBIOLOGICAL RISK (ONEHEALTH4FOOD)

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A result of the degradation of the environmental health is the emergence and reemergence of microbiological hazards directly affecting safety of consumed foods. The new microbial risks may not be mitigated or prevented with a traditional approach or managed as a separate factor, regardless of other health problems but rather it requires to consider a global perspective and multisector strategy, based on the One Health concept, putting together information from different disciplines. The OneHealth4Food is a scientific network formed by researchers in the field of Food Safety and One Health, creating a collaborative and synergistic framework, within food context, which will settle the methodological principles of application of an integrated risk assessment process in the food chain. In so doing, it pursues to develop and adopt strategies of detection, prevention and control to tackle emergent and reemergent threats arising from the current societal challenges.

The Network will address the search for new information sources intended to study emergent foodborne pathogens and the collection and analysis of mathematical models, which will be included in a specific tool, making models accessible and applicable. Besides, the role of Next Generation Sequencing (NGS) and Whole Genome Sequencing (WGS) as monitoring tools and early warning systems for known and emergent microbial hazards will also be explored. Another utmost aspect is the development of methodologies for risk ranking based on a quantitative approach and incorporating uncertainty description. Finally, different thematic workshops will be developed, dealing with specific topics and case studies, in which the output from the Network activities will be put into practice.

The network OneHealth4Food brings together the current knowledge from prestigious Universities and Research Public Institutes including researchers who have participated or participate in national and international bodies in charge of Risk Assessment such as the European Food Safety Authority (EFSA) and Spanish Nutrition and Food Safety Authority (AESAN). Hence, the Network proposed herein will have a high impact on the decision-making process and the application of control measures by food authorities and producers. All in all, OneHealth4Food will contribute, from an One Health perspective, to reduce the impact of the future foodborne diseases in a global world

Flash communications

EFFECT OF PRE-HARVEST TREATMENT WITH ELICITORS ON THE HYGIENIC-SANITARY QUALITY AND FUNGAL POPULATION OF DRIED FIGS

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Traditional crop of dried figs involves staggered ripening, progressive drying at different times, and soil contact, which can favour mould growth especially those belonging to *Aspergillus* section *Nigri* and *Flavi*. These fungi are known producers of ochratoxin A and aflatoxins, respectively, whose maximum content in dried figs is legislated due to their toxicity. Elicitors are becoming one of the best alternatives to chemical pesticides to counteract fungal contamination in fruits. Elicitors are responsible for plant growth and are involved in plant defense responses, including the control of pathogen proliferation. Due to their advantages, elicitors have been tested in other fruit crops, but there is a lack of knowledge on dried figs.

The objective of this work was to evaluate the effect of pre-harvest application of two elicitors (γ-aminobutyric acid, GABA, and melatonin, MT) applied twice with different concentrations (MT: 0.1 y 0.5 mM; GABA: 10 and 50 mM) on microbial load, fungal population and mycotoxin production of `Calabacita´ dried figs. Dried figs were harvested in two harvesting dates (F4 y F5) in 2022 compared with an untreated control and 20% tween control. Results showed that elicitor type, concentration and harvesting date have different influences on microbial load, fungi population and mycotoxin production. Mould counts remained similar to the control when GABA 10 mM was applied for both harvesting dates. The most frequent mould genus detected was *Aspergillus* sp. The main species was *A. niger*, major OTA producer, although this was not detected in some batches. *A. flavus*, known as aflatoxin producer, was detected under the detection limit, in line with the non-detection of aflatoxins.

These findings will be useful in developing new strategies to efficiently control toxigenic fungus spoilage in dried figs. Nevertheless, comparison with another crop year is necessary to establish the behaviour of elicitors in dry figs.

PERSISTENCE OF FOODBORNE PATHOGENS DURING HYDROPONIC CULTIVATION OF LETTUCE ON NUTRIENTS BASED ON SOURCE-SEPARATED URINE

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Hydroponic (soilless) cultivation of crops such as leafy greens represents an important industrial food production technology. Recirculation and process control of the nutrient solution offer advantages such as precision fertilization, improved water and nutrient use efficiency, and reduced environmental impact. Furthermore, hydroponics in protected environments reduce the risk of contamination from foodborne pathogens which often originate from soil. Improved resource utilization is essential in the circular economy, and plant nutrients recovered from organic waste such as source-separated urine represent sustainable alternatives to traditional, mineral fertilizers.

One key consideration relates to the fate of pathogens in the crop cultivation process. Here, we investigated the survival and growth of known foodborne pathogens in a hydroponic lettuce cultivation system using a nutrient solution based on source-separated, mineralized, human urine.

The experiment was conducted in six 4,5 L deep-water batch culture systems containing three plants of *Lactuca sativa* each. The seeds were allowed to germinate and establish for one week before the nutrient was added to the water. Simultaneously, five foodborne pathogens (*Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enterica*, *Escherichia coli* and *Bacillus cereus*) were spiked in the nutrient solution at approximately 104 CFU/mL. Pathogens growth and survival was analysed from the nutrient solution by conventional plating on general and selective growth media weekly over one month. The fate of pathogens was further analysed by additionally sampling the edible plant parts, roots, and biofilm inside the cultivation container, at the day of lettuce harvest.

The results show that all pathogens, except *Salmonella enterica,* decreased to below the detection limit (< 10 CFU/mL) in the nutrient solution over time, but at different rates. *L. monocytogenes* and *E. coli* were however still detected in the roots at harvest. *S. enterica* increased slightly over time in the nutrient solution to >1,0*105 CFU/mL and was also detected in all sample types at the day of harvest. *S. enterica* was the only pathogen that was detected on the edible part of the plants.

This experiment demonstrates that common foodborne pathogens can survive in a nutrient solution derived from human waste during the cultivation of lettuce and eventually contaminating the product.

PREDICTIVE MICROBIOLOGY AND MICROBIAL RISK ASSESSMENT

Keynote 9.1

FROM ANALYSES OF THE RISK ANALYSIS TO FOOD SAFETY RECOMMENDATIONS

Heidy M. W. den Besten, Wieke van der Vossen-Wijmenga, Marcel H. Zwietering
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Listeriosis cases have been associated with consumption of ready-to-eat cooked meat products. A risk assessment can provide insights in factors affecting the number of cases and provides guidance for recommendations to reduce the disease burden. This presentation will discuss a stepwise risk assessment for ready-to-eat cooked meat products, from exposure assessment –with a focus on actual consumer practices– to risk characterisation, and compares different approaches to analyse the risk analysis. Decisions made in the different steps of a risk assessment affect the final risk estimation.

The risk assessment discussed in the presentation serves as example risk analysis that uses complementary, transparent approaches to analyse the risk. The pros and cons of various approaches will be discussed: usage of databases for parameter estimation, deterministic versus stochastic risk characterisation, analysis of the disease cases versus analysis of the probability of illness, and homoscedasticity versus heteroscedasticity of variances for variables.

This analyse of the risk analysis sheds light on relevant aspects to analyse within risk assessments, and also prioritizes the most important factors contributing to the risk. From these primary risk factors, key recommendations could be derived for targeted communication to consumers to reduce listeriosis.

Keynote 9.2

ADVANCEMENTS IN PREDICTIVE MICROBIOLOGY IN FOODS: BRIDGING DISCIPLINES FOR A SAFER FOOD FUTURE

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University of Córdoba, Spain

Predictive microbiology has emerged as a crucial tool in ensuring the safety and quality of food products. Over the years, significant advancements in statistical software and computing have significantly moved the discipline forward, enabling the development of sophisticated mathematical models and computational tools that cover a wide range of microbial phenomena (growth, inactivation, cross-contamination, etc.), foods and microorganisms. The publication of *The Thin Book* by Pérez-Rodríguez & Valero in 2013 marked a milestone in predictive microbiology, summarizing the field's progress and highlighting the predictive microbiology track to the digital area. The journey of predictive microbiology began with the development of mathematical models aimed at predicting microbial growth and inactivation kinetics in different food matrices.

These models, initially based on empirical data, have evolved to incorporate complex factors influencing microbial behaviour, including environmental conditions, food composition, and microbial interactions. Recent innovations in predictive modelling techniques, such as mechanistic models and artificial intelligence algorithms, have enabled more accurate predictions, empowering food industry stakeholders to make informed decisions regarding food safety and shelf-life extension. Latest achievements in predictive models have not only enhanced our understanding of microbial behaviour in foods but also opened doors to interdisciplinary applications. The integration of omics technologies, including genomics, proteomics, and metabolomics, with predictive microbiology holds promise for personalized food safety solutions. By unravelling the molecular mechanisms underlying microbial

adaptation and virulence, omics-driven predictive models enable targeted interventions and the development of precision food safety strategies.

Additionally, the advent of metagenomics has revolutionized our understanding of microbial communities in complex food ecosystems, paving the way for ecosystem-based approaches to food safety management. Moreover, the use of dedicated predictive microbiology models with digitalization technologies has revolutionized food safety management systems. Real-time monitoring of microbial populations along the food supply chain, coupled with predictive modelling, facilitates proactive risk assessment and intervention strategies, better addressing foodborne illness outbreaks. Furthermore, the utilization of big data analytics and machine learning algorithms enables rapid identification of microbial hazards and optimization of processing parameters, enhancing both efficiency and safety in food production.

In the face of climate change, predictive microbiology offers invaluable insights into the impact of environmental factors on microbial proliferation and survival in foods. Shifts in climate variables patterns (temperature, humidity, wind conditions etc.) pose challenges to microbial food safety. Predictive models tailored to climate-related variables empower food producers to adapt their practices and mitigate the risks posed by changing environmental conditions, ensuring the resilience of the food supply chain. This presentation delves into the evolution of predictive microbiology, highlighting its multifaceted contributions across various domains such as digitalization, climate change, omics, and beyond. Characterization of population dynamics, as well as the integration of multidisciplinary information (climate change, molecular information, energy resources, etc.) into predictive microbiology models, remain the major challenges to be addressed in the coming years.

Oral 9.1

ESTIMATING STRAIN VARIABILITY IN THERMAL INACTIVATION OF *CAMPYLOBACTER JEJUNI* BASED ON WHOLE GENOME DATA

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Campylobacter jejuni is one of the broadest-spreading foodborne pathogenic bacteria. The quantitative microbiological risk assessment (QMRA) helps us to understand and control microbial risks, and considering strain variability with predictive models is essential in QMRA. Strain variability has been described based on statistical hypotheses such as that model parameters follow probability distributions. Recently, the application of genomic data on predictive microbiology has attracted attention, and the genome-based models could predict bacterial behaviors of unknown strains.

This study aimed to develop a predictive model for the reduction behavior of *C. jejuni* based on whole genome data and to predict the behaviors of *C. jejuni* reported in genome banks. Thermal tests for sixteen strains of *C. jejuni* at 55 °C were conducted, and the observed reduction behaviors were described with the modified Weibull model using the most probable curve method. The sequenced whole genome data of the sixteen *C. jejuni* and the genomes from the genome bank (679 genome data) were analyzed with whole genome multi-locus sequencing typing (wgMLST). The dimensions of allele data were reduced with the uniform manifold approximation and projection (UMAP).

From the preprocessed whole genomic data, a genome-based predictive model was developed with xgBoost for parameters of the modified Weibull model. The RMSEs of parameters from the genome-based model were 0.29 (delta) and 0.15 (power) by full cross-validation. The wgMLST and UMAP can preprocess whole genome data for predictive modeling. The genome-based model predicted the reduction behaviors of 695 genomes. The genome-based model prediction of the strain variability was similar to the prediction derived from a multivariate normal distribution model but a little smaller. The genome-based model parameter predictions depended on the

input parameters' maximum and minimum values. The dependency could be a limitation for whole genome-based predictive models of strain variability using machine learning.

Oral 9.2

EXPLORING THE BIODIVERSITY OF A TARGETED MICROBIAL CORE OF LACTIC ACID BACTERIA: CONNECTING EXPERIMENTAL DATASETS WITH PREDICTIVE MODELS

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Microorganisms represent a biodiversity reservoir in every ecological habitat, with lactic acid bacteria (LAB) encompassing a diverse array of genera capable of colonizing different niches. The adaptability of LAB to various environmental conditions is attributed to the flexibility of their metabolism. Due to advancements in DNA sequencing and bioinformatics, we can now delve into the potential metabolic functionality of microbial genomes. Despite the swift evolution of sequencing technology, systematic studies on phenotype profiling have lagged behind due to technical and throughput limitations.

This work aims to investigate the phenotypic biodiversity of a selected microbial core (SMC) of LAB to explore the microorganisms' metabolic functionalities by exploring genotypic and phenotypic biodiversity in microbial collections. The SMC treated in this work consists of 150 strains of LAB of food origin belonging to the University of Parma Culture Collection (UPCC).

Strains of the SMC were genotypically characterized by Amplified Fragment Length Polymorphism (AFLP), a fingerprinting technique that provides resolution to the subspecies level. Metabolic capabilities of the strains were tested on an array of 70 different substrates (including sugars, amino acids, carboxylic acids, etc.) by using GENIII plates (Biolog). At the same time, genotypic and phenotypic data were retrieved from the publicly available database BacDive (https://bacdive.dsmz.de/). The experimental and *in silico* data were used as a data set for training predictive machine learning models.

In particular, we applied classification algorithms for predicting the phenotypic traits encoded by the bacterial strains according to their phylogenetic profiles (experimental or simulated AFLP). As a result, we were able to correctly classify a good portion of phenotypic traits with an average accuracy of 0.75 and by using 80% of the data set for the training in a 5-fold testing process.

A combination of genotypic and phenotypic data can provide an insight into the metabolic capabilities of industrially relevant strains, disclosing their potential application for the biotransformation of various substrates, including wastes and by-products of food origin. The application of Artificial Intelligence to investigate the biodiversity of microorganisms represents a powerful approach to expand the existing knowledge on bacterial physiology and developing novel industrial applications of LAB.

Oral 9.3

MODELING THE GROWTH AND DYNAMICS OF THE UROPATHOGENIC *ESCHERICHIA COLI* (UPEC) IN SUGARCANE JUICE

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Freshly prepared fruit juices (e.g., sugarcane juice) without sterilized processes before consumption are vulnerable to foodborne pathogens contamination, including uropathogenic *Escherichia coli* (UPEC), which is an emerging foodborne pathogen responsible for 65-75% of urinary tract infections (UTI). However, the growth kinetics of UPEC in freshly prepared fruit juices is still unclear. This study aimed to assess the effect of storage temperature on UPEC growth in sugarcane juice via predictive modeling.

The growth curves of UPEC were generated by inoculating diluted and undiluted sugarcane juice with a four-strain UPEC cocktail and incubating at 4, 10, 15, 20, 30, and 40°C. Changes in the UPEC growth were analyzed using three primary models and two secondary models to estimate

the kinetic parameters and the effect of temperature. Model performance was evaluated by comparing statistical indices, including root mean square error (RMSE) and proportion of prediction error (pPE).
Growth of UPEC was observed in both diluted and undiluted juice samples at temperatures between 15 to 40 °C. Moreover, the statistical indices implied that the integration of the reparameterized Gompertz and the Ratkowsky square-root models was adequate to describe the UPEC growth in both types of sugarcane juice. The estimated nominal minimum temperature (T_0) of UPEC in sugarcane juice and diluted ones was 14.6 and 15.0 °C, respectively. The prediction performance was validated under isothermal (35°C) and dynamic conditions (15 to 37°C with 4 h cycles). Validation results showed the model appropriately estimated UPEC growth under isothermal conditions in both sugarcane juices (RMSE, 0.19-0.79 log CFU/mL; pPE, 0.82-1.00). The prediction for the dynamic condition in sugarcane juice (RMSE, 0.48 log CFU/mL; pPE, 0.82) and diluted ones (RMSE, 0.32 log CFU/mL; pPE, 1.00) was acceptable, with pPE > 0.7.
The developed model, therefore, may be useful for simulating the effects of storage temperature on the growth of UPEC in sugarcane juice and diluted ones and for microbial risk assessments evaluating food safety.

Oral 9.4

THERMAL INACTIVATION OF *BACILLUS LICHENIFORMIS, BACILLUS SUBTILIS* AND *BACILLUS CEREUS* SPORES IN A PEA-BASED MILK ALTERNATIVE

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The use of plant-based ingredients is not new to the food industry, however, the way in which they are processed and used to produce dairy alternatives should be evaluated to verify if conventional processing is adequate to ensure microbiological safety and stability of the newly developed recipes. The objective of the study was to model and compare the thermal inactivation of spores of three *Bacillus* species in a pea-based milk alternative. *Bacillus licheniformis* CTCPA 3107001, *Bacillus cereus* F4810/72 and a *Bacillus subtilis* strain isolated from pea-based drink were selected as model organisms as they are commonly involved in spoilage and safety incidents. Experiments were performed in a pea-based milk alternative with the methodology of thermal treatment with capillary tubes in oil bath. Heat treatments were conducted in duplicates for *B. cereus* and triplicates for *B. licheniformis* and *B. subtilis.* Spores were inoculated to the matrix with initial concentration of 9, 8 and 6 log CFU/mL for *B. licheniformis, B. subtilis* and *B. cereus*, respectively. Samples inoculated with *B. cereus* were heat-treated at four temperature levels between 90-100 °C, while those inoculated with the other two microorganisms were heat-treated at five temperature levels between 97-110 °C.
Fittings were performed to estimate the D-values and then the Z_T-values by linear regression. Amongst the three species, *B. subtilis* was the most heat resistant, while *B. cereus* the most heat sensitive. Z_T-values were 8.98, 9.99, 6.07 °C and the predicted $D_{135°C}$ were 0.03, 0.05 and 8.6 × 10-5s for *B. licheniformis*, *B. subtilis* and *B. cereus,* respectively. The inactivation times required in the tested plant-based matrix were below the established practices even for the most resistant amongst the tested species. This is important as these microorganisms are highly prevalent in raw ingredients.
To our knowledge, this is the first attempt to compare heat inactivation of spores of spoilage and pathogenic *Bacillus* spp in pea-based milks. These results can be combined with prevalence likelihood data to support the designing thermal treatment regimes.

Oral 9.5

INSIGHTS INTO PORK SPOILAGE UNDER MODIFIED ATMOSPHERES: UNRAVELING AND MODELING THE RELATIONSHIP BETWEEN *BROCHOTHRIX THERMOSPHACTA* LEVELS AND VOLATILE METABOLITE PRODUCTION

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Food spoilage keeps causing much loss and waste, leading to huge economic and ecological costs. Understanding the bacterial spoilage would help to develop efficient storage strategies. Specific spoilage organisms (SSOs) in meat, such as *Brochothrix thermosphacta*, are able to produce volatile organic compounds (VOCs) and off-odors depending on the packaging atmospheres. However, the relationship between bacterial growth, metabolite accumulation and gaseous conditions still remains unclear. This study aimed to investigate the *B. thermosphacta* behavior under different atmospheres by establishing a VOC production model.

Solid pork simulation media were inoculated with *B. thermosphacta* (initial level: 4.0-4.5 log CFU/g), stored at 4 °C under air, high-CO_2 (60% O_2/40% CO_2), and low-O_2 (100% N_2) conditions, and characterized every 1–2 days by microbiological plating and selected-ion flow-tube mass spectrometry (SIFT-MS). VOCs showing an exponential increase during storage were selected for modeling. An exponential model was used to fit VOC concentrations (ppb_v) against the bacterial counts (log CFU/g) of each individual media sample, resulting in a production factor and the microbial count corresponding with the start of the VOC increase.

The *B. thermosphacta* numbers at the stationary phase were ca. 8.9 log CFU/g under air but were lower (ca. 8.0 log CFU/g) under high-CO_2 and low-O_2 atmospheres. The model used could successfully predict the production of specific VOCs. For instance, acetoin was largely generated under O_2-rich atmospheres (> 400 ppb_v under air, and > 200 ppb_v under 60% O_2/40% CO_2), and its production factor was two times higher than that under 100% N_2. Acetoin started to increase exponentially when the *B. thermosphacta* counts reached around 106 CFU/g.

Determining the metabolism type of SSOs under different atmospheres aids in optimizing packaging technologies and enhancing quality control to reduce food wastage. The developed model could be used to predict the early production of VOCs or other metabolites in the different scenarios associated with food aroma/flavor development.

Flash communications

DECISION SUPPORT TOOL FOR THE SAFETY OF PLANT-BASED MEAT ANALOGUES: A NEW GROWTH AND GROWTH BOUNDARY MODEL FOR *LISTERIA MONOCYTOGENES* WITH NOVEL MODULES FOR ORGANIC ACIDS AND STRAIN VARIABILITY

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The development of ready-to-eat plant-based meat analogues poses new food safety challenges for food business operators (FBO). Particularly, *Listeria monocytogenes* is a pathogen of prime concern in this category.

The aim of this study was (i) to assess the growth of *L. monocytogenes* in plant-based meat analogues and (ii) to develop a growth and growth boundary model for *L. monocytogenes*. For this, a novel model matrix was developed to simulate various plant-based meat analogues. Different *L. monocytogenes* strains were independently inoculated in the model plant-based matrices with aw 0.94-0.99, pH 5.3-6.6, 0-2.5 mM of undissociated lactic and acetic acid at 5-15 °C. Growth observations were compared with predictions made by the *L. monocytogenes* model for chilled meat products included in the Food Spoilage and Safety Predictor (FSSP). The FSSP model, that is based on the gamma

approach, was reproduced with R software, and extended to incorporate the effect of undissociated acetic acid and to address the effect of strain variability. Strain variability was quantified by characterizing cardinal growth values of 22 *L. monocytogenes* strains (food isolates and reference strains) for each parameter studied, i.e., aw, pH, organic acids (OA) and temperature.

Results generated in the plant-based matrices so far, showed that the growth of *L. monocytogenes* was systematically underestimated in the predictions made by the FSSP model. Bias factors of 1.6 and 2.6 were obtained for products formulated without and with organic acids, respectively. The characterization of minimum inhibitory concentration (MIC) values of OA unraveled that for all *L. monocytogenes* strains, lactic acid MIC values (4.3-6.1 mM) were systematically higher than the value included in the FSSP model (3.79 mM). In case of acetic acid, a large strain-variability in MIC values (5-12 mM) was found, highlighting the need to include in strain variability in the model.

This study provides an extended growth and growth boundary model for *L. monocytogenes* accounting for strain variability and product formulation that can help FBO to overcome the challenges in the development and innovation of new plant-based meat analogues.

ENHANCED FOOD PRESERVATION FOR A SUSTAINABLE FUTURE THROUGH A NOVEL GAS-WASHING BOTTLE INCUBATION SYSTEM: MODELING *LISTERIA MONOCYTOGENES* GROWTH UNDER CONTROLLED ENVIRONMENTAL CONDITIONS

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Microbial hazards play a crucial role in the shift to advanced sustainable packaging, particularly addressing safety concerns linked to *Listeria monocytogenes*. Given its psychrotrophic nature and potential to cause foodborne infections, with the highest mortality rate in the EU, assessing the safety and shelf life of refrigerated foods is imperative. This involves predicting the survival and growth of this pathogen under various modified atmosphere packaging (MAP) conditions, which requires data collection under well-controlled conditions. However, dynamic gas exchanges may occur during storage. Thus, this work aims to analyze and model *L. monocytogenes* growth in a liquid medium under well-controlled gas concentrations, pH, and temperatures, employing an innovative gas-washing bottle incubation system (GBIS) to keep the desired atmosphere constant.

L. monocytogenes was inoculated (3.0 log CFU/mL) into neutral (7.8) and acidified (6.2) brain heart infusion (BHI) and investigated at different temperatures (4 °C and 7 °C) for 13 days under different modified atmospheres (CO_2%/O_2%: 0/0, 20/0, 40/0, 60/0, 60/2, 60/20, 0/20). Three BHI broth-filled bottles, interconnected in series, were subjected to daily gas flushing to maintain a stable atmosphere. pH measurements were conducted every four days, while sampling was done before each flushing to obtain growth curves. Primary growth models (Logistic, Gompertz, and Baranyi) were applied to fit these curves.

GBIS proved successful in investigating the effect of well-controlled atmospheres as well as constant pH and temperature on *L. monocytogenes* growth. The growth rate decreased as CO_2 concentration increased, whereas it decreased with lower pH and temperature. The influence of O_2 level on growth rate was observed at 7 °C, particularly pronounced with lower pH in the absence of CO_2. All primary models indicated the slowest *L. monocytogenes* growth under CO_2-rich atmospheres, along with lower temperature and pH. GBIS emerges as a promising tool to advance the understanding of microbial growth under precisely controlled gas atmospheres, allowing the exploration of numerous variable combinations in one setup. Beyond facilitating quantitative data collection for developing predictive models, this approach holds the potential to design optimum MAP systems, thereby ensuring the food safety of products subjected to modified atmospheres within the context of sustainable preservation.

SAMPLING AS USEFUL AND RISK BASED STRATEGY TO AVOID LISTERIOSIS CASES FROM "RAKFISK", A TRADITIONAL READY-TO-EAT, HIGH RISK FISH PRODUCT WITH INCREASING POPULARITY

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The trade of the traditional fish product "rakfisk" has grownfrom home production to a trade with more than 50 producers with regional and national distribution in Norway. However, the product is a high-risk product for listeriosis. The objective of the present study was 1. To build a farm-to-fork risk model, assess the consequences of contamination in the differentsteps, and 2. To develop tools to reduce the risk. The studies included analyses of more than 700 kg of naturally contaminated Rakfisk.

Rakfisk is produced from freshwater trout and salt, w/wo a starter culture added. Closed boxes (5-30 kg each) are allowed to mature for several months, until a rich flavour and a softtexture is developed. Rakfisk is a ready-to-eat product.

It has been assumed that *Listeria monocytogenes* grow well in rakfisk during maturation, which means that the boxes would either be *Listeria*-free or contain high amounts of *Listeria*. Concentrations below 100 cfu/g have been seen in surveillance programs, but assumed to be due to cross contamination from contaminated to not contaminated boxes during repacking into small packages for sale in retail.

The results of the present study did not support these assumptions. On the contrary, the risk model and verification studies indicated that the growth of *Listeria* during maturation early in the production phase allow significant growth, while following maturation period and period after repacking showed only limited growth. Temperature control and/or short time from slaughter until start of the maturation period werefound essential for keeping the concentration of *L. monocytogenes* low.

Analyses of fish positions in the box after maturation revealed that the concentration of *L. monocytogenes* was very heterogenous inside the box, but sampling of brine from more places in the box was effective for detection of positive boxes. Sampling therefore appear as good and realistic strategy to avoid that contaminated Rakfisk are placed on the market. Combining brine samples from different places in the box into one sample was effective for correct detection of contaminated boxes.

The conclusion was that it is possible to produce safe Rakfisk and verify the safety by sampling.

EXPLORING THE ROLE OF PH ON THE MICROBIAL RESPONSES OF FOODBORNE PATHOGENS IN FOOD PRODUCTS STORED UNDER DIFFERENT TEMPERATURES

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Hurdle technology is a well-recognized approach that considers multiple factors, such as pH and temperature, to inhibit microbial growth and extend food shelf life. Understanding how these factors affect the responses of main foodborne pathogens in foods are crucial for designing effective measures to increase food safety.

This study was aimed to assess the influence of pH of food products and the storage temperature (°C) on the fate of

Listeria monocytogenes, *Salmonella* spp., *Bacillus* spp. and *Escherichia coli*. Growth, survival and reduction microbial data (log cfu/mL) in high acid (pH ≤ 4.6) and slightly acid and neutral (pH > 4.6) food products stored at different temperatures (< 45 °C) were systematically extracted from ComBase database (in total 121 primary studies). A series of microbial inactivation models were assessed based on their fitting capacity by performing (non)linear regression analysis. Hereafter, the time for 3-log reductions (L3R, min) of the studied microorganisms was estimated for all the applied models.

Multivariate correspondence analysis was performed to identify patterns and homogeneous groups within the dataset, considering the different food categories assessed. Regression analysis was also used to explain the relationship between the studied variables, i.e., L3R, pH and storage temperature during treatments. L3R times ranged between 24.74-778.39 and 61.41-644.17 min in products belonging to sauces and beverage categories (pH ≤ 4.6), respectively. In contrast, L3R times ranged between 17.77 and 386.60 min in milk products (pH > 4.6). Within the high-acid food category, regression analysis showed a statistically significant relationship between the storage temperature and the L3R times ($p \leq 0.05$), meanwhile this relationship was not significant in slightly acid and neutral products ($p > 0.05$). These results indicate that the low pH combined with temperature may lead to faster log reductions in high-acid foods. This study deepens into the significant contribution of hurdle technology to extending the shelf life and safety of foods by comprehensively understanding how pH and temperature influence the response of key foodborne pathogens. Further research is needed to gather more data for a more informative assessment of these interactions across different food categories.

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PREDICTING AND OPTIMISING THE DYNAMICS OF BACTERIA UNDER ANTIMICROBIALS IN THE FOOD INDUSTRY THROUGH A NEW BATTERY OF DYNAMIC MATHEMATICAL MODELS

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Predictive microbiology is a powerful tool for predicting bacterial responses to different factors like temperature or pH, which is still at an early stage when the stressor is an antimicrobial compound. Actually, the mechanisms of action of antimicrobials, and therefore the resulting bacterial dynamics, are particularly diverse in food applications including antibiotics in food production, disinfectants to avoid cross-contamination or food preservatives and sanitisers for extension of food safety and quality.

In this talk, we aim to discuss four relevant mechanisms when studying the antimicrobial effect for which we have been developing mathematical models. The ultimate goal is to find mathematical models with sufficient predictive capabilities for allowing optimisations: finding the antimicrobial concentrations or conditions for maximising food safety and quality.

The first selected mechanism consists of distinguishing between different types of antimicrobials. A very diverse spectrum of bacterial responses can be observed when the antimicrobial is bactericide (killing the population), bacteriostatic (inhibiting growth), or even both depending on the concentration. As an example, we present a model of *E. coli* and *B. cereus* dynamics under different concentrations sub-inhibitory concentrations of carvacrol.

The inoculum effect is another aspect, usually disregarded when modelling. Nevertheless, it is common to observe that the effect of the antimicrobial depends not only on the antimicrobial concentration but also on bacterial densities. We will present a mathematical model able to describe this effect for disinfection of *L. monocytogenes* with Benzalkonium.

Another relevant mechanism is the minimisation of any type of resistance/tolerance of the bacterial population

to the antimicrobial, which could lead to the selection and regrowth of more tolerant subpopulations in heteroresistance populations. In this context, we will show the modelling conditions needed to describe regrowth of bacteria under ciprofloxacin.

Last we would like to stress how relevant is to expand further these models to consider either the food matrix or the operational conditions. Here we will illustrate how predictive microbiology models of antimicrobial effects are critical if combined with models describing for example the diffusion of the antimicrobial in active packaging or the influence of fluid dynamics on produce washing.

SALT REDUCTION AND TEMPERATURE ABUSE IMPLICATIONS ON *LISTERIA MONOCYTOGENES* BEHAVIOUR IN *CHOURIÇO*

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Reducing sodium in cured meats, like *chourição,* is essential to address health concerns related to excessive salt intake. However, reformulation presents challenges, as salt reduction may facilitate the growth of undesirable contaminants.

In this study, the main objective was to evaluate the impact of a new formulation of reduced salt *chourição* on the survival of *Listeria monocytogenes.*

For that, slices of traditional and 25% (w/w) salt-reduced *chourição* were inoculated (n = 3) with a cocktail of seven *L. monocytogenes* strains (~3 log CFU/g) and stored for 60 days at 4 and 10 °C (temperature abuse). *Listeria monocytogenes* counts were determined, and the Baranyi and Roberts model was used to describe pathogen growth.

In traditional *chourição*, the pathogen maximum growth rate (μ_{max}; log CFU/g day-1) at 10 °C (0.21 ± 0.03) was 1.5 times higher than μ_{max} at 4 °C (0.14 ± 0.04). In salt-reduced *chourição*, μ_{max} at 10 °C (0.40 ± 0.24) was approximately 3 times μ_{max} at 4 °C (0.13 ± 0.02), highlighting a greater impact of temperature on microbial behaviour when salt is reduced. At 4 °C, pathogen μ_{max} in traditional (0.14 ± 0.04) and in salt-reduced *chourição* (0.13 ± 0.02) were similar. However, at 10 °C, μ_{max} was higher in salt-reduced *chourição* (0.40 ± 0.24), compared to traditional (0.21 ± 0.03), probably due to the combined effect of higher temperature and lower salt content. In salt-reduced *chourição*, storage at 4 and 10 °C led to shorten lag phases (18 and 12 days) in comparison to that of traditional *chourição* (23 days at 4 °C).

Product reformulation requires careful consideration so that food safety is not compromised. Various factors, including temperature, are important to hinder pathogen proliferation and guarantee the microbiological safety of foods when reducing the preservative effect of one hurdle (in this case, salt content), as illustrated by this study.

TECHNOLOGIES FOR FOOD PRESERVATION AND SUSTAINABILITY

Keynote 2.1

ADDRESSING FOOD SAFETY AND AGRI-FOOD CHAIN SUSTAINABILITY CHALLENGES THROUGH FOOD MICROBIOLOGY RESEARCH

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Food microbiology has an evident role to play in food safety. However, its relationship with the sustainability of the agri-food chain is no less significant and has become the subject of considerable interest. In this sense, the discipline of food microbiology offers considerable potential for improving food safety and the sustainability of the agri-food chain through the study of pathogenic, spoilage and beneficial microorganisms. Over the past 10 years, my research group has developed a series of projects in which microorganisms are either the problem to be addressed or a tool employed to guarantee food safety and concurrently improve the sustainability of the agri-food chain. The line of research we have been working on the longest is the evaluation of the antimicrobial efficacy of non-thermal methods aimed at improving food preservation. In this regard, we have assessed mechanisms of microbial inactivation and resistance related to thermal treatments, high hydrostatic pressure, pulsed electric fields, ultrasound, plasma-activated water, and essential oils (current project: ARMADA-PID2021-123404NB-I00). The contribution of this project to sustainability stems not only from the optimization of new technologies but also from the synergism that emerges when such technologies are applied in combination with natural antimicrobials such as essential oils or bacteriocins, which help optimize their antimicrobial efficacy. Food safety is thus improved as well as sustainability while reducing the intensity of treatments, including equipment usage time and energy costs.

Microorganisms can also be a useful tool in detecting other hazards, such as the presence of antibiotic residues in food. Our group, in collaboration with Zeulab S.L., has developed various biological screening methods within the framework of the European project INTERREG POCTEFA-TESTACOS for detecting antibiotic residues in food and/or in live animal blood. These methods are based on the growth inhibition of *Geobacillus stearothermophilus* in the presence of antibiotics above the maximum levels of antibiotic residues allowed in food. This project's contribution to improving the sustainability of the agri-food chain is based, among others, on reducing antibiotic use, preventing antibiotic resistance, and avoiding the slaughter of non-compliant animals, thereby reducing the harmful environmental impact of breeding livestock that ends up as waste.

Spoilage microorganisms can also cause food safety problems, as can microorganisms that produce biogenic amines, including histamine. In the context of the recently launched European project INTERREG POCTEFA-TESTACOS+, we are determining the presence of histamine in cured cheeses while identifying the responsible microorganisms. As a final step, in collaboration with OX-CTA S.L., we are proposing novel, more sustainable cleaning and disinfection products that not only limit the presence of those microorganisms (thus limiting histamine accumulation in cheeses) but also generate zero waste.

Finally, spoilage microorganisms also hamper the sustainability of the agri-food chain by reducing the shelf life of foods, thus increasing food waste and reducing food availability. Our most recent project (SERENADE-HORIZON EUROPE G.A. 101072846), in collaboration with BSH Electrodomesticos España S.A., among others, aims to develop smart containers using eco-friendly materials and sensors capable of predicting the shelf life of ready-to-eat meals stored under refrigeration in supermarkets and household appliances. Our group is currently studying the relationship between microbial growth and changes occurring in the volatilome within the headspace of airtight bowls containing ready-to-eat food, with the aim of identifying the most representative volatiles generated during food spoilage. They can be detected by sensors designed to

predict the shelf-life of meals, thereby avoiding unnecessary food waste.
All these actions not only give rise to innovative solutions that help improve food safety but also directly contribute to increasing the overall sustainability of the agri-food chain.

Keynote 7.1

HIGH PRESSURE-BASED TECHNOLOGIES FOR ENHANCED FOOD PRESERVATION AND SUSTAINABILITY

Carol Tonello-Samson
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High Pressure Processing (HPP) is an innovative technology in the food industry that uses high pressure, typically up to 600 MPa, to preserve food products. This non-thermal technology does not involve heat. Consequently, one of the significant advantages of HPP is its minimal impact on the food's sensory and nutritional attributes. This makes HPP particularly suitable for preserving a wide variety of food products, including fruit and vegetable-based products, meats, seafood, and dairy products.
In addition to increasing the shelf-life of food, HPP is effective in controlling a broad range of pathogens, including bacteria and viruses, thereby ensuring food safety. However, HPP cannot inactivate spores, so products processed with this technology need to be stored under refrigerated conditions. Recent developments in the industry have introduced the combination of pressure and heat in a process called High Pressure Thermal Processing (HPTP). This process can achieve product sterility while exposing products to a lower overall thermal load than conventional heat sterilization.
HPP is not only beneficial for food safety and quality but also contributes positively to environmental sustainability. Compared to traditional thermal processes, HPP can lead to substantial energy savings as it requires less energy per kg of food, while HPTP requires less heat input. Moreover, by extending the shelf life of processed products, HPP significantly reduces food waste. This extended shelf life also means less frequent transportation, leading to a reduction in carbon emissions. However, it should be noted that HPP requires food to be packaged in flexible and waterproof materials, commonly plastic, which can be detrimental to the environment. Developments in plastic packaging materials, such as recycled materials and bioplastics, and advancements in HPP machinery, such as the ability to process beverages in bulk, are addressing this issue.
In conclusion, HPP and HPTP ensure food safety and preservation while also promoting a more sustainable food industry. Their benefits extend beyond the consumer to the environment, making them outstanding solutions in the food processing industry.

Keynote 7.2

USE OF ESSENTIAL OILS IN NANOEMULSION TO INACTIVATE MICROORGANISMS AND IMPACT OF QUANTIFICATION OF MICROBIAL ACCLIMATION TO STRESS ON THE APPARENT RESISTANCE TO STRESS FACTORS

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Foodborne outbreaks affect fresh and mildly processed fruits and vegetables that are a fundamental part of a balanced diet. Processing of many fruits and vegetables usually includes a washing stage, intended to to reduce notably the microbial load and eliminate dirtiness present on their surface. Due to its low cost and usability, chlorine is extensively used in the food industry as a disinfectant.
Despite this, and because of its health adverse effects (potential carcinogenesis and recent restrictions in the legislation), there is a present and on-going challenge in finding suitable alternatives that can control foodborne pathogens. Even though the antimicrobial effects of essential oils are known, their application for the fresh vegetable industry remains challenging due mainly to their hydrophobic nature and high volatility.
To overcome this problem, nanoemulsions present an alternative that can increase their miscibility, effectiveness

and stability. Essential oil nanoemulsion were prepared and their application as washing solutions was tested. Cherry tomatoes were artificially contaminated with pathogenic/spoilage microorganisms followed by dipping in different washing solutions such as water, chlorinated water and nanoemulsions at different concentrations and for different contact times. This treatment proved to be very efficient against all microorganisms tested, reaching up to 2 log cycles inactivation.

However, the use of different inactivation strategies such as essential oils or mild heating, either separately or combined, can induce adaptation/acclimation strategies in pathogenic bacteria. This adaptation involves bacterial cells activating a range of stress response mechanisms that enhance their chances of surviving. The so-called "stress acclimation mechanism" considers that bacterial cells adapt dynamically to the initial (sub-lethal) stages of the treatment, activating a stress response that increases their survival rate. The use of mutants can help to elucidate the mechanisms behind this effect.

A model has been proposed by our group to quantify the impact of this acclimation phenomenon in bacteria. It can be relevant for microbial inactivation during processing of certain products, where a lower reduction may be obtained due to the dynamic increase in resistance. The impact of factors such as nanoemulsions on the biological food safety are analyzed.

Oral 2.1

BIOPROTECTIVE EFFECT OF LACTIC ACID BACTERIA ON COLD-SMOKED RAINBOW TROUT AGAINST *LISTERIA MONOCYTOGENES* BIOFILM CELLS AT DIFFERENT REFRIGERATION TEMPERATURES

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Listeria monocytogenes is a recognized foodborne pathogen responsible for illnesses resulting from consuming contaminated ready-to-eat (RTE) products such as fishery products, posing significant challenges to society, economies, and public health. Reported cases in Europe in 2022 have increased 15.9% from previous year. It is an ubiquitous and persistent microorganism given its ability to form biofilms in different contact surfaces in food industries.

In this study, transfer and growth potential of *L. monocytogenes* biofilm cells were assessed in cold-smoked rainbow trout together with the inhibitory potential of different lactic-acid bacteria (LAB) strains against *L. monocytogenes* growth. Portions of 10 g of cold-smoked rainbow trout were inoculated by spraying with two different LAB cocktails: *i) Lb. lactis* and *Ln. carnosum* and *ii) Lb. plantarum* and *Lb. pentosus*. *L. monocytogenes* biofilms of three strains (LMG23773; 12MOB102LM; 12MOB107LM) were grown during 120 h on stainless steel (SS) coupons (25 cm2) in modified TSB medium and subsequently transferred to the fish portions.

Samples were vacuum packaged and stored during 21 days at different refrigeration temperatures (i.e. isothermal profile at 5 °C and dynamic conditions from 4 °C to 20 °C). Results showed a high transfer rate of *L. monocytogenes* biofilm cells, reaching more than 84% of cells transferred from SS to the food, and their growth potential was from 4 log CFU/g to nearly 7 log CFU/g. The inhibitory effect of the LAB cocktail of *Lb. lactis* and *Ln. carnosum* was observed at both storage conditions since the *L. monocytogenes* concentration remained at 4 log CFU/g during the study period. On the contrary, the inhibitory capacity of *Lb. plantarum* and *Lb. pentosus* against *L. monocytogenes* was only observed at 5 °C storage, reducing the initial concentration by 1.5 log CFU/g; however, at dynamic storage conditions, no significant differences were observed against the control samples (without LAB inoculation).

The utilization of biopreservation strategies could prove to be an effective solution to mitigate the risks associated with *L. monocytogenes* contamination in RTE fishery products.

Oral 2.2

DECIPHERING THE BACTERIAL INTERACTION NETWORK WITHIN SEAFOOD MICROBIOME TO DEVELOP A SUSTAINABLE BIOPRESERVATION STRATEGY

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Seafood products and their storage conditions are suitable for the development of microorganisms originating from fish and seawater or resulting from contamination during the food chain processing. They are thus highly susceptible to spoilage due to biochemical and microbial degradations and are considered as potential vectors of human pathogens (e.g *Listeria monocytogenes*). Biopreservation is a microbiome engineering strategy based on bacterial competition. It consists in fighting against undesirable bacteria by inoculating protective microorganisms or their metabolites exhibiting antimicrobial activities in food. Despite the undeniable inhibition effect of selected lactic acid bacteria (e.g *Carnobacterium* spp.) against *Listeria monocytogenes*, the associated sensory impact of the biopreservative strain addition and its effect on spoilers varies according to the composition of the product microbiota. This microbiota is closely linked to the processing and the storage conditions.
To understand the effect of the addition of protective culture on all bacterial species of microbiome, omics strategies were combined to establish the bacterial interaction network and assess the competitions related to the production of antimicrobial compounds. One hundred strains representative of salmon microbiome diversity were selected from the laboratory collection. Miniaturized cross inhibition between all strains, representing a total of 10 000 assays, were performed. Inhibition profiles of strains were then related to the presence of antimicrobial biosynthetic clusters, thanks to sequencing, assembly and annotation of all genomes. The results provided an exhaustive description of the bacterial interaction network and antimicrobial diversity. In fact, between 0 and 23 antimicrobial biosynthetic clusters could be identified per genome. Half of them encoded peptide molecules like unmodified bacteriocins, ribosomally synthesized and post-translationally modified peptides (RiPPs) and non ribosomal peptides (NRPs).
Then, culturomic and transcriptomic approaches were combined to determine the abiotic and biotic factors that impact the synthesis of active peptide during salmon storage. Three bioprotective *Carnobacterium* strains harbouring different bacteriocin equipments were firstly investigated.

Oral 2.3

BIOLOGICAL GROWTH CONTROL OF LISTERIA MONOCYTOGENES IN SALMON APPLYING PROTECTIVE CULTURES OF LACTIC ACID BACTERIA

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Listeria monocytogenes is a serious food safety challenge for the salmon industry due to its ubiquitous nature, ability to grow at low temperatures, persist in processing facilities and, thereby, its ability to contaminate ready-to-eat-(RTE) salmon products.
Using protective lactic acid bacteria (LAB), biopreservation can be a powerful tool to prevent the growth of *L. monocytogenes*. However, there is a knowledge gap related to the intra-species tolerance towards biopreservation. *L. monocytogenes* comprises four separate deep-branching lineages, subdivided by multilocus sequence typing (MLST) into sequence types (STs). Some STs are more prevalent in seafood production environments, and there is also variation in their resistance towards acid and other environmental stress factors.
Thus, the present study aimed to investigate the intraspecies variation of *L.monocytogenes* tolerance towards biopreservative LAB strains. A total of approximately 50 *L. monocytogenes* strains of various STs (1, 3, 6, 8, 14, 37,121, 398) originally isolated from salmon production facilities and salmon products will be evaluated for their ability to grow in the presence of selected LAB strains in a model system at 15 °C.
So far, 29 *L. monocytogenes* strains have been evaluated *in vivo* in the presence of selected LAB strains belonging to genus *Carnobacterium*. All *L. monocytogenes* strains

were entirely inhibited by *C. divergens* 468, while C. *maltaromaticum* 55 showed high or complete inhibition (> 6 log reduction) of 14% of the *Listeria* strains, medium inhibition (3-6 log reduction) for 79%, and low inhibition (<3 log reduction) for 7%.

Furthermore, a selection of LAB and *L. monocytogenes* strains will be tested *in situ* to validate the bioprotective properties of LAB towards the most resistant *L. monocytogenes* strains. Inoculated *C. divergens* 468 (log 7 CFU/g) effectively inhibited the growth of the selected *L. monocytogenes* strain (initial concentration log 4 CFU/g) in vacuum-packed fresh salmon at 4 °C stored for 16 days.

Our study contributes to unravelling the potential of applying LAB biopreservation as a hurdle against *L. monocytogenes* in raw RTE-salmon products.

Oral 7.1

EFFECT OF HIGH HYDROSTATIC PRESSURE (HHP) COMBINED WITH BLUEBERRY EXTRACT OR NISIN ON FOOD SAFETY AND SHELF LIFE OF BEEF AND PLANT-BASED PATTIES

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The interest in plant-based meat alternatives has become increasingly prominent in recent years while HHP stands out as a promising technology to ensure the safety and extend the shelf life of food products. However, their physicochemical properties, such as high pH, pose challenges that require intense processing conditions to address food safety hazards. In search for a more optimized process, the combination of HHP with clean label additives is proposed.

The primary objective is to investigate the potential synergism between HHP with nisin or blueberry extract to enhance food safety by the control of *Listeria monocytogenes* and to extend the shelf life of both, beef and plant-based burgers.

Plant-based and beef burgers containing no additives, nisin (500 IU/g) or blueberry extract (4%) were either inoculated with a 5-strain cocktail of *L. monocytogenes* (7 log CFU/g) or remained uninoculated for shelf-life assessment. Patties were high-pressure processed at 600 MPa for 3min and stored under moderate temperature abuse (7 °C) for 100 days. Key parameters, including *L. monocytogenes* counts in inoculated samples, aerobic plate counts (APC), lactic acid bacteria and total anaerobic counts were monitored throughout the storage period. The experiment was performed in two independent repetitions.

Following HHP, *L. monocytogenes* remained below the detection limit (1 log CFU/g) in beef burgers containing nisin and blueberry extract until day 100 of storage, whereas growth was observed after 32 days in control samples without additives. More than a 5-log reduction of *L. monocytogenes* was achieved immediately after the HHP in plant-based patties (with or without additives). The pathogen remained below 3 log CFU/g until the last day in patties containing blueberry extract while growth was observed at the day 50 in control burgers. Concerning the shelf life, total mesophilic bacteria growth was noticed at the day 29 in beef samples without additives or treated with nisin. In contrast, APC remained below 3 log units until the end of the study in the plant-based patties treated with additives.

This research contributes valuable insights into the potential applications of combining HHP with natural antimicrobials and highlights the difference between animal and plant matrices for risk assessment purposes.

Oral 7.2

HEAT RESISTANCE CHARACTERIZATION OF ANTIMICROBIAL ACTIVITY OF CELL-FREE SUPERNATANTS OBTAINED FROM POTENTIAL PROTECTIVE CULTURES

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The use of protective cultures (PC) as an alternative to traditional preservatives for ensuring food safety and increasing shelf life is being advocated as an increasingly demanded option among industries embracing the 'clean label' trend. The use of PC in fermented foods is widespread.

Nevertheless, there are certain drawbacks associated with their use. For instance, due to microbial metabolism, foods may undergo organoleptic changes; furthermore, its addition to products intended for heat treatment is limited due to the sensitivity of cells to heat. One potential solution to these limitations is the utilization of cell-free supernatants (CFS) from Qualified Presumption of Safety (QPS) microorganism cultures as biopreservatives.

The current study is focused on exploring the ability of CFS to maintain their antimicrobial activity when applied to foods subjected to heat treatments.

For that purpose, the anti-*Listeria monocytogenes* activity of CFS from three strains of *Bacillus subtilis*, *Lactiplantibacillus plantarum*, and *Pediococcus acidilactici*, and the anti-*Lentilactobacillus parabuchneri* activity of CFS of one strain of *Levilactobacillus brevis*, were determined after pasteurization and sterilization treatments.

None of the applied pasteurization treatments (70 and 90 °C, up to 2 h) resulted in a significant loss of antimicrobial activity ($p > 0.05$). Therefore, if incorporated into a food subjected to pasteurization, it would be expected the CFS to maintain their antimicrobial efficacy. Regarding the sterilization treatments, the CFS from *L. brevis* exhibited the highest resistance, retaining its complete antimicrobial activity even after 2 h at 121 °C. In contrast, the *B. subtilis* CFS proved to be the most sensitive, with a significant reduction ($p < 0.05$) in its antimicrobial activity after 1 h, at sterilization temperatures, 110 and 121 °C. Nevertheless, when conventional treatments like botulinum cooking (121°C/3min) or milk sterilization (110°C/ 20 min) were applied, the estimated losses of efficacy would just range between 3-15% and 10-20%, respectively, as a function of the CFS used.

Thus, the CFS of these strains would be also suitable for their use in products subjected to sterilization treatments, ensuring the retention of an adequate level of antimicrobial activity.

Oral 7.3

OPTIMIZATION OF THE OHMIC-HEATING PROCESS TO PREPARE *BIFIDOBACTERIUM ANIMALIS* PARAPROBIOTIC BASED ON THE SPECTROPHOTOMETRY AND FLOW CYTOMETRY ANALYSES

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Objective

Paraprobiotics are known as an inactivated form of probiotics, which have been reported to have several health-promoting benefits (HPBs). The aim of this study was obtaining paraprobiotic from *Bifidobacterium animalis* Bb-12 (BB) with the highest HPB through the ohmic heating (OH) process.

Methods

According to the conditions determined by the statistical method of Response surface methodology (RSM), 27 bacterial suspensions of BB were treated through OH at different factors, including electric field, OH temperature, cell concentration, and OH time. The optimization of OH process was done based on some properties of obtained BB paraprobiotics (BBP) including cell surface hydrophobicity (CSH), cell auto- and co-aggregation (CAA and CCA) by spectrophotometer, and membrane integrity (MI) by flow cytometry. The analyses of metabolite compounds of BB and BBP was done by using GC-MS and HPLC. In addition, the SEM analysis was applied to visualize the cell damages of BB induced by the OH process.

Results

The linear model was more accurate for CCA, and the quadratic model for other responses. The electric field had the most effect on the MI of BBP; however, for other responses this was for OH temperature. The surface characteristics

(CSH, CAA, and CCA) of BBP obtained by OH were more than BB (control). The surface characteristics were increased as OH electric field and OH temperature increased. This could be attributed to thermal and electroporation effects of OH, which are considered as the main OH mechanisms and change the cell permeability by destruction of membrane structure and making pore in its structure. The changes in the membrane structure were confirmed by SEM analysis. In the both samples, lactic and acetic acids were detected as the most amount of characterized metabolites. Most of the chemical compounds in BB and BBP were close to each other; however, BBP was including higher amounts of some compounds, such as uridine 5'-diphospho-N-acetylglucosamine.

Conclusions

The OH treatment including electric field of 8 V/cm, OH temperature of 88 °C, cell concentration of 8 log CFU/ml, and OH time of 3 min was considered as the optimum OH condition to prepare BBP.

Oral 7.4

IMPACT OF SUPERCHILLING TECHNIQUE ON THE MICROBIOLOGICAL QUALITY OF FRESH SALMON

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Superchilling is an innovative method for food preservation at temperature between refrigeration and freezing. It addresses the challenges of maintaining food safety while improving process sustainability. Refrigeration increases the shelf life by slowing down or even stopping the growth of spoilage microorganisms. However, it can allow the growth of psychrotrophic bacteria such as the foodborne pathogen *Listeria monocytogenes*. Freezing stops bacterial growth, but it affects the food organoleptic qualities (texture, water loss, colour). Moreover, long-term storage at negative temperature requires high energy inputs. The aim of superchilling is to combine the advantages of the two techniques: a longer shelf life compared to refrigerated products, a better preservation of organoleptic qualities and a lower energy consumption compared to freezing.

Superchilling is a two-step technique: an initial pre-freezing phase, and a storage phase at 1 to 2 °C below the initial freezing temperature. The objective of this study is to understand the impact of different conditions of superchilling on the growth of salmon microbiota compared to refrigeration. Salmon samples were chilled at various temperatures: (i) 2°C, the standard refrigeration temperature used in fish preservation, (ii) -0.8°C, the salmon initial freezing temperature; (iii) –1.8 °C and –2.8 °C, two superchilling temperatures to respectively obtain 30% and 40% ice content. Only the samples stored at the two latter temperatures were subjected to initial pre-frozen step at –23 °C during 13 min. The growth of natural salmon microbiota was followed by plating during 13 days. In parallel, genomic DNA extractions were performed over time to evaluate the evolution of microbiota diversity by 16S rRNA metabarcoding.

The bacterial population of salmon samples increased over time when stored at 2 °C and -0.8 °C. Bacterial diversity decreased over time, probably due to the specific growth ability of psychotropic bacteria. Growth under superchilling conditions at –1.8 °C and –2.8 °C was significantly reduced. This work provides promising results to understand the impact of superchilling on the evolution of salmon microbiota and thus to optimise the management of food quality and safety.

Oral 7.5

QUALIFICATION OF *PEDIOCOCCUS ACIDILACTICI* AS A SURROGATE FOR *SALMONELLA* IN POWDERED INFANT FORMULA DRYED BY THE NEW PULSE SPRAY DRYING TECHNOLOGY

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Pulse spray drying (PSD) is a new technology based on pulsating gas (air) flow providing higher energy efficiency by drying at higher temperatures for shorter times.
This study aimed *(i)* to qualify *Pediococcus acidilactici* as a surrogate for *Salmonella* considering the influence of temperature and water activity (a_w) of powdered infant formula (PIF) and *(ii)* to evaluate the survival of *P. acidilactici* during the PSD of PIF.
PIF with adjusted a_w (0.45, 0.75 and 0.98) was individually inoculated with *S. enterica* serovar Senftenberg (ATCC 43845) and *P. acidilactici* (ATCC 8042) at *ca.* 7 log CFU/g and heated at different target temperatures (60-90 °C) up to 30 min. Log-linear Bigelow-type model was used to estimate the inactivation kinetic parameters (D and z). For pilot plant experiment, reconstituted PIF (40% total solids) was inoculated with *P. acidilactici* at 6 log CFU/g and dried by PSD at three outlet temperatures (80, 90 and 100 °C). Primary (main chamber) and secondary (cyclone) powders were collected at 30 and 45 min of the drying process.
Thermal resistance of both microorganisms followed a log linear Bigelow type model showing a significant impact of the matrix a_w. The decimal reduction time (D) of *P. acidilactici* was longer than that of *S.* Senftenberg, e.g. about 1 to 0.5 units for a_w 0.75 and 0.45, respectively at the reference temperature (70°C). Thus, for low moisture matrix the surrogate was more resistant than the pathogen. For rehydrated PIF (a_w 0.98), although D values at ≤ 65 °C were higher for *Salmonella* than for *P. acidilatici*, the *z* value for the surrogate doubled that of the pathogen indicating its higher resistance to the temperature increase under high moisture conditions.
Regarding PSD, temperature had a significant impact on microbial inactivation. While for treatments at 90 and 100 °C about 3 log reduction was achieved on average, less than 2 log reduction was observed when drying at 80 °C. Small or no relevant effect of the sampling time and type of powder were observed.
This study qualified *P. acidilactici* as potential non-pathogenic surrogate to assess the impact of spry drying technology on infant formula at pilot plant and industrial scale.

Flash communications

INVESTIGATING POSSIBLE NEW PRODUCTS FROM KIWI WASTE: HOW TO CLOSE THE LOOP

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In the food chain, various wastes are generated for different reasons, such as discarded products in the field, residues from processing, or products that are part of a surplus production and remain unsold. All these products represent what is called food loss and food waste. To achieve the goals set by the European Green Deal and move towards a circular economy, these wastes can be valorized into value-added compounds.
In this study, we considered the kiwifruit supply chain, in particular, kiwifruit discarded in the field for size and those not sold because represent a surplus. The valorization technique was fermentation, as it is known to lead to the formation of different compounds, including flavorings and bioactive compounds. To optimize the valorization process, a multidisciplinary approach was chosen to reach multiple fields of application.
An experimental design (DoE) was set up to identify the starter able to grow in the kiwi and to optimize the parameters of the fermentation process. Once the fermented biomass was obtained, it was analyzed for different purposes. Firstly, the volatile component was analyzed to identify the variation of aromatic compounds that could be further recovered by distillation and used in food/cosmetic industry. The second part of the project focused on the application of fermented biomass as biofertilizers used in different tomato crop cultivation systems and the analysis of the plant's vegetative

parameters in response to the treatment as well as the soil microbial community to identify possible changes.
From the results obtained with DoE, it was possible to identify the optimal conditions of the process and the analysis of factors influencing different responses for flavor production. The application of fermented biomass as a biofertilizer showed differences depending on the application used (in field or soilless) leading to differences in plant development. The soil microbial community analysis also shows differences depending on the treatment used.
This approach made it possible to evaluate several ways for valorizing residual kiwi biomass and the results suggest that fermentation leads to different final products with a high added value, gaining the opportunity to occupy multiple market sectors.

ASSESSMENT OF *CAMPYLOBACTER* REDUCING EFFECT OF SPRAYING SLURRY ICE ON POULTRY SAMPLES

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The *Campylobacter* level on neck skins of broiler carcasses in the EU must comply with a process hygiene criterion of a maximum of 1000 CFU/g in 10 of 50 carcasses from 2025 (currently 15 of 50), which challenges the poultry industry significantly. This study aimed to assess the *Campylobacter*-reducing effect and the impact on the shelf life of chicken carcasses by spraying slurry ice onto carcasses with an IceGun® device, which can be installed on the existing cooling chain in poultry slaughterhouses.
The IceGun® was installed in a Danish slaughterhouse specialising in free-range and organic broiler flocks. From August to September 2023, 45 carcasses were randomly selected from a single flock on four different days. Twenty carcasses were air-chilled conventionally; twenty-five were air-chilled and sprayed with slurry ice (IceGun®) on the breast, leg, and inner cavity at different time points. After 160 minutes of chilling, neck and leg skin samples were collected to enumerate *Campylobacter* by culture. The carcasses were packed in a modified atmosphere (MAP) and refrigerated for up to 2 weeks for testing for bacterial load assessment or sensory quality evaluation. Three isolates per sample were preserved by freezing for subsequent whole-genome sequencing analysis.
There was a significant reduction in *Campylobacter* levels on treated neck skins compared to untreated neck skins (0.24 log10 CFU/g), while no difference was seen on leg skins. No significant difference in *Campylobacter* counts was seen between treated and untreated MAP-packed carcasses. During the 2-week storage period, there was an increase in the total viable counts and *Pseudomonas*. The treated carcasses had higher pH (0.2) and salt content (0.05%) throughout the 2-week storage period. No noticeable difference in sensory parameters was seen between treated and untreated carcasses. The genetic strain variations will be further explored through whole-genome sequencing (WGS).
IceGun can significantly reduce the *Campylobacter* on broiler carcasses measured on the neck skins without any noticeable effect on the shelf-life. However, the reduction is slight, and combinations with other decontamination strategies are required to control *Campylobacter* in the broiler chain.

NON-*SACCHAROMYCES* KILLER YEASTS FOR THE SUSTAINABLE BIO-MANAGAMENT OF POSTHARVEST FUNGAL DISEASES IN FRUITS

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Postharvest fungal infections pose substantial issues in agricultural crop management, resulting in economic losses and food waste. Synthetic fungicides can treat many postharvest illnesses, however chemical residues and the development of resistant fungi, which are also hazardous to the environment and human health. Reducing pesticide use was one of the United Nations' Sustainable Development Goals since pesticide contamination is considered ecological catastrophe. As the demand for sustainable agriculture grows, other options like biological control are gaining attention. Based on both in vitro and in vivo testing, Non-*Saccharomyces* killer yeasts, such as *Metschnikowia* spp., *Hanseniaspora* spp., *Rhodotorula* spp., *Meyerozyma* spp.,

and *Pichia* spp. have so far been shown to be effective biocontrol agents against postharvest fungal disases in fruits. Research on non-*Saccharomyces* killer yeasts has shown that they are effective against a wide range of postharvest diseases, mostly caused by *Botrytis cinerea*, *Penicillium* spp., Aspergillus spp., *Fusarium* spp., and *Alternaria* spp. The antifungal mechanism of action of killer yeasts can be determined by the lytic enzyme activity, inhibition of spore germination, biofilm activity, iron depletion, diffusible metabolites, wound-site colonisation, mycocin, and volatile organic compounds (VOCs). production.

Furthermore, non-Saccharomyces killer yeasts can inhibit mycotoxin-producing fungi in fruits and provide bio-degradation and adsorption mechanisms. Likewise, their compatibility with other postharvest treatments, including as cold storage and modified environment packing, can increase their potential for integration into existing disease management strategies. However, strain selection, formulation development, application methods, and regulatory approval are all challenges that must be overcome. The application of these non-*Saccharomyces* killer yeasts an offer sustainable, green and eco-friendly options for the management of postharvest fungal diseases in fruits and many crops. The key issue is the translation of laboratory tests to the field and their adaptation to large-scale commercial applications.

LACTOFERMENTED FRUIT PURÉE AS A NEW PRESERVATIVE SOLUTION FOR FOOD AND PET FOOD APPLICATIONS

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Leveraging on natural raw materials to provide innovative ingredients for food and beverages, pet food and aquafeed is key to fulfilling the increasing needs of the Food industry, while ensuring food quality. This research project aims to develop fruit or vegetable-based antimicrobial fermentates, active against a wide spectrum of microbial targets related to food spoilage and foodborne illnesses, using lactic acid bacteria (LAB).

In a first screening phase, thirty different LAB strains were cultured in nine different plant substrates in Erlenmeyer flask. Fermentation performance was measured after 48 hours of fermentation. In a second phase, best LAB strains were selected for each of the nine substrates to carry out a second screening step in a 1L pH-regulated bioreactor. Antimicrobial activity of pasteurized fermentates was determined by susceptibility tests in Petri dishes against eleven bacteria and ten yeasts and molds targets (food spoilage and pathogens). In a final phase, focusing on one specific strain/substrate combination, different parameters were optimized to reduce fermentation time while maintaining the antimicrobial properties of the product. These parameters served as a basis for 100L scale-up trials to produce first prototypes for application testing.

The data collected along the screening phases show a large disparity in the ability of LAB strains to grow and produce antimicrobial fermentates from different plant substrates. Certain plant substrates have been shown to be more inclined to produce antimicrobial fermentates than others when fermented with the same LAB strains. Interestingly, antimicrobial activity is not fully correlated with the organic acids content of fermentates, suggesting the production of antimicrobial metabolites during fermentation.

In the case of a specific fruit puree substrate, one strain of *Lc lactis subsp. lactis* showed high activity against ten of the eleven bacterial targets (MIC <1.25%). More precisely, six of the eleven targets were inhibited at dosage lower than 0.15%. The optimization and scale-up work carried out with this strain/substrate combination made it possible to reduce the duration of the fermentation process by half while maintaining antimicrobial performances. The prototypes produced will be used both for evaluations in food and pet food applications.

COMBINATION TECHNOLOGIES FOR INCREASED SEAFOOD SAFETY AND SHELF LIFE; BACTERIOSTATIC EFFECTS OF NISIN, CHITOSAN AND MODIFIED ATMOSPHERE PACKAGING ON *LISTERIA MONOCYTOGENES* AND SELECTED PATHOGENIC AND FOOD SPOILAGE BACTERIA IN BROTH AND COLD SMOKED SALMON

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The bacteriocin nisin is generally regarded to be significantly more effective against Gram positive (G+) bacteria compared to Gram negative (G-) bacteria, whereas chitosan is reported to be more effective against G- bacteria. Modified atmosphere packaging (MAP) typically inhibits obligate aerobe bacteria and is less effective against facultative anaerobes, including e.g. the pathogen *Listeria monocytogenes*, and *Photobacterium phosphoreum*, which is considered specific spoilage organism (SSO) of several MAP seafood products.

Our rationale was to study combination technologies, including nisin, chitosan and MAP, as a broad-spectrum hurdle technology for seafood preservation. First, the *in vitro* effects of nisin and chitosan, alone and in combination, were assayed against three genetically diverse *L. monocytogenes* strains, and one strain each of *Staphylococcus aureus*, *Escherichia coli*, *Vibrio cholerae* (non-O1), and *P. phosphoreum*. The minimum inhibitory concentration (MIC) of nisin was between 2 and 8 µg/mL in G+ bacteria, and ≥ 512 µg/mL in G- bacteria, except for *P. phosphoreum* which displayed an atypically low MIC for G- bacteria of 2 to 4 µg/mL. The MIC for chitosan was 0.03 to 0.06% (w/v) for *L. monocytogenes*, 0.06% for *P. phosphoreum*, and 0.12% for *S. aureus*, *V. cholerae*, and *E. coli*.

Interactive effects of nisin and chitosan combined, as expressed by the fractional inhibitory concentration (FIC) index, were between 1.1 and 1.5 for G+ bacteria and *P. phosphoreum*, which is defined as an additive effect, and 0.4 and 0.5 in *V. cholerae* and *E. coli*, respectively, defined as synergy. The *in vitro* effect of MAP (CO_2:N_2 of 100:0, 66:33; 33:66 and 0:100) combined with nisin and chitosan, respectively, was tested on *L. monocytogenes*. There was a clear inhibitive effect of CO_2, which was significantly enhanced by nisin, but not by chitosan. Second, challenge studies with *L. monocytogenes* inoculated on cold smoked salmon (CSS) were performed to demonstrate the inhibitory effects of nisin and chitosan alone and in combination in real produce. The effects of the treatments on colour and texture of CSS were also investigated.

NATURAL PRESERVATIVES AND FOOD SAFETY: CHALLENGES AND OPPORTUNITIES

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Contamination of food products by pathogenic microorganisms contributes to most of the outbreaks of foodborne illness. Synthetic chemical preservatives are often used to inhibit microbial growth in foods. However, there are concerns over the carcinogenic potential of dietary nitrate and nitrite intake. The past few decades have seen an extensive search for novel technologies alternative to synthetic chemical preservatives to reduce the level of contamination of foods by pathogenic and spoilage microbes.

This review provides a general overview of natural preservatives with potential applications in the food industry, including phages and antimicrobial peptides (AMPs) of plant or insect origin, or from phages (endolysins) and bacteria (bacteriocins). We attempt to elaborate the challenges facing the development of novel natural preservatives as antimicrobial hurdles, taking into consideration the sharp contrast between extensive studies in this particular field and very limited industrial use even though there are some good candidates with similar or even superior potency and spectrum as compared with nisin, an AMP approved by European Union and many other countries back in 1980s.

With the availability of several AMP databases such as APD3, Norine and PhaLP, it is possible to develop more effective AMPs as candidate food preservatives by rational design and proper expression strategies to address efficacy (antimicrobial activity and spectrum) and cost, the two major concerns of the food industry. More specifically, we emphasize the importance of having streamlined

approaches and methodological guidelines in the research and development of natural preservatives for commercial exploitation.

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THE POTENTIAL OF HIGH PRESSURE PROCESSING TO CONTROL PATHOGENS IN DURIAN

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Durian (*Durio* spp.) is a prized tropical fruit in Southeast Asia, and its pulp is highly valued for different culinary preparations. However, poor handling practices can lead to microbiological contamination. Considering the physicochemical characteristics of the pulp (pH 5.5-7.0; a_w 0.97), control measures must be implemented to eliminate these hazards. In this regard, High Pressure Processing (HPP) is a promising nonthermal technology with the potential to ensure food safety with a minimal impact on quality.

To select suitable HPP conditions, durian pulp was inoculated with a five-strain cocktail of *Staphylococcus aureus,* a species known for its pressure resistance. Samples were processed at 600 MPa with either a single pulse of 10 min, two pulses of 3 min each, or three pulses of 2 min each. As a result, 3.86-log_{10}, 3.94-log_{10} and 3.04-log_{10} reductions were achieved by each treatment, respectively. Hence, the single pulse of 10 min was chosen for efficiency reasons.

To evaluate the effectiveness of the 600 MPa for 10 min treatment in controlling other pathogens, individual five-strain cocktails of *Escherichia coli* O157:H7, *Salmonella enterica*, *Listeria monocytogenes,* and *S. aureus* were studied. To assess any impact of typical distribution conditions for durian pulp, half of the samples were frozen for 7 days at –18 °C immediately after HPP. As a result, 5.03-log_{10} and 5.02-log_{10} reductions were achieved for *E. coli* O157:H7 and *S. enterica* following HPP, whereas the reductions for *S. aureus* and *L. monocytogenes* were 3.90-log_{10} and 4.50-log_{10}, respectively. Freezing did not affect *E. coli* O157:H7 and *S. enterica*, but inactivation of *S. aureus* and *L. monocytogenes* slightly increased to 4.42-log_{10} and 4.66-log_{10} reductions, respectively.

Microbiological quality indicators were evaluated in durian samples processed under the same conditions. A reduction in the load of total aerobes (1.3-log_{10} reduction), lactic acid bacteria (1.8-log_{10} reduction) and *Enterobacteriaceae* (1.46-log_{10} reduction) was observed. Moulds and yeasts were not detected before or after treatment (< 100 CFU/g).

In conclusion, HPP technology emerges as a promising tool for controlling pathogens and improving the microbiological quality of durian products. However, further research is needed to optimize processing conditions for industrial implementation.

IDENTIFICATION OF GENES INVOLVED IN RESISTANCE OF *E. COLI* K12 DURING HIGH PRESSURE PROCESSING IN BROTH AND A PLANT-BASED MATRIX

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Introduction

The application of High-Pressure Processing (HPP) as a mild preservation food technology has increased during the last decade. However, there is still limited knowledge on the molecular mechanisms of piezotolerance, especially in foods, which is relevant for a more targeted HPP approach to ensure safety of HPP treated products.

Purpose

The objective of this study was to identify individual genes involved in piezotolerance using the *E. coli* KEIO mutant collection and investigate their role in a plant-based food matrix.

Methods

In total, 50 *E. coli* K-12 isogenic mutants (KEIO collection) were grown to stationary phase (37 °C, 16-18 h) in Luria Bertani (LB) broth along with the WT K-12 and individually exposed to relatively mild HPP (300 MPa, 5 min) for identification of genes involved in piezotolerance. Selected

piezosensitive mutants (2) and the WT, were grown as previously and individually inoculated (~7.0 log CFU/g) in a plant-based matrix (10 g) that was vacuum-packed and pressurized at 300 MPa for 10 min. Samples were plated in duplicate on selective (Sorbitol MacConkey agar) media (n = 4) and incubated at 37 °C for 48 h. Significant differences ($P \leq 0.05$) were evaluated by one-way ANOVA and post-hoc Tukey's HSD test in GraphPad Prism (v.10.1.2).

Results

In general, the inactivation of the mutants in the plant-based matrix was relatively low. The pressure sensitivity of the two mutants including a well-described one (Δ*rpos*) was similar to that of the parental strain (1.10, 0.19 SD) in the specific matrix demonstrating that the role of the matrix is highly important in piezotolerance.

Significance

The identification of genes involved in piezotolerance may differ between broth and food systems. The discovery of cellular HPP targets in a plant-based matrix as presented in this study, is a realistic approach for development of efficient HPP decontamination strategies.

APPLICATION OF BACTERIOPHAGES FOR THE BIOCONTROL OF *SALMONELLA TYPHIMURIUM* IN NATURAL CASINGS USED FOR TRADITIONAL FERMENTED MEAT PRODUCTS

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The study aimed to investigate the application of bacteriophages for the biocontrol of *Salmonella* Typhimurium in natural pork casings (NPC) used for traditional fermented meat products at different temperatures.

NPC pieces samples (2 × 2 cm2) were rehydrated with three consecutive washes in sterile distilled water. Subsequently, the samples were drained and inoculated with *ca.* 4.54×106 cfu/g of *S.* Typhimurium CECT4594. Then, the samples were immersed in sterile distilled water to simulate a new washing process containing a commercial solution of SALMONELEXTM lytic bacteriophages with *ca.* 9.22 × 109 pfu/mL. The samples were homogenized for 10 s in the phage-containing solution, drained again, and stored at 10 and 25 °C for 48 h. *Salmonella* viable cells and phage concentration were determined in the NPC and the simulated washing water. Control samples were prepared by inoculating the pathogen or phages individually. Experiments were performed in duplicate.

At 10 °C, a reduction of *ca.* 1.15 log in *Salmonella* was observed in the casing after washing in the phage-containing solution after 48 h. In the washing water, the pathogen concentration remained *ca.* 1.05 log during the 48 h. At 25 °C, washing did not reduce *Salmonella*, reaching *ca.* 9.01 × 107 cfu/g after 48 h. A slight reduction of the pathogen was observed (> 0.5 log) during the first 3 h, followed by an increase in pathogen concentration over the remaining storage period, reaching 1.52 × 104 cfu/mL. At both temperatures, phage concentrations in the casing and wash water remained *ca.* 9.41 × 109 pfu/g/mL, like the control samples. On the other hand, at 25 °C, pathogen growth was observed in control samples, reaching *ca.* 1.05 × 108 cfu/g. Washing the NPC with SALMONELEXTM demonstrated efficacy in reducing *Salmonella* at 10 °C in 48 h, while at 25 °C washing process failed to reduce the presence of the pathogen in the casings.

These results suggest that the effectiveness of phages in reducing *Salmonella* in NPC is temperature-dependent and may be less effective at higher temperatures. This study highlights the importance of considering temperature conditions when using phages for the biocontrol of *Salmonella* in NPC used for the stuffing of traditional fermented meat products.

THE EFFECT OF MARITIME PINE BARK EXTRACT ADDITION ON THE MICROBIOLOGICAL QUALITY OF DRIED HORSE MACKEREL FILLETS

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The Atlantic horse mackerel (*Trachurus trachurus*) is an abundant, sustainable and undervalued fish species. The present study is focused on enhancing the value of horse mackerel by developing a ready-to-eat dried smoke-flavoured product with reduced salt content and extended shelf-life. Thus, the major goal is evaluating the preservative impact of incorporating pine bark extracts from *Pinus pinaster* Aiton subsp. *atlantica* in the fillets brining step.

Three batches of fish fillets were produced: B1 – Dried fillets just brined; B2 – Dried fillets brined with liquid smoke; and B3 - Dried fillets brined with liquid smoke and pine bark extract. Fish fillets were brined for 30 min, followed by 3 hours drying at 70 °C in a mechanical chamber with convection. After blast chilled, samples were vacuum packed and stored at 4 °C for 85 days. Sampling was performed weekly for evaluating quality and safety parameters, namely, total microbial count, *Enterobacteriaceae*, *E. coli*, yeasts and moulds, LAB, *S. aureus*, spores of sulfite-reducing anaerobes, *Listeria* spp., *Salmonella*, *Bacillus cereus* and *Bacillus* spp.

The total microbial count of B1 samples increased during storage, exceeding the upper borderline limit (< 107 cfu/g, HPA, 2009) after 29 days of storage. B2 samples at day 64 presented *Enterobacteriaceae* counts above the upper borderline limit (6.0 × 104 cfu/g, HPA, 2009). Regarding B3 samples, all microbiological indicators were undetectable or present in numbers below the limit values during the 85 days of storage.

The results indicated that the application of maritime pine bark extracts, as a brine ingredient, to produce dried smoke-flavoured horse mackerel fillets effectively extends the shelf life of such fish products.

USING SUPERCRITICAL CO_2 TO INCREASE THE SAFETY AND PRESERVATION OF FOODS

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Supercritical carbon dioxide (SC-CO_2) can be used to increase the safety and prolong the preservation of food products at low temperature. The process was successfully applied for the inactivation of microorganisms in both solid and liquid food products. Recently, SC-CO_2 has been also used for the simultaneous pasteurization and drying of the food, showing promising results as alternative drying technology. Different food matrices can be dried, including fruits, herbs, and meats. The process ensures microbial safety while preserving nutritional and sensory qualities. SC-CO_2 drying operates under low temperatures and high pressures, effectively inactivating pathogenic microorganisms such as *Salmonella enterica*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7, with varying efficiency across different food products. The technology can be integrated with high-power ultrasound (HPU).

The combined SC-CO_2 + HPU technique demonstrates a significant improvement in drying efficiency, showing greater reduction in weight and lower water activity levels compared to SC-CO_2 alone. This method maintains the color, shape, and nutritional integrity of the food, similar to conventional drying technologies. The ability to inactivate ≥ 6 log CFU/g of pathogens, notable in plant-based food within the first 15 minutes, and in meats after at least 45 minutes, highlights the process's effectiveness.

This approach to food drying could represent a significant advancement in addressing food security challenges, warranting further exploration in other food matrices for industrial applications including the valorization of food waste for the development of novel ingredients. Specifically, this last aspect is under study through the Project "Upcycling pea waste side streams for developing future food ingredients" UPea funded by MUR through the Next Generation EU, PNRR M4C2I1.1, PRIN 2022 prot. 2022P5C3E – CUP C53D23004980006.

FOOD MYCOLOGY

Keynote 5.1

UNDERSTANDING THE BIODIVERSITY AND PHYSIOLOGY OF THE INDIGENOUS MICROBIOTA IN VEGETABLE FERMENTATIONS BRINED WITH LOW SALT TO ENABLE INNOVATIVE PRODUCTS FROM SURPLUS VOLUMES

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Utilization of surplus vegetables in healthy snacks, veggie-centric menus, and beneficial fermented foods are desirable to feed a growing consumer population. Because cucumber is free of antimicrobials and starch, we used it as a model system for vegetable fermentations to understand indigenous microbial diversity and physiology.

We identified twenty-three bacterial genera in the various stages of cucumber fermentation including lactic acid bacteria (LAB) and ɣ-proteobacteria. We used culture dependent techniques to develop and characterize a collection of 1,400 specimens and metagenomic analysis of total genomic DNA isolated from commercial fermentation cover brines to understand microbial diversity. Several *Enterobacter* spp. were detected to up to 3 log of CFU/mL at pH values of 6.2 to 4.04 ± 0.15. Such genus was found to proliferate faster than the prevailing LAB and produce sufficient carbon dioxide to produce bloater defect in a cucumber fermentation model system free of salt and the competing indigenous microbiota; but were inhibited by cover brines containing low salt (2 %).

The LAB *Leuconostoc*, *Lactococcus* and *Weissella* spearhead low salt cucumber fermentation instead of *Lactobacillus* species typical of the higher salt (> 6%) counterpart. *Leuconostocaceae* may produce over 40% carbon dioxide in a cucumber fermentation model system and cause bloater defect if it is not outcompeted by the leading lactobacilli. *Lactiplantibacillus pentosus*, *Levilactobacillus brevis*, *Pediococcus ethanolidurans* and some strains of *Lactiplantibacillus plantarum* represent most of the total colony counts during the peak of a cucumber fermentation regardless of salt type. Comparative genomic and phenotypic analyses *L. pentosus* and *Lev. brevis* revealed that: (1) two *L. pentosus* clades, A and B, and three subclades, A.I, A.II, and A.III exist in cucumber fermentations at varied time points, and (2) *Lev. brevis* autochthonous to cucumber lack citrate lyase genes and the associated catabolic function.

The CRISPR *loci* found in *L. pentosus* isolates confirmed recent evolutionary history and the subspeciation described above, suggesting a functional intraspecies diversity in cucumber fermentations. All the *Lev. brevis* genomes encode for 1,2-propanediol metabolosomes like those used by pathogens to colonize the human gut. Autochthonous and allochthonous *Lev. brevis* were found to remove plant sugars and spoilage implicated metabolites, features useful in enhancing the microbial stability of fermented vegetables. Growth and acid production by *L. plantarum* and *L. pentosus* restrains *Lev. brevis* proliferation, which additionally may be more susceptible to bacteriophage infection than the former. The incidence of *L. plantarum* and *Lactococcus lactis* in the bacterial collection of fermentation isolates was minimal (n = 9 and n = 4, respectively), but these species have specific properties valuable to produce relishes and bloater free pickles. *Levilactobacillus namurensis*, *Levilactobacillus zymae*, *Lentilactobacillus buchneri*, *Lentilactobacillus farraginis*, and *Lentilactobacillus rapi* have been detected in commercial cucumber fermentations during long term storage (> 90 days). *Lentilactobacillus buchneri* was found to degrade lactic acid in cucumber fermentation and contribute to spoilage characterized by increasing pH. The yeasts, *Pichia manshurica* and *Issatchenkia occidentalis*, also contribute to lactic acid degradation and rising pH spoilage. The LAB found in cucumber fermentation during long term storage can degrade a variety of organic acids and some encode for putative propanediol dehydrogenases associated with microcompartments. The root cause of microbial instability in low salt cucumber fermentations is still under study to enable the manufacture of novel fermented vegetable products employing unique plant-derived lactic acid bacteria.



POSTERS

FERMENTED FOODS

P-001

PEDIOCOCCUS PENTOSACEUS BACTERIOCINOGENIC STRAINS ARE PREDOMINANT AND WIDELY REPRESENTED IN CHEESE FROM BULGARIAN MIDDLE SCALE DAIRY FACILITY

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In addition to fermentation importance of the lactic acid bacteria (LAB) for the formation of the dairy products, LAB are fundamental to cheese biopreservation associated to the production of different antimicrobial metabolites, including organic acids and bacteriocins. The aim of the current study was to prospect LAB from different type cheese obtained from same dairy facility under the hypothesis that specific bacteriocinogenic strains, with activity against *Listeria monocytogenes*, can be predominant in artisanal cheese production facilities.

Goat, sheep, and cow feta cheese were obtained from dairy plant located near Belogradchik, Bulgaria. Based on the preliminary screening for bacteriocinogenic LAB, from initial 147 selected isolates with antimicrobial potential, 18 were confirmed to be producers of bacteriocins expressing proteinaceous antimicrobial compounds, which are stable at temperature treatments between 8 and 100 °C for 60 min and to 121 ° for 15 min, in addition to pH ranging from 2.0 to 10.0 and to the presence of 1% NaCl, SDS, and Tween 20 and 80. Preselected isolates were confirmed as safe (γ-hemolytic, no antibiotic resistance, mucus degradation properties, neither proteolytic or gelatinase activity). Isolates of interest were differentiated by repPCR and further identified according to recommendations, including 16S rRNA partial gene sequencing, as belonging to the species *Pediococcus pentosaceus*, *Pediococcus acidilactici*, *Latilactobacillus curvatus*, *Lactiplantibacillus plantarum* and *Lacticaseibacillus paracesei*.

Strain ST0401KOC, from the predominant *P. pentosaceus* cluster was further evaluated regarding its bacteriocinogenic properties. Bacterial growth of this strain, cultured in MRS at 37 °, reached levels corresponding to OD 600 nm of 5.32, pH decreases from 5.72 to 4.09 and the production of bacteriocin against *L. monocytogenes* 402 released bacteriocin activity of 51200 AU/mL detected between 9 and 21 hours of cultivation. Addition of cell free supernatant from *P. pentosaceus* ST0401KOC to actively growing *L. monocytogenes* 402 cultured at 37 °C resulted in growth inhibition over 12h period. Alternative biocontrol strategies based on the use of bacteriocinogenic LAB could serve as an important tool to address safety issues in cheese dairy environments aligning with current trends in society, industry, and academia, which seek to replace traditional preservation methods that employ common chemical agents.

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P-002

FERMDB: THE LARGEST INTEROPERABLE DATABASE AND MAP OF FERMENTED FOODS TO DATE

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Fermented foods and beverages have been a part of the human diet for thousands of years, and play an important nutritional, cultural, and economic role in most human societies. Differences in ingredients, local practices, and environmental conditions can impact microbial communities in fermented foods resulting in distinct flavors, textures, and nutritional properties of final products.

Despite their ancient roots, omnipresence, potential health benefits, recent hype, and the plethora of tools to study microbial diversity, the microbial and functional diversity of most fermented foods currently remains unknown, while changing lifestyle practices threaten the loss of some fermented foods. Here we (i) introduce FermDB, the largest interoperable database and map of fermented foods to date (https://bokulich-lab.github.io/FermDB/), intended as an extensible community resource for studying food fermentations; (ii) provide a consistent fermented food ontology for classification of these foods; and (iii) estimate global production values and biomass of some of the most common fermented foods to contextualize the social and economic importance of microorganisms for human food production. These resources are an important next step on the path toward conserving microbial biodiversity of traditional foods and studying their associated health benefits.

P-003

DYNAMIC CHANGES OF FERMENTATION PROFILES DURING PINEAPPLE JUICE FERMENTATION WITH SINGLE AND CO-CULTURES OF ALLOCHTHONOUS AND AUTOCHTHONOUS YEASTS

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The allochthonous yeasts are typically used as starters to produce fruit wines, whereas the autochthonous yeasts, non-*Saccharomyces* yeasts have been reported to be wild yeasts that yield distinctive flavors and high quality utilized for traditional wine fermentation. Phu Chawa pineapple (*Ananas comosus* L. Merr. var Phu Chawa) is a new hybrid variety pineapple of Thailand. Its flesh has a sweet and sour taste, deep yellow colour and strong flavour with total soluble solid of 16-22 °Brix which is high potential for quality fruit wine production.

The objective of this research was to investigate the changes of fermentation profiles of Phu Chawa pineapple juice during fermentation with single of *Sacchromyces cerevisiae* (SC) and *Meyerozyma guilliermondii* (MG), and co-cultures of *Sacchromyces cerevisiae* and *Meyerozyma guilliermondii* (SM).

The results indicated that yeast population of SC rapidly increased approximately 3 log cycles in day 2 of fermentation. MG had slower assimilation rate of nutrient in pineapple juice than those of SC. The fermentation profile of SM was relative similar to those of SC throughout fermentation days.

P-004

LACTICASEIBACILLUS PARACASEI TS35: A PROMISING PROBIOTIC FOR FUNCTIONAL FOOD PRODUCTION

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The incorporation of probiotics into foods has made a significant contribution to the development of the functional food industry. This research aimed to obtain a novel probiotic strain for use in the production of functional food. The probiotic properties of *Lacticaseibacillus paracasei* TS35, endogenous in shalgam juice from Türkiye, were assessed after phenotypic and molecular identification.

The strain exhibited susceptibility to ampicillin, chloramphenicol, erythromycin, and tetracycline, and was non-hemolytic, thereby confirming its safe status. *L. paracasei* TS35 survived at pH 2, 3, and 4, and 0.3% bile salt concentration for 4 h, with a survival rate of 109.66%. The isolate survived well to pepsin (for 4 h) and pancreatin (for 6 h) exposure with survival rates of 114.61% and 106.17%, respectively. *L. paracasei* TS35 demonstrated an auto-aggregation capacity (8.76%) and a strong hydrophobicity (53.69%) towards xylene.

Moreover, the cell free supernatant of the strain exhibited antibacterial activity against *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 1103, *E. coli* O157:H7 ATCC 43895, *Listeria monocytogenes* Scott A, *Salmonella* Typhimurium NRRL B-4420 and *Staphylococcus aureus* 6538P. On the other hand, *L. paracasei* TS35 showed the highest acetate (8135 µg/mL), butyrate (1025 µg/mL) and propionate (14629 µg/mL) production.

Hence, this work affirms the use of *L. paracasei* TS35 as a safe probiotic candidate. The *in vivo* assays should be performed to better characterize the probiotic potential of the strain.

P-005

USE OF *BACILLI* AS BENEFICIAL FERMENTATION ORGANISMS IN NON-CONVENTIONAL APPLICATIONS

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Traditional fermentations of soy, tubers and condiments recruit *Bacillus* species as fermentation microbes that contribute to food quality by secretion of antifungal lipopeptides, hydrolytic enzymes, and exopolysaccharides. This communication outlines several examples demonstrating the beneficial impact of bacilli in food fermentations:

- Antifungal activity of lipopeptides: bacilli produce lipopeptides with antimicrobial activity. The *in situ* activity of the lipopeptide iturin A from *B. velezensis* and *B. amyloliquefaciens* in model *daqu* fermentations inhibited the growth of moulds during fermentation and thus impacted community assembly in this spontaneous fermentation (2).
- Protease activity of *Bacillus* spp. The selection of starter cultures of plant-based cheese analogues requires innovative approaches for selection of starter cultures. For products from pasteurized ingredients, protease activity must be derived from fermentation microbes. The use of bacilli as adjunct cultures in production of soy cheese substantially increased proteolysis. *B. velezensis* FUA2155 significantly ($P<0.05$) increased the accumulation of glutamate during ripening of soy cheese.
- Bacilli as beneficial fermentation microbes in baking. Enzymes of *Bacillus* spp. are widely used in baking applications to increase bread volume. The use of a small proportion (2.5%) of *B. velezensis* FUA2155 or *B. amyloliquefaciens* Fad We ferments with wheat flour or wheat bran increased the volume of white wheat bread and of wheat bread with wheat bran, by more than 50%. A *Bacillus* ferment prepared with buckwheat did not increase bread volume. The impact of bacilli was mediated by partial hydrolysis of arabinoxylans, which are present in wheat but not in buckwheat.

The use of bacilli in food processing can contribute to replace additives and ingredients to achieve a more sustainable and health-beneficial food supply.

P-006

SELECTION OF EXOPOLYSACCHARIDE-PRODUCING LACTIC ACID BACTERIA STRAINS FOR THE STABILIZATION OF FRUIT PREPARATIONS

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Fruit preparations are intermediate products in the food industry and used in various applications. These preparations consist of fruit pieces embedded in a fruit puree. To prevent the settling of fruit pieces within the food mixture, hydrocolloids, typically polysaccharides, are incorporated.

In the current study we identified suitable bacterial strains, which are able to produce exopolysaccharides inside the fruit matrix (*in situ*) with potential stabilizing effects. The testing procedure included confirmation of phenotypic slime formation on solid media (Loop-test), PCR-based detection of the *gtf* gene encoding a glycosyltransferase involved in β-glucan production, immunological detection of a β-glucan polysaccharide capsule, and a high-throughput assay for EPS production in fruit purée or in a mixed fruit-vegetable purée.

More than 200 bacterial strains, including isolates from eight different fruit sources were examined. Among these 50 strains were capable of slime production on MRS agar, of whom 11 carried the *gtf* gene. Among these, six strains produced EPS in strawberry and peach purées mixed with beetroot or carrot purée, respectively. Theses strains belonged to the species *Pediococcus parvulus*, *Lacticaseibacillus paracasei*, *Levilactobacillus brevis*, *Pediococcus claussenii,* and *Furfurilactobacillus rossiae.*

Upon further investigation, these identified strains exhibit potential to serve as a starter culture for stabilizing fruit preparations.

P-007

CHANGES IN THE CHEMICAL PROFILE OF *OPUNTIA FICUS-INDICA* CLADODES AFTER LACTIC ACID FERMENTATION

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Opuntia ficus-indica L. is a plant native from the Americas and also found in Europe, India, the Middle East, and Africa. Its fruits are commonly consumed fresh or transformed. Cladodes, although they are also consumed by humans food in some countries, are often considered by-products and are mainly used as feed or discarded. Several studies have reported that cladodes are a rich source of bioactive and functional compounds with antioxidant activity, making them a valuable candidate for the production of fortified foods. The use of lactic acid fermentation on plant by-products rich in (poly)phenols can be a viable strategy for their valorisation. This process can improve the quantity and quality of bioactive molecules compared to raw by-products.

This work aimed to develop and optimize the lactic acid fermentation of cladodes to obtain a product with a modified polyphenol profile and high antioxidant activity and also to enrich the aromatic profile. To achieve this goal, various bacterial strains from the University of Parma Culture Collection (UPCC) were used to investigate the fermentative processes and chemical profile transformations in *Opuntia ficus-indica* cladodes. A Design of Experiment (DoE) was applied to identify the optimal fermentation conditions using different quantitative and qualitative factors for a total of 46 samples. The total phenolic content (TPC) of fermented cladodes was evaluated using the Folin-Ciocalteu assay and HPLC-DAD. The antioxidant activity was measured using the ORAC assay, and the aromatic profile was determined using the HS-SPME/GC-MS technique.

The results obtained in this study demonstrate that *Opuntia ficus-indica* cladodes can be used as a fermentable matrix, resulting in increased values for antioxidant activity and total phenolic content after fermentation, as well as a modified aroma profile. Furthermore, the strains used for medium to long fermentation times were found to be particularly effective in modifying the antioxidant activity, TPC and aromatic profile of the samples. These samples, from a health-promoting perspective, could be considered as new sources of phenolic compounds natural with high antioxidant activity, to be used for applications such as nutraceuticals, biopharmaceuticals, cosmetics, as well as natural food additives, demand from consumers.

P-008

PHYTATE DEGRADATION IN WHEAT, BUCKWHEAT, SOY, AND RICE FLOURS BY *LACTOBACILLI* AND YEAST ISOLATED FROM AFRICAN AND ASIAN TRADITIONAL FERMENTED FOOD

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Phytic acid (myo-inositol 1,2,3,4,5,6-hexakisphosphate) is a phosphorus reservoir found especially in cereals, legumes, and oilseeds. This compound forms complexes with essential minerals such as K, Mg, P, Ca, Fe, Zn, and Mn, thus reducing their bioavailability. Therefore, due to its negative impact on intestinal mineral absorption, phytic acid is considered an antinutritional substance, as it can modify functional characteristics in food proteins. In seeds, the aleurone layer contains approximately 90% of the grain's phytic acid, with the germ holding about 10%.

Consequently, flours, especially those from whole grains, are rich in phytic acid. Therefore, increasing the use of bran and whole wheat flour in food formulations may compromise the nutritional quality of end products by limiting mineral bioavailability. Microbial fermentation has been described as a strategy to reduce the adverse effects of phytic acid in food

products. The present work aimed to thoroughly investigate the impact of different yeasts and lactic acid bacteria, isolated from Asian and African traditional fermented foods, on different flours (wheat, buckwheat, rye, and soy flours) and doughs prepared with selected cereal meals on the levels of phytate during fermentation and breadmaking. Eleven yeasts belonging to *Pichia kudriavzevii, Saccharomyces cerevisiae*, *Pichia fermentans*, and *Kluyveromyces marxianus* and two lactobacilli belonging to *Lactiplantibacillus plantarum* and *Lacticaseibacillus paracasei* have been selected.

The comparison of selected yeasts and lactobacilli in the degradation of phytic acid with different flours as substrates (wheat, buckwheat, soy, and rice) showed that the degradation ranged from 16 to 77%. The best-performing strain resulted in P. kudriavzevii TY1322 with 77% of phytic acid degradation.

The degradation of phytate for bread production using single yeast was 52.06% ± 4.25, while when a mixed inoculum yeast-lactobacilli was used, the degradation improved up to 60,02% ± 0.21. Lactobacilli can achieve a strong phytic acid degradation both by lowering pH and providing more suitable pH conditions for phytate degradation by endogenous phytases and by a direct source of microbial phytases.

Since the potential benefits of bread incorporating various grains (e.g., soy, buckwheat, and rye) are increasingly evident, this research could have an important implication for human health.

P-009

MICROBIAL PHYTATE DEGRADATION IN SOY MILK FERMENTED PRODUCTS

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Due to the rising incidence of dairy intolerance and the growing popularity of vegan/vegetarian diets, there is an increasing need for alternatives to dairy products. One commonly employed substitute in vegetarian and vegan food items is soybean, known for its mineral and protein content. However, soybean can contain antinutrient such as phytate (inositol hexaphosphate, IP6), which limits the absorption of nutrients. Microbial phytases, enzymes that hydrolyze phosphate groups from IP6, have gained considerable attention in solving this issue. The breakdown of IP6 by these enzymes releases chelated minerals like Fe and Zn and solubilizes IP6, thereby enhancing the availability of minerals for intestinal absorption. The objective of this study was to assess 10 strains of Lactic Acid Bacteria (belonging to *Lacticaseibacillus paracasei*, *Lactobacillus delbruecki* subsp. *delbruecki*, *Lactobacillus helveticus*, and *Lactiplantibacillus plantarum*) and 22 yeasts (previously isolated from Tajikistan yogurt and togwa, a Tanzanian traditional fermented cereal- and cassava-based product). The study aimed to evaluate their ability to ferment soy milk, individually or in mixed cultures, and their phytase activity. All strains belonging to *Lacticaseibacillus paracasei* showed the ability to ferment soy milk and thicken the resulting product. *Kluvieromyces marxianus* and *Pichia kudriavzevii* were identified as suitable candidates among the yeasts.

The strong phytate-degrading ability previously observed in vitro for *P. kudriavzevii* TY1322 was confirmed during soy milk fermentation. Furthermore, the phytate degradation was enhanced when *P. kudriavzevii* TY1322 was co-cultured with *Lacticaseibacillus paracasei* AB3 and *K. marxianus* AL3 or BL8. These findings suggest that these strains are promising candidates for phytate degradation in the fermentation of soy-based food products.

P-010

QUANTIFYING DAIRY PROPIONIC ACID BACTERIA IN RAW CHEESE MILK: A TIME-CONSUMING CHALLENGE

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Monitoring of dairy propionic acid bacteria (dPAB) in cheese milk is essential, because low levels of dPAB (>10 CFU) can cause severe cheese defects, that result in significant financial losses for the producers. Microbiological dPAB quantification methods commonly used in the dairy industry are extremely time-consuming due to their slow growth and the lack of a selective medium, requiring a final identification step after cultivation. PCR-based methods are faster and highly specific, but they are costlier, require trained personnel, and may have higher detection limits.

The objectives of this study were to obtain an overview of the dPAB contamination levels in raw milk, to determine which method is best suited to detect (low level) dPAB contamination, and to identify farm characteristics (e.g. milking system) potentially favoring elevated dPAB levels. Therefore, we analyzed a total of 426 raw milk samples. Initially, 88 samples were pre-screened to identify the best-performing culture medium. The samples were analyzed on two commonly used media: lithium-glycerol agar (LGA) and yeast extract-lactate agar (YELA). We then analyzed 338 samples on LGA and with a multiplex qPCR-assay targeting dPAB.

The pre-screening showed that the media performance of YELA was significantly inferior to LGA, 44% of the samples were not quantifiable on YELA due to non-target overgrowth, whereas dPAB levels ≥ 4.0 log dPAB/mL raw milk were quantified on LGA. The dPAB levels of the 338 samples ranged from no dPAB in 1 mL raw milk (LGA, PCR) to 4.2 (LGA)/5.1 (PCR) log dPAB/mL raw milk. We observed no growth on LGA in 4.4% of the samples analyzed, but 20.7% of the samples were presumably dPAB free when analyzed by multiplex qPCR. Statistical analysis showed a correlation between the dPAB levels quantified by LGA and by multiplex PCR, but dPAB contamination appears to be a highly farm-specific problem. We conclude that LGA is advantageous for low levels dPAB detection in raw milk, while multiplex PCR is well-suited for quantification and simultaneous identification at higher concentrations. Therefore, especially quantification of low levels of dPAB remains a time-consuming challenge.

P-011

COMPARATIVE STUDY OF MICROBIAL SPECIES PERFORMANCE IN *AMAZAKE* PRODUCTION

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In this study, we investigated the dynamic changes in reducing sugar content, enzymatic activities, metabolite profiles, and consumer perceptions during the fermentation of amazake, using *Aspergillus oryzae* and *Bacillus amyloliquefaciens*. Notably, the enzymatic activity of α-amylase and protease, as well as the reducing sugar content, were higher in *A. oryzae* amazake samples (AAO), throughout the fermentation process, at 55 °C. Additionally, *B. amyloliquefaciens* amazake samples (ABA) were analyzed at 70 °C, to assess the thermal stability of the enzymes secreted by this bacterium.

As anticipated, α-amylase activity and reducing sugar content increased at this higher temperature, while protease activity was inhibited. Furthermore, liquid chromatography-mass spectrometry (LC-MS/MS) analysis, indicated that levels of amino acids and organic acids were distinctly higher in AOA compared to the ABA at 55 °C for 16 h of fermentation. A multivariate principal component analysis (PCA) plot based on the non-targeted compounds in LC-MS/MS datasets indicates a highly distinguished pattern for AAO and ABA. Intriguingly, observed physicochemical differences did not align with consumer perceptions, as both amazakes were equally accepted. Consumers described AAO as more floral and fruity, while ABA was characterized as nutty and starchy.

In conclusion, the study underscores the potential of *Bacillus amyloliquefaciens* as a microorganism for fermenting amazake, producing optimal parameters and distinct organoleptic characteristics.

P-012

EVALUATION OF SPONTANEOUS FERMENTATION WITH BACKSLOPPING METHOD FOR THE PRODUCTION OF ALCOHOLIC GINGER BEER

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Ginger beer is a fermented beverage made from the rhizomes of *Zingiber officinale* and originated in England in the mid-17th century as a low-alcohol beer. This drink can be produced using a microbial consortium called the Ginger Beer Plant (GBP) or follows the backslopping method, consisting of a small amount of fermented material as inoculum in a new batch. In addition, an alcoholic version can be obtained with commercial yeasts in a controlled fermentation. In this last preparation, the grain wort or sugar substrate (mainly sucrose) could be diluted for low-alcohol beer production. However, the most common commercial ginger beer is made with carbonated water and ginger syrup/extract.
Few studies have focused on the production of ginger beer, and there are no investigations on the use of non-*Saccharomyces* yeasts in controlled fermentation. The aim of this study was the analysis of the fungal community involved in ginger bug activation and beer fermentation using the backslopping method. Two different ginger rhizomes, labelled A and B, were used for the fermentation trials.
During the process, native yeasts were isolated at different times (24, 36, 48, 72 and 96 hours during the activation and 0, 7 and 14 days during the fermentation). Furthermore, the sugar consumption measured the weight loss and the pH of ginger bug and ginger beer were analyzed. A strain of *Candida* sp. and *Wickerhamomyces* sp. dominated in the ginger A and B batch samples, respectively, during activation and fermentation. No significant sugar consumption was observed in both. This occurrence could be explained by the inability of these native strains to ferment sucrose. Subsequently, these two batches were inoculated with a commercial strain of *Saccharomyces cerevisiae* and *Torulaspora delbreuckii* to complete the fermentation.
In conclusion, this preliminary study evidenced that native yeasts occurring in the spontaneous fermentation obtained by the backslopping method can not ensure the production of alcoholic ginger beer. In this case, the use of starter yeasts seems necessary. Hence, the evaluation of the effects of S. cerevisiae and non-*Saccharomyces* yeasts, such as *T. delbrueckii,* on chemical and sensory traits deserves further investigations.

P-013

LACTIC ACID FERMENTATION AS A TOOL TO IMPROVE AROMA OF MICROALGAL FOOD PRODUCTS: THE CASE OF *CHLORELLA VULGARIS*

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The request for food products enriched with microalgae is growing. The biomasses of these microorganisms are well known for their good nutritional composition, especially in proteins, but are characterized by an undesired taste and smell. These characteristics are hurdles for the formulation of food and beverages and producers in the sector are constantly looking for solutions. Lactic acid fermentation has been known for centuries for its high capacity to metabolize different matrices, and consequently to produce new molecules with potential bioactivities or to modify the volatile fraction of the fermented matrices.
This study aims to evaluate the impact of lactic acid fermentation on the volatile fraction of different *Chlorella vulgaris* biomasses. Fermentations of 4 different *C. vulgaris* powder, each characterized by distinct compositions in carbohydrates, lipids, photosynthetic pigments, and proteins, was conducted utilizing lactic acid bacteria (LAB) strains from the bacterial species *Lacticaseibacillus rhamnosus, Lcb. paracasei, Lcb. casei, Leuconostoc citreum, Lactobacillus delbrueckii subsp. bulgaricus,* and *Lactiplantibacillus plantarum*. Each *C. vulgaris* biomass was hydrated with 75% of sterile water and fermented for 72 hours by inoculating 7 Log CFU/g of the selected LAB strains.

The characterization of the volatile fraction was conducted on all the samples by HS-SPME/GC-MS Analysis. The results have underlined variable volatile fractions produced consequently to fermentation and in relation to the microalgae compositions, especially considering the amount of proteins and chlorophylls. In particular, the fermentation process significantly influenced the concentration of those compounds responsible for aldehydic/ethereal, buttery/waxy (acetoin and diacetyl), alkane and fermented aromatic notes. In most of the tests performed, after fermentation, the undesirable notes of the microalgal biomasses were reduced making the biomasses more acceptable to consumers.

P-014

MICROBIAL EVALUATION OF THE *ATHINOLIA* GREEK OLIVE CULTIVAR USING CLASSICAL MICROBIOLOGICAL AND AMPLICON-BASED METAGENOMICS APPROACHES

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The Mediterranean diet is based on the consumption of fermented table olives and olive oil. According to the International Olive Council statistics, Greece is the country with the highest olive production growth rate while it is the second table olive producer after Spain. Athinolia cultivar (also known as mastoid and tsounati) is mostly cultivated in southern Laconia, in the municipality of Monemvasia, in southeast Peloponnese. It has a unique character of fruitiness with dominant notes of citrus and almond, a powerfully fresh, harmonious, and well-balanced taste, and a golden green color.

Taking the exceptional characteristics of Athinolia into consideration, the aim of this study is the evaluation of the microbiota during a spontaneous Greek-style fermentation using classical microbiological and amplicon-based metagenomics analyses. Classical microbiological analysis was performed for enumeration and isolation of lactic acid bacteria (LAB), *Enterobacteriaceae*, and fungi from olive and brine samples throughout fermentation. Rep-PCR was used for grouping LAB and fungi isolates at specific fermentation time points, while 16S rRNA gene and ITS DNA region sequencing were performed for the identification of isolates at the species level. Additionally, the bacterial and fungal diversity were evaluated through amplicon-based metagenomics analysis, using total DNA extracted from olive and brine samples at the same fermentation time points.

According to the results of the classical microbiological analysis for the Athinolia cultivar, bacteria and fungi counts were similar between olive and brine samples, ranging from 5.33 to 8.26 log CFU g–1 or mL–1. In the case of *Enterobacteriaceae*, the counts were up to 7.7 log CFU g–1 or mL–1 at the beginning of the fermentation and eliminated after 60 days for both olive and brine samples. Throughout the work, 166 bacteria and 245 fungi were isolated and identified using molecular techniques. Although the number of identified LAB and fungi genera was low, a vast diversity was revealed by the metagenomics analysis. It is worth mentioning, that this is the first report on the microbiota of Athinolia olive cultivar using the metagenomics methodology.

P-015

ENHANCING FOLATE LEVELS IN DIFFERENT FERMENTED MILKS THROUGH BIOFORTIFICATION AND CONTENT EVALUATION DURING STORAGE USING HPLC-MS/MS ANALYSIS

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Folate is the natural form of vitamin B_9, involved in several biological processes, as well as red blood cell production and is particularly important in stages of rapid growth and development (pregnancy and infancy). This essential vitamin is naturally present in many foods and humans can only obtain it through the diet. For this reason, folate deficiencies are common and it is necessary to obtain folate from exogenous sources to prevent related diseases (neural tube defects and megaloblastic anaemia). The recent food market trends aim

to develop functional food with increased nutritional value mainly obtained by natural sources. Among these, biofortification appears to be a promising strategy to increase natural folate content, particularly when applied to fermented foods. The aim of this study was to enhance folate levels in fermented milks produced starting from cow milk (yogurt and kefir-like product) and almond milk, through biofortification. Over 100 lactic acid bacteria (LAB) strains of different origin were screened for their ability to produce folate. First, the selected strains were analysed by PCR for the identification of genes involved in folate synthesis. Then, the amount of folate produced by LAB in skim milk was analysed using the UHPLC-MS/MS technique. Based on these results, the best performer strains were identified and used to ferment milks testing different strain combinations. The shelf-life of the fermented products was studied by performing microbiological (LAB counts and pH measurement) and chemical analyses (folate quantification using UHPLC-MS/MS) at different times (0, 7, 14 and 25 days). Total folic acid was calculated for all samples. In addition, six folate metabolites (10-Formyl-THF, 5,10-MethenylTHF, 5,10-Methylene-THF, 5-Methyl-THF, Dihydrofolate and Tetrahydrofolate) were calculated via a semi-quantitative approach.

The results obtained showed that folate production is strongly strain-specific and blend-specific. The final level of total folate metabolites in the fermented cow milks resulted to be much higher with respect to those of almond-based alternative. The amounts of folate obtained can't meet the daily requirement in either the general population or at-risk classes, but these kinds of products can be considered as an aid to fight folate deficiency.

P-016

PATHOGEN BEHAVIOUR IN FERMENTED FOODS: A DESCRIPTION OF VARIOUS FOODBORNE PATHOGENS SURVIVAL IN CHARACTERISED FERMENTATIONS OF KEFIR AND SAUERKRAUT

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Fermented foods are becoming increasingly popular due to their reported health benefits, increased consumer awareness and unique sensory profile. Their production and variation is increasing commercially and domestically. This rapid (and sometimes uncontrolled) increase could lead to food safety issues. Experiments were performed on the fermented foods kefir and sauerkraut in relation to the artificial inoculation of 5 different bacteria (*Staphylococcus aureus, Salmonella enterica* serovar Senftenberg, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Clostridium sporogenes*) into the food, along with characterising the fermentation microorganisms and their microbial community from sequencing and their physiochemical properties. The foods were co-inoculated with the pre-mentioned pathogens individually at the start of fermentations and sampled for the pathogen, lactic acid bacteria (LAB), yeasts, pH, lactic acid content and microbial diversity throughout the fermentation and storage period.

Milk kefir was fermented with 2% kefir grains over 48 hours at 25 °C and held in storage at 4 °C for 4 weeks or until the pathogen was undetectable. In these experiments, *S. aureus* and *S.* Senftenberg were unable to survive the fermentation process while *L. monocytogenes* and *E. coli* O157:H7 decreased but showed increased survival at low numbers for a considerable amount of time in storage. *C. sporogenes* spores persisted at inoculation levels until the end of storage. Two types of sauerkraut fermentations with 2% salt were carried out, with and without a commercial starter culture at 25 °C over a week or until the pathogen was undetectable. In sauerkraut, the starter fermentations were able to cause a large (~5 log cfu/g) decrease in non-spore forming bacteria to undetectable levels about 1-2 days faster than the non-starter fermentations. *S. aureus* had the lowest survival compared to the other bacteria, while the others

had similar levels of survival and were still detected in the non-starter fermentations at Day 5 (*L. monocytogenes* and *S.* Senftenberg) and Day 7 (*E. coli* O157:H7). Spores of *C. sporogenes* displayed their characteristic resistance and survived past the normal fermentation time with and without starters.

This demonstrates that the fermentation process in acidic foods like kefir and sauerkraut are efficient in reducing the amount of non-spore forming pathogens, however this effect does vary depending on type of pathogen and food.

P-017

IMPACT OF PARTIAL SUBSTITUTION OF SODIUM CHLORIDE BY POTASSIUM CHLORIDE ON THE FERMENTATION PROFILE OF CV. KALAMATA NATURAL BLACK OLIVES

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Sodium chloride is a basic raw material in the fermentation of natural black olives in amounts ranging from 6-8%. Given the link between sodium intake and cardiovascular diseases, the table olive industry has undertaken considerable effort to reduce the amount of sodium in table olive processing.

Thus, the purpose of this work was to investigate the effect of partial substitution of NaCl by KCl on the fermentation profile of cv. Kalamata black olives to produce a reduced sodium food. For this purpose, natural black olives of cv. Kalamata were fermented according to industrial practice in 7% (w/v) NaCl. In addition, three substitution levels of NaCl by KCl were undertaken, namely 25, 50, and 75%. The changes in the microbial association (lactic acid bacteria-LAB, yeasts, enterobacteria), pH, salt content, and titratable acidity were monitored during fermentation.

Results showed that all substitution levels resulted in vigorous lactic acid fermentations, due to the dominance of LAB populations. Specifically, the growth rate of LAB was higher in KCl brines, namely 0.406, 0.440, and 0.554 h-1, at 25, 50, and 75% substitution, respectively, compared to control fermentation (0.315 h-1). No significant differences were observed in yeast populations among different salt compositions. Enterobacteria were detected in lower pH values (3.8) in brines containing KCl, compared with control fermentation (4.5). A rapid decrease of pH was observed within the 25 days of fermentation in the brines containing KCl, reaching a value of 3.7 compared to control fermentation in which the pH was 4.7 for the same time period. Salt level was maintained at 7% throughout fermentation by periodic additions of NaCl or mixtures of NaCl/KCl.

In conclusion, potassium chloride can be effectively used in table olive fermentation to produce a reduced-sodium final product, and thus meet consumers demand for lower dietary sodium intake.

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P-018

SPONTANEOUS VEGETABLE FERMENTATIONS: A FOOD SAFETY PERSPECTIVE

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Spontaneously fermented vegetables are increasingly set available in small-scale retail and (pop-up) catering shops. This (re)discovery of alternative and traditional food networks is driven by changing consumer preferences, e.g. towards plant-based and (perceived) healthy diets, together with increased environmental awareness. Microbiological food safety is of low concern for low-acid fermented vegetables. However, the exclusion of starter cultures induces process variation (acidification rate and final acidity) by relying on the natural present lactic acid bacteria. This research explores unpasteurized, spontaneous fermented vegetables their lactic acid bacteria, together with potential consumer exposure to (i) foodborne pathogens, and (ii) biogenic amines.

A market survey was conducted in Belgium and Austria to identify unpasteurized, spontaneous fermented vegetable products. Selected products were sampled seasonally and analysed for biogenic amines and several microbiological parameters: (i) linked to the fermentation process (lactic acid bacteria), (ii) linked to process hygiene or reliability (*E. coli*, *Enterobacteriaceae*), and (iii) relevant foodborne pathogens (*L. monocytogenes*, *Salmonella* spp., *Y. enterocolitica*, and STEC). Challenge tests were performed to assess the growth potential of *L. monocytogenes* during vegetable fermentation, considering different food handling scenarios.

This research showed that spontaneous fermentation is highly variable. A rapid pH drop to a level below 4.2 is the major microbiological safety determinant for vegetable fermentations. Most market samples complied with a pH level lower than 4.2. Persisting *Enterobacteriaceae* might indicate either a slow pH decrease with related favourable initial growth conditions or a too short fermentation time. Challenge tests strengthened the importance of the fermentation time for pathogens to be no longer detected.

Combining the use of well-selected starter cultures with an end-of-production pasteurization step is recommended to ensure the safety of fermented vegetables. However, traditional spontaneous fermentation fully relies on (i) the level and composition of the indigenous microbiota, being inherently high in variability, and (ii) the fermentation practices. This research aims at providing guidance on proper fermentation practices, ensuring food safety.

P-019

METAGENOMIC AND METABOLOMIC PROFILE OF MOSCATO WINE PRODUCTION

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Microbial communities composition and activity during the different stages of wine production chain play a decisive role on the overall wine quality and consumer appreciation. For this reason, the knowledge of factors that drive the association between the microbial diversity and wine chemical composition is essential to produce high quality wines.

In this study, 3 different inoculation protocols were used to ferment vitis *vinifera* L. cv. Moscato grape must, including a mixed fermentation with *Lachancea thermotolerans* and *Saccharomyces cerevisiae* (either in co-inoculation or 48 h sequential inoculation) while a pure fermentation with *S. cerevisiae* was performed as control. Fermentations were performed in the experimental wineries of the University of Turin (Italy) and West Attica (Greece), using the same experimental plan and fermentation conditions.

Production process through different stages of wine fermentation was explored by applying different analytical methods based on metagenomic and metabolomic approaches. Shotgun metagenomics analysis revealed a wide spectrum of yeast and bacteria that are affected by the inoculation protocol applied.

On the other hand, metabolomic profiling focusing on target compounds, showed that it is possible to distinguish the different inoculation protocols both in Greece and Italy according to the different metabolic profile and mark the presence of specific molecules related to specific yeast metabolic activities.

P-020

MICROBIOTA DYNAMIC OF GREEN COFFEE FERMENTATION IN APPLE JUICE AND ITS IMPACT ON COFFEE VOLATILE PROFILE

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The consumption of coffee and coffee based beverage is globally widely diffuse. Commonly, coffee cherries are fermented to obtain green coffee beans which, once dried, are shipped in the different countries where they are roasted, processed and sold. Nowadays to obtain blending with new aroma characterizations, the coffee industries are starting to try a re-fermentation by using different liquid matrices as source of precursors for the correct microbial development.
In this study, the microbiota dynamics and the volatile component of a spontaneous green coffee fermentation were compared with one co-inoculated with *Pichia kluyveri* and *Lactiplantibacillus plantarum*. A commercial apple juice was selected as liquid phase precursor for the fermentation to try to give a flavour characterization on the final coffee sensorial profile.
Samples from the two different fermentations were collected at different times (24, 30, 48, and 72 h) and analysed for the microbial load and pH trend. The acidification rate of the two fermentations among the time followed the same trend. The results showed for lactic acid bacteria (LAB) a load after 72 hours, reaches around 9 Log CFU/g for both spontaneous and co-inoculated fermentations although after 24 hours LAB load was significant lower for the spontaneous fermentation. GC-MS analyses for the volatile composition and shotgun metagenomic sequencing showed differences in the microbial function and development.
The results will give an overview and a better understanding about a potential new coffee fermentation process which could open to new applications for the food industry.

P-021

INVESTIGATING NITRIC OXIDE SYNTHASE ACTIVITY IN COAGULASE-NEGATIVE *STAPHYLOCOCCI*

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Through the use of arginine, the current study investigated nitric oxide synthase (NOS) activity in thirty-five staphylococcal strains from five distinct coagulase-negative staphylococci (CNS) species. The NOS pathway is increasingly being investigated as it could be exploited as an alternative to nitrite in meat processing, also considering the implication of synthetic curing agents in the generation of carcinogenic compounds. The significance of the NOS pathway lies in its capacity to degrade L-arginine into L-citrulline and nitric oxide, leading to the formation of nitrosomyoglobin, which imparts a pinkish-red colour to fermented meats.
This exploration involved an in-depth analysis of the NOS activity in the CNS, employing both genotypic and phenotypic methods. Indeed, PCR assays were carried out using four different primer pairs to evaluate the presence of *nos* genes, while a UHPLC-HRMS approach was used to quantify the concentrations of L-arginine and L-citrulline contained in the arginine-enriched meat simulation media fermented by the investigated bacterial cultures.
Among the strains studied, sixteen were found to harbour the gene encoding NOS. Strains from the *Staphylococcus saprophyticus*, *Staphylococcus equorum*, and *Staphylococcus carnosus* species exhibited phenotypic NOS-like activity. This was evidenced by the degradation of L-arginine and the production of L-citrulline (values ranged from 8.73 to 23.70 mg/L) in meat simulation media fermented under microaerobic conditions. However, a considerable species- and strain-level variability in L-citrulline production was observed among staphylococci.
Notably, *S. equorum* L33, *S. saprophyticus* L43 and L49, and *S. carnosus* TM300 were the strains exhibiting the highest L-citrulline production (22.01, 22.30, 21.00, and 23.70 mg/L, respectively), compound linked to nitric oxide release. Moreover, *S. equorum* L33 showed the best safety profile,

displaying absence of genes responsible for producing amines and transferable antibiotic resistance, as well as inability to form biofilms.
This investigation underscores the potential of using meat-associated CNS to promote colour development in nitrite-free meats, offering a promising area for enhancing the visual appeal of such products.

P-022

PARAMETERS INFLUENCING WATER KEFIR GRAIN GROWTH DYNAMICS BY IMAGE ANALYSIS

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Water kefir is a fermented beverage that is produced by adding water kefir grains to a sucrose solution with dried fruits. The approximately 1-10 mm large grains consist of a bacterial exopolysaccharide matrix and are mainly colonised by lactic acid bacteria, acetic acid bacteria and yeasts. The production depends on the successful cultivation and propagation of the existing grains: During fermentation cycles, the mass of the grains increases, but growth dynamics between the start and end points of fermentation and the parameters that can influence this process have not yet been analysed. Monitoring growth is potentially relevant for the industrial production of water kefir, as no defined starter cultures are available. Our aim was therefore to establish a method for the continuous documentation of grain growth and thus enable a kinetic description of the involved processes.
We developed a documentation system that takes high quality pictures, which are then further processed with Fiji image-analysis software. Kefir grains were selected by size and incubated in standardized water kefir medium at room temperature for 72 h. The number of and increase in white pixels in the pictures against a black background represent a measure of the two-dimensional grain size and growth, respectively.
The developed method enables the visualization of grain growth curves as a function of white pixels over fermentation time. These are comparable to classic bacterial growth curves, as they exhibit lag, exponential, and stationary phases. The influence of the number of starter grains and their size could be evaluated based on curve progression and total growth. It was found that an increasing number of grains and larger grain sizes have a negative effect on the overall growth rate. However, the exponential and stationary growth phases tend to be reached faster as the number of grains increases. In ongoing experiments, the influence of the sugar content and the type of water in the medium is being investigated.

P-023

MICROBIAL COMMUNITY OF MEAT ANALOGUES FERMENTED USING MEAT STARTERS

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The purpose of this study was the exploration of changes in the microbiota of dry fermented meat analogues manufactured replacing traditional ingredients from animal origin with sustainable alternatives from plant origin. The most common substitutes of ingredients of animal origin are texturized plant proteins and vegetable fats. However, the use of new ingredients may affect proliferation of the commercial meat starters, and also change the dynamics of the autochthonous microbiota found in traditional fermented meat products. In this study, we have investigated the microbial community of hybrid dry fermented sausages manufactured replacing part of the animal ingredients with sustainable ones.
Hybrid sausages where prepared replacing 50% of pork meat and back-fat with texturized pea protein and coconut fat. The batter was inoculated with a bacterial commercial starter, and the manufactured sausages were soaked in a bath containing *Debaryomyces hansenii*. Fermentation and drying were done at controlled temperature and relative humidity until reaching a 35-40% weight loss. Samples were taken at the beginning and at the fermentation and drying stages of the process. Microbial counts were done on PCA, MRS, AMS, VRBGA and RB media plates. Analysis of the microbiota was done from DNA extracted from the sausage samples. Bacterial community was explored by sequencing ribosomal regions V4-V5 16S rDNA, and fungal community by sequencing of ITS1. Bioinformatics analysis was carried out using mothur.

Counts of total mesophilic bacteria and lactic acid bacteria were slightly higher in the hybrid sausages than in the traditional ones. On the contrary, staphylococci and fungi counts decreased in the hybrid sausages during the curing stage. Enterobacteria were absent at all stages of the manufacturing process.
Metagenomics of the 16S rDNA showed a codominance of genera *Latilactobacillus* and *Staphylococcus* at the initial stage of the manufacturing process. However, *Latilactobacillus* dominated the fermentation and curing stages. Similarly, metagenomics of the its1 region of the rDNA revealed a higher presence of *Debaryomyces* in the traditional sausages. In conclusion, dry fermented meat analogues could be manufactured using a meat starter, although the selection of fungal starters might be necessary to ensure the colonization of the sausage surface.

P-024

DIRECTING FLAVOUR FORMATION BY LACTIC ACID BACTERIA IN HYBRID DAIRY-PLANT INGREDIENT EMULSIONS

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To increase the sustainability of food supply there is a move towards the increased use of plant proteins. This shift comes with many technological challenges, as current food production processes are mostly optimized for animal, e.g. dairy, proteins. In addition, the creation of full vegan dairy-mimic products may come with challenges related to (off-) flavor or (off-)taste, antinutritional factors, and insufficient nutritional quality. The combination of plant-based ingredients with dairy in hybrid products can offer the best of both worlds to consumers by providing optimal taste and health benefits, through increasing the intake of plant-based foods and limiting animal product consumption.
We here investigate the potential to reduce off-flavor and to produce desired cheese flavors in milk to which plant protein was added (hybrid "milk") by applying fermentation with lactic acid bacteria (LAB). For this, pea, mungbean and chickpea proteins were selected as target plant proteins. Standardized hybrid "milk" formulations were prepared by adding a dispersion of plant protein to UHT dairy milk. Fifty LAB strains of the genera *Lactococcus*, *Lactobacillus*, *Leuconostoc* and *Streptococcus* were selected for the fermentations focused on cheese flavor formation, which was assessed by GC-MS analysis.
The results showed strain-specific differences in their potential to form desired cheese flavor compounds and/or to reduce undesired flavors in dairy-plant hybrid emulsions. The data suggest that combinations of strains might allow further improvement of sensorial properties and it demonstrates the potential of fermentation to facilitate the development of innovative plant-dairy hybrid products.

P-025

THE METAOLIVE PROJECT: META-OMIC APPROACHES FOR TRADITIONAL TABLE OLIVE FERMENTATION

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The microbiota of table olives and their brines (4 to 8% NaCl) is usually dominated by lactic acid bacteria (mostly *Lactiplantibacillus* spp.) and yeasts (e.g., *Saccharomyces cerevisiae, Candida boidinii, Wickerhamomyces anomalus*) which are able to stand the highly selective conditions (high NaCl and phenols, low nutrients) and cause desirable changes but their role is not always clear-cut.
The project MetaOlive will significantly advance the state of the art on the microbiology of fermented olives in the Mediterranean area.
Using a multi-omic approach, MetaOlive partners will:
- Create the widest and most comprehensive map of the microbiome and volatilome of commercial table olives in the Mediterranean area, depicting a variety of traditionally produced table olives in different countries and including different production technologies and parameters;
- Understand spoilage dynamics in commercial table olives of the Mediterranean area;
- Carry out an in-depth analysis of the sources of contamination and of the microbial dynamics during

fermentation and storage of the Italian PDO "Oliva di Gaeta", identifying the main reasons of the ecological success of beneficial microorganisms during olive fermentation and highlighting genes and functions relevant for the fermentation and for successful interactions.

The outputs of MetaOlive project will contribute to the understanding of key ecological processes in microbial ecology of table olive (e.g., contamination, dispersal, selection) and to the knowledge of the genetic determinants of the success of microbial strains in olive fermentation. The results will also be valuable for stakeholders potentially contributing to the identification of markers for olive varieties tracking and providing information on the role and genomes of beneficial and detrimental microorganisms, their sources and means for their control.

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P-026

THE POTENTIAL OF FERMENTATION WITH *PLEUROTUS OSTREATUS* TO DEVELOP PROTEIN-RICH SNACKS FROM FAVA BEANS (*VICIA FABA* L.)

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The high intake of fast carbohydrates and unsaturated fats, particularly through snack consumption, plays a significant role in the rise of childhood obesity and overweight nowadays. Snack development based on legumes seems to be an effective strategy to boost plant-based protein intake in kids' diets. Although legumes are an excellent source of proteins, they are characterized by a lack of certain essential amino acids in their content and the presence of anti-nutritional factors that may have adverse physiological impacts. Solid state fermentation (SSF) with *Pleurotus ostreatus* is recommended as a sustainable approach to enhance legumes' nutritional and functional qualities.

Therefore, this study aims to explore the effects of SSF on the nutritional attributes of fava beans (*Vicia faba L.*) and its effect on the functional characteristics of bars based on fermented fava beans as a new snack targeted at children and adolescents. From the analysis of the nutritional composition of the beans, fermentation with the fungus resulted in a reduction of carbohydrates from 63 to 58% dry basis and an increase in the protein fraction from 30 to 36% dry basis. In addition, fermentation also increased essential amino acids by 25%. Furthermore, the ACE inhibitory capacity of the fermented fava beans was 40%, and for the unfermented, only 4%. Additionally, it was found that fermentation had a beneficial impact on the functional characteristics of developed products, maintaining the same tendency (increased protein content, essential amino acids content, and ACE inhibitory capacity).

Using fermented fava beans positively affects the sensorial characteristics of bars compared with samples with unfermented legumes. The developed snack based on the suggested mixture of ingredients is characterized by high overall acceptability, pleasant taste and odor, and required

texture. Comparing the color of the bars, the L* value was 42.80 for the unfermented sample and 37.34 for the fermented one. In addition, the mean b* value was also higher for the unfermented sample by 11.8%. In conclusion, fungus fermentation improves the nutritional quality of fava beans, making them a beneficial ingredient for producing snacks with enhanced functional characteristics.

P-027

ADVANCING LACTIC ACID PRODUCTION: COMPARATIVE ISOTHERMAL MICROCALORIMETRY OF *LACTOBACILLUS PLANTARUM* GROWTH ON BAMBOO *VERSUS* BACTERIAL CELLULOSE SUBSTRATES

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Isothermal microcalorimetry (IMC) is a powerful analytical tool that measures the real-time metabolic heat flow of reproducing bacteria, offering a unique window into microbial fermentation, pivotal for D-Lactic acid (D-LA) production, a biologically important molecule with extensive industrial applications, primarily produced through bacterial fermentation.

This study applied IMC to investigate the growth and fermentation dynamics of *Lactobacillus plantarum* with various concentrations of bacterial and bamboo cellulose, focusing on the effect of these substrates on lactic acid production. *Lactobacillus plantarum* was cultured in de Man, Rogosa, and Sharpe broth (MRSB), supplemented with 0.1-0.5% bacterial or bamboo cellulose, and monitored via IMC at 35 °C to record bacterial growth and lactic acid fermentation in real-time. Calorimetric data was analyzed to investigate the impact of cellulose supplementation on bacterial metabolism and lactic acid production.

The study revealed that within the 0.1-0.5% range, bamboo cellulose significantly modifies *L. plantarum*'s metabolic profile when co-fermented with glucose. A delayed and broadened thermal peak suggested prolonged metabolic activity, likely due to bamboo cellulose serving as an additional nutrient source or influencing glucose metabolism. Crucially, this co-fermentation setup yielded higher lactic acid levels, peaking at 8.16%, underscoring cellulose's potential as an adjunct in lactic acid fermentation.

The study demonstrates IMC's utility in real-time tracking of bacterial fermentation, with the results emphasizing bamboo cellulose's capacity to boost *L. plantarum*'s lactic acid production, a finding with considerable implications for optimizing industrial fermentation processes. The observed extended metabolic activity and increased lactic acid yield due to cellulose addition point towards a promising strategy for leveraging biomass resources more sustainably and efficiently, particularly for high-value products like D-LA.

P-028

NUTRITIONAL ENHANCEMENT OF OKARA VIA SOLID-STATE FERMENTATION: SYNERGISTIC EFFECTS OF *CORDYCEPS MILITARIS* AND *LACTOBACILLUS PLANTARUM* ON DIETARY FIBER COMPOSITION AND FUNCTIONAL PROPERTIES

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This study investigates the enhancement of soluble dietary fiber (SDF) in okara, a by-product of soy milk and tofu production, using solid-state fermentation with Cordyceps militaris and *Lactobacillus plantarum*. Despite its high cellulose content, okara is often discarded, representing a significant loss of potential resources. Fermentation, employing enzymes produced during microbial growth, modifies the structure and function of dietary fibers, aiming to improve their physiological activities.

Our research compared the effects of single and mixed strains fermentation on the physicochemical and functional properties of SDF in okara. We found that fermentation with Cordyceps militaris for seven days optimized the conversion of insoluble dietary fiber to SDF, increasing from 9.78 to 15.09%. Fermentation with *Lactobacillus plantarum* also enhanced SDF content, rising from 9.6 to 11.67%. However, mixed fermentation was less effective, yielding only a

13.40% SDF. The fermentation process notably improved the hydration properties of dietary fibers, as evidenced by the significant increase in the water solubility index, from 0.18 to 0.30% with *Cordyceps militaris*, and to 0.25% with *Lactobacillus plantarum*. Furthermore, post-fermentation analysis showed increased total phenolic and cordycepin content in the products.

These findings suggest that solid-state fermentation with either *Cordyceps militaris* or *Lactobacillus plantarum* can substantially enhance the value of okara by improving its SDF content and physicochemical properties. This enhancement not only marks a significant step in utilizing food industry by-products but also indicates the potential of fermented okara as a functional food ingredient. It contributes to the development of sustainable, health-promoting food products and underscores the importance of innovative methods in the valorization of food waste.

This approach could aid environmental sustainability and open new avenues in the food and nutraceutical industries. Overall, both single and mixed fermentation methods improve the availability of dietary fiber in okara, allowing its use as a functional ingredient in various food products.

P-029

CHEESE WHEY TO ECO-PLASTICS: REVOLUTIONIZING SUSTAINABLE PHA PRODUCTION

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Polyhydroxyalkanoates (PHAs) are biopolyesters synthesized by microorganisms, offering potential as eco-friendly substitutes for conventional plastics due to their complete biodegradability and desirable mechanical properties. They can be synthesized by bacteria as energy reserves in carbon-rich yet nutrient-deficient environments, such as cheese whey, a major dairy byproduct. This study aimed to isolate microbial strains able to produce PHA from whey, addressing both environmental concerns and the need for sustainable waste management in the food industry.

Over 200 bacterial strains belonging to species known for PHA production were isolated and screened using PCR-based techniques to remove clones and assess the presence of *pha* synthetase, resulting in the selection of 56 *Bacillus* spp. and 41 *Pseudomonas* spp. strains. These strains were tested for lactose utilization on Lactose Nitrogen Poor Agar (NPA) to identify PHA producers. Sudan black staining method was used to assess the presence of PHA, using *Cupriavidus necator* as a positive control. Results showed that 25% of the strains could produce PHA from lactose as the sole carbon source, with 1 *Bacillus* spp. and 1 *Pseudomonas* spp. strains exhibiting high PHA production.

Selected strains were further evaluated in fermentations using cheese whey as the growth substrate, successfully consuming all lactose. Extraction methods minimizing chemical solvents were employed, yielding PHA with purity levels and dry weight yields of 22.46% and 21.06%, surpassing the positive control (*C. necator* 10.04%). These findings indicate promising industrial potential for PHA production, offering a sustainable solution to plastic pollution and waste management challenges.

P-030

EXPLORING THE POTENTIAL OF NATIVE BRAZILIAN FRUITS FOR THE PRODUCTION OF NOVEL LOW-ALCOHOL FERMENTED BEVERAGES

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Brazil has vast biodiversity, with several native fruits rich in bioactive compounds, such as phenolic compounds. Generally, these fruits are cultivated by small farmers and processed into pulp, which can be used to make drinks. Fermentation is widely used to produce fruit-derived alcoholic beverages. However, in recent years, there has been a decline in alcohol consumption in many countries and an increase in demand for drinks with a healthy appeal. In this context, low-alcohol drinks, such as kombucha, have gained popularity. Kombucha is a tea fermented with microorganisms that easily adapt to

different substrates. The microorganisms present are mainly acetic acid bacteria that oxidize ethanol into acetic acid.
Therefore, our proposal is to explore native Brazilian fruit pulps to produce new fermented drinks with low alcohol content using kombucha microorganisms. Thus, a kombucha with green tea (5 g/L) and sucrose (70 g/L) was fermented for 6 days at 25 °C, and 10% of this solution was used as an inoculum. Different aqueous extracts were prepared, using 10% of red araçá, butiá, or jaboticaba fruit pulp and 70 g/L of sucrose as a substrate for fermentation, which occurred at 25 °C. The dynamics of the fermentations were monitored at times of 0, 2, 5, 7, and 10 days. The physicochemical characteristics of pH, brix, total titratable acidity, and color were evaluated. Furthermore, substrate consumption (sucrose, glucose, and fructose), metabolites produced during fermentation (acetic acid and ethanol), and total phenolic content were measured.
Preliminary results point to an adequate adaptation of the inoculum to the substrates, with biofilm formation typical of kombucha microorganisms, sucrose hydrolysis, with low ethanol production, and moderate acetic acid production, with different values for each fruit. Furthermore, the total phenolic content increased after fermentation of the extracts. Finally, the pH and acidity values found indicate a way to produce safe drinks from a microbiological point of view and with sensory quality.
As perspectives, we hope that the evolution of this work will contribute to increasing the demand for native Brazilian fruits, promoting biodiverse production systems, and generating knowledge and technology.

P-031

DELVING INTO THE POTENTIAL OF *LACHANCEA THERMOTOLERANS* IN GREEN TABLE OLIVE FERMENTATION

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Table olives are one of the most popular commercial fermented vegetable products. Although its consumption and its production are widespread all around the world, it is in the Mediterranean coast (specifically in countries like Spain, Italy or Greece) where its production and cultivation are concentrated. Thus, this industry is searching in the last years for new solutions for their common problems. Among them, the low initial survival of the commercial inoculums which are currently available. Because of that, current investigations are looking for the potential use of new microorganisms with a high biotechnological interest which can reduce or eliminate these fermentation problems.
In this work, the possible impact of individual inoculation of different strains of *Lachancea thermotolerans* (*Lt*) and subsequently the inoculation of *Lactiplantibacillus plantarum* (*Lp*) was studied. During fermentation, the evolution of physical-chemical and microbiological parameters was studied in all fermenters. Furthermore, a metagenomic analysis was carried out to assess the impact of the use of these strains in the diversity of this fermentation process. Finally, a sensory analysis of each packaged final product was carried out.
In all the fermenters where the consortium between *Lt* and *Lp* was inoculated, an effective imposition of the yeast was demonstrated. In addition, a rapid acidification was also detected in those fermenters, which resulted in a rapid proliferation of lactic acid bacteria. In addition, most of the table olives obtained with *Lt* showed the best ratings in the sensory analysis.
Thus, the different *Lt* strains studied show a good biotechnological potential with a high level of implementation. Before considering these yeast strains for its possible use on industrial scale, future tests should be carried out to determine their performance in larger fermenters.

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P-032

METABARCODING ANALYSIS REVEALS A DIFFERENTIAL BACTERIAL COMMUNITY PROFILE ASSOCIATED WITH 'TORTA DEL CASAR' AND 'QUESO DE LA SERENA' PROTECTED DESIGNATION OF ORIGIN CHEESES

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A detailed understanding of the microbial ecology of artisanal cheeses is a crucial factor for implementation practices that can help to update their quality.

In this study, the bacterial communities of Protected Designation of Origin (PDO) 'Queso de la Serena' and 'Torta del Casar' cheeses were characterised by 16S rRNA gene met-abarcoding sequencing over 60 days of ripening at three dairies for each cheese variety. Both PDO cheeses presented a distinctive and unique bacterial community, suggesting that the manufacturing practices of each PDO shaped the microbiota. *Lactococcus lactis* and, to a lesser extent, *Leuconostoc mesenteroides* were the key lactic acid bacteria (LAB) species that drove the ripening process of PDO 'Torta del Casar', whereas in PDO 'Queso de la Serena', *Leu. mesenteroides* was the main LAB species, followed by *Lactococcus raffinolactis* and *Lc. lactis*, depending on the dairy. Furthermore, a wide range of non-starter LAB, Gram-negative bacteria and Gram-positive catalase-positive cocci were found, with a variable presence and abundance between dairies. In this regard, the dairy was a strong contributing factor to the variability in the composition of the bacterial community in each cheese type. In contrast, the impact of the ripening process was limited.

Overall, this study enhances the understanding of the bacterial dynamics that occur during the ripening of these traditional PDO cheeses, highlighting the need to improve the standardisation of the raw materials and manufacturing practices to minimise inter-dairy variability, ensuring final quality and their unique sensory traits.

P-033

SOURCE OF ISOLATES AND PROBIOTIC ACTIVITY OF YEASTS FOR SELECTION AS POTENTIAL CANDIDATE STRAINS

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Probiotics are vital and beneficial organisms which offer health benefits to host organisms. The properties of probiotics must exhibit *in vitro* characteristics to survive during the passage through the human gastrointestinal tract, tolerance to low pH and bile salts and efficiently adhere to intestinal epithelial cells with special health promotion feature such as antioxidant. Many studies have investigated on the bacterial probiotics but not on the yeast. Yeast holds several advantages in comparison to bacteria as they are normally resistant to antibiotics, which are necessary for their resistance during antibiotic treatment, and no transfer of genes related to antibiotic resistance. Sources of yeasts found in natural habitats and also present in many traditional fermented foods and beverages but impact of isolation source on strains having key probiotic properties has still been rarely reported.

The study aimed to evaluate feasible sources for isolation of yeast having probiotic properties for further selection as probiotic candidate strains with antioxidant activity. Yeast was isolated from rice wine, palm wine, soy source and shrimp paste during early to final stages of natural fermentations and from fresh toddy palm sap and lychee peel. Yeast colonies from each source were randomly selected by Harrison's disc methods and 10% representative were further selected based on colony morphology. Based on number of isolates, lychee peel and fermented shrimp harbor the most isolated numbers with various morphologies, respectively.

The number of yeast isolates with tolerance to gastrointestinal fluids were mainly isolated from fermented shrimp and lychee peel. However, when subjecting isolates to hydrophobicity

test, mainly yeasts from lychee peel were still had these properties while yeasts obtained from the other sources had significantly lower. In addition, yeast isolates from lychee showed higher antioxidant activity compared to the other sources.
This demonstrated that yeast in natural habitats with high stress such as low nutrients, low Aw, exposing to sunlight and UV like on fruit peels could be a key factor associated to yeast adaptation leading to a tolerance strain with potential probiotic activity. Thus, this could be principal information for further searching and development of new yeast probiotics.

P-034

LACTOBACILLI, BEST ALLIES OF MENTAL HEALTH: THE POWER OF COMPARATIVE GENOMICS

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Modulation of gut-brain axis is an evolving concept to improve human health. Some strains of lactic acid bacteria (LAB) classified as psychobiotics can produce neuroactive molecules, capable of modulating mood and cognition in humans. To simplify psychobiotic strains screening we used comparative and functional genomic analysis on LAB genomes to identify the most widespread species in fermented foods with prevalence of genes possibly related to psychobiotic activities.
We selected and downloaded from the NCBI public database 16 genes related to neuroactive molecules and genomes of 25 LAB species. For each of these genomes, the genes were predicted using the PROKKA software. We carried out the mapping against functional genes using DIAMOND software. Finally, we statistically processed the results using Rstudio software.
This approach has revealed the presence of species-specific patterns. In particular, the *Levilactobacillus brevis* species appears to be the one with the greatest number of genes related to the production of neurotransmitters. Furthermore, we found a low presence of the serotonin production pathway among the main fermenting species.
Our results demonstrate the importance of computational biology and how comparative genomics becomes valuable to support in vitro/vivo tests to launch new products with benefits on mental health.

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P-035

TRADITIONAL FERMENTED FOODS AS A SOURCE OF BENEFICIAL MICROBES: A META-ANALYSIS

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Fermentation is an optimal strategy to extend the shelf-life of perishable products. Furthermore, it is a sustainable process that increases the concentration of vitamins, essential amino acids and minerals, improving the nutritional value. Also, Fermented Foods (FF) can be a vehicle of beneficial microorganisms. The purpose of this meta-analysis is to provide a worldwide map of the traditional dairy- and non-dairy FFs and of their associated microbiota, also focusing on potential beneficial outcomes for human health.
Studies describing the microbiota of traditional FFs through metataxonomic approaches were selected. Metagenomic reads from each study were downloaded from the NCBI SRA database and analysed through DADA2.
Our results indicate that traditional FFs are a valuable source of beneficial microorganisms, mostly belonging to the Lactic

Acid Bacteria group, with probiotic activities. In addition, we highlighted that most of the currently approved probiotic strains have been firstly isolated from traditional FFs, thus emphasizing the nutritional and beneficial value of these foods for human health. Furthermore, literature suggests that probiotic strains from fermented foods might interact with the human microbiota, not only by establishing themselves, but also by competing for nutrients with potentially hazardous and pathogenic species.

This meta-analysis highlights the wide distribution of traditional FFs worldwide and that they are a valuable source of probiotic microorganisms, which might not only establish in the human gut and exert beneficial activities, but also compete for nutrients with undesirable species.

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P-036

ISOLATION AND CHARACTERIZATION OF POTENTIAL NEXT GENERATION PROBIOTICS FROM THE HUMAN GUT

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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 and Collinsella aerofaciens. We also have characterized oxygen resistance of the anaerobic strains and the presence of genes related to the production of beneficial metabolites.

P-037

IMPACT OF PULSE PROTEINS ON GUT MICROBIOME AND HUMAN HEALTH: A META-ANALYSIS

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Plant-based diets (PBDs) are characterized by high consumption of fruit, vegetables, pulses and whole grains, and limited meat intake. They are associated with improved health outcomes, lower mortality rates, and reduced environmental impact. In contrast to the animal protein-rich Western diets, pulses serve as the primary protein source in PBDs and are correlated with beneficial health effects.

Recent studies have shown that dietary protein source and intake significantly affect gut microbiome's composition and function. However, the physiological mechanisms underlying the health benefits of pulses, particularly their interactions with the gut microbiome, are not fully understood.

Therefore, a comprehensive literature screening was conducted, focusing on pulses, plant-based diets and gut microbiome, to evaluate the influence of pulses and their proteins on gut microbiome. The selection criteria included randomized controlled trials (RCTs) on humans with available raw 16S data (FASTQ or FASTA) and diet metadata. Raw data were downloaded and processed through QIIME 2 pipeline.

Consumption of pulses positively affects the gut microbiota, enhancing fiber-degrading and anti-inflammatory bacteria. A higher intake of pulses was associated with elevated levels of *Faecalibacterium* and *Eubacterium*, bacterial genera known for producing beneficial metabolites for gut health and exhibiting anti-inflammatory properties. Additionally, a greater consumption of pulse proteins positively correlated with an increase in health-promoting bacterial genera.

Pulses may enhance gut microbiome composition and promote human health, although results vary depending on individual dietary habits and pulse types. Further research, including human clinical trials, is needed to explore the mechanisms by which the consumption of pulses and their proteins affects the gut microbiota, host metabolism, and overall health outcomes.

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P-038

EXPLORING PROBIOTICS POTENTIAL: REBALANCING GUT HEALTH IN NON-CELIAC GLUTEN SENSITIVITY

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Non-celiac gluten/wheat sensitivity (NCGS/WS) is a clinical entity characterized by intestinal and extraintestinal symptoms induced by gluten ingestion in the absence of wheat allergy or celiac disease. Impaired intestinal barrier and permeability and gut dysbiosis have been advocated as key factors by several studies. Indeed, the causal association between dysbiosis and NCGS/WS is still unclear due to the controversial results.

Fifty individuals with suspected NCGS/WS were subjected to the double blind-placebo-controlled crossover gluten challenge test. Twenty-seven participants were gluten responsive (NCGS) and randomized into two arms: Experimental arm: 6 weeks with daily intake of probiotics (*L. plantarum*, *L. paracasei* and *L. salivarius*) or Control arm: 6 weeks with daily intake of placebo. All participants were undergoing a gluten-free diet for 4 weeks (sampling point T1), after which gluten was reintroduced into their diets for 2 weeks (sampling point T2). Shotgun metagenomics and GCxGC-ToFMS were used for gut microbiome and volatilome analysis. Food frequency and general health-related quality of life questionnaires were also analyzed.

Taxonomic profile of the gut microbiome showed probiotic colonization, a reduction in *Bacteroides* which impairs immune function and an increased in beneficial *Bifidobacterium* and *Paraprevotella*. At pathway level, in experimental arm, we observed an increased in Carotenoid and glutathione biosynthesis genes with cytoprotective activity may further contribute to mucosal and gut barrier health, such as stabilizing tight junctions. Volatilome showed a difference between arms for the following class: linear and branched alkanes, ketones and aldehydes short chain fatty acids due to presence of the probiotics. In conclusion the

loss of gluten tolerance may have been regained through the colonization of the intestine by the selected probiotics.

P-039

PROBIOTICS ADMINISTRATION TO TREAT DIFFERENT SKIN CONDITIONS: A META-ANALYSIS

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Skin is the largest organ and the outermost layer of our body. It has two main functions: a physical barrier to prevent the invasion from pathogens or other toxic substances and an immunological barrier reducing the effects of injuries and infections. The human skin is also an ecosystem because it is the house of millions of microorganisms (bacteria, fungi, viruses). Bacteria are the most abundant microbe on the skin, they are important to control the balance of the microbiota and the transient microbial colonization and to assist the host's innate immunity. There is a delicate balance between the host and the microbiota indeed a disruption of this equilibrium can cause different types of diseases, disorders, or infections. In addition, aging skin, wrinkles, pigmentation, and dryness are problems that plague skin people. Recently, a lot of studies demonstrated that oral and topical use of probiotics can have great potential in preventing and treating skin diseases including eczema, atopic dermatitis, acne, allergic inflammation, or skin hypersensitivity. These probiotics can be also used as a cosmetic product to improve some skin conditions.

The purpose of this study is to highlight the application of different types of living bacteria to treat skin disorders and skin conditions. Thirty-eight articles concerning the use of probiotics and nine articles about the use of postbiotics for the treatment of different skin conditions were analyzed. The taxa mainly used are *Bacillus* (n = 6), *Lactobacillus* (n = 20), and *Bifidobacterium* (n = 7) strains for the treatment, for example, of atopic dermatitis (n = 8), eczema (n = 5), acne vulgaris (n = 8), skin aging (n = 7), and other skin conditions. The results obtained are promising.

P-040

A MICROBIOME-BASED PRECISION NUTRITION APPROACH TO MAXIMIZE ANTICARCINOGENIC CONJUGATED LINOLEIC ACID (CLA) PRODUCTION BY HUMAN GUT MICROBIOTA

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Colorectal cancer (CRC) is the second deadliest cancer for both sexes globally and also the third most commonly diagnosed cancer despite advances in prevention, diagnosis, and treatment. Scientific evidence demonstrates gut microbiota's fundamental role in CRC initiation, development, and metastasis. In this sense, conjugated linoleic acid (CLA) is a metabolite produced by the intestinal microbiota with an anticancer effect in CRC mediated by proliferation and apoptosis pathways and inflammation.

Scientific evidence indicates that CLA could be a potential therapeutic against CRC. Although different nutritional and technological strategies have been developed to increase the amount and bioavailability of CLA in humans, up to date, only those based on intestinal microbiota bacteria are promising.

The present research aims to propose a microbiome-based precision nutrition approach that favours the future practical application of individualizing dietary intake to maximize CLA production by the human intestinal microbiota.

P-041

BIOACCESSIBILITY OF ISOTHOCYANATES FROM MUSTARD (*SINAPIS ALBA*) AND THEIR INTERACTIONS WITH COLONIC MICROBIOTA

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White mustard (*Sinapis alba*) is a vegetable belonging to the *Cruciferae* family. It contains a group of phytochemicals so-called glucosinolates (mainly sinalbin and glutropaeolin) that when hydrolyzed in the digestive tract, produce, among other compounds, isothiocyanates (ITCs) (4 – hydroxybenzyl – ITC and benzyl – ITC respectively). ITCs are produced via the catalytic activity of plant myrosinase (present in cruciferous) and β-thioglucosidases in the gut microbiota. ITCs are of great interest for human health due to their anti-inflammatory and anti-cancer properties, and antimicrobial capacity.

The objective of this research was to study the bioaccessibility of ITCs from white mustard and its effect on the intestinal microbiota. For this purpose, a bioaccessibility protocol (INFOGEST method) was used. This procedure replicates the physiological conditions occurring in the human digestive tract, including enzymes, pH, temperature and the duration of white mustard in the mouth (oral phase), stomach (gastric phase), and small and large intestine. For the *in vitro* colonic fermentation, faeces of volunteers of the same age group were homogenised to obtain a faecal inoculum in phosphate buffer. The non-solubilised residue coming from the small intestine was incubated with this faecal inoculum and 10 mL of fermentation culture medium at 37 °C for 20 h under anaerobic conditions. Finally, digested samples were centrifuged at 4000 rpm for 15 min.

The concentration of 4 – hydroxybenzyl – ITC and benzyl – ITC present in the supernatant (bioaccessible fraction) and the final residue was analysed by GC – MS. The effect of colonic fermentation on ITCs was quantified, observing changes in its content, although no trend was observed for the different initial ITC levels tested. Interestingly, ITCs impacted the microbiota composition, although results exhibited a high variability across experiments. Additional experiments were conducted to determine the antimicrobial activity of ITCs on foodborne pathogens (*Listeria monocytogenes*, *Escherichia coli*, *Salmonella* spp., etc.), showing positive results in both Gram + and – bacteria; however, it varied depending on the type of ITC and bacteria strain.

Results demonstrated the bioactive and prebiotic properties of ITCs derived from mustard, highlighting its potential as a natural source of these beneficial compounds.

P-042

UNRAVELING THE NEUROACTIVE POTENTIAL OF *GRANA PADANO* AND *PARMIGIANO REGGIANO* CHEESE MICROBIOME

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Grana Padano (GP) and Parmigiano Reggiano (PR) are among the finest Italian traditional Protected Designation of Origin (PDO) ripened cheeses. GP and PR undergo to a strong proteolysis during the long ripening process (12 months or more). During ripening, the microbiome metabolizes amino acids, producing compounds responsible for flavor, as well bioactive molecules.

This study explores GP and PR microbiome, to identify key genes involved in the production of psychobiotics, such as gamma-aminobutyric acid (GABA) and other bioactive compounds. We sampled PDO GP (n = 42) and PR (n = 63) and assessed the microbiome by high-throughput shotgun metagenomic sequencing.

Our findings revealed a rich diversity of microbial communities in both GP and PR cheeses, with notable representation of bacterial species known for their proteolytic activity. Importantly, we identified the genes involved in the biosynthesis of neuroactive molecules, including GABA, suggesting a putative link between the consumption of these cheeses and mental health.

This study contributes novel insights into the microbial dynamics and functional attributes of GP and PR cheeses,

shedding light on their potential as sources of bioactive compounds with implications for human health. The identification of a psychobiotics potential in traditional ripened cheeses opens avenues for further research into the potential neurological benefits associated with their consumption.

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P-043

CULTIVATING KNOWLEDGE: INVESTIGATING THE EFFECTS OF FERMENTED FOOD CONSUMPTION THROUGH UNEXPLORED MICROORGANISMS

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Coffee is habitually consumed by billions of people worldwide and has well-established metabolic and health-related benefits. However, it remains unclear how it interacts with the human microbiome. In a multi-omic, multi-cohort study, we recently unveiled that the coffee-microbiome link is strongly reproducible across cohorts and driven primarily by the abundance of the Lawsonibacter asaccharolyticus species, suggested to intervene in the chlorogenic acid pathway according to metabolomic data. Here, we aim at better characterizing the metabolic importance of *L. asaccharolyticus* by studying and validating its association with coffee consumption *in vitro*.

To this end, single cultures of the only *L. asaccharolyticus* strain available in culture collection were supplemented with increasing concentrations of different types of coffee, namely brewed and instant coffee, to assess their effect on bacterial growth. The same conditions were applied to *Escherichia coli* and *Bacteroides fragilis*, chosen as representatives of facultative aerobe and anaerobe commensals of the human gut microbiome that do not result in any statistical association with coffee. In addition, possible influence of caffeine was assessed by comparing the previous conditions with decaffeinated coffee supplementation.

As a result, supplementation of low concentrations of coffee increased the growth of *L. asaccharolyticus* by up to twofold compared to the control. No clear-cut differences were observed between powdered, brewed, and instant coffee, and decaffeinated coffee yielded results similar to normal coffee. Conversely, coffee inhibited growth of *E. coli* and *B. fragilis* in a dose-dependent manner.

Altogether, in contrast with antimicrobial properties of coffee observed in other species, our findings corroborate the hypothesis that coffee enhances the growth of *L. asaccharolyticus* and causally explains the metagenomic and metabolomic correlations observed in human cohorts. These results pave the way for further experiments to study the molecular mechanisms through which *L. asaccharolyticus* exploits coffee for its growth.

FOOD MICROBIOTA AND IMPACT ON HUMAN MICROBIOME

P-044

PREVALENCE AND PERSISTENCE OF *LISTERIA MONOCYTOGENES* IN A READY-TO-EAT FOOD PROCESSING INDUSTRY

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Listeria monocytogenes is an important zoonotic foodborne pathogen responsible for listeriosis, a severe disease in high-risk groups with a 20-30% fatality rate. In 2021, it ranked fifth among reported zoonoses in the European Union (EU), with 2.183 cases and 23 outbreaks.

Ready-to-eat (RTE) meats ranked second in prevalence of this pathogen in the EU in 2021 (average 2,3%). This is particularly relevant in Spain, where the meat sector has the highest turnover of any food and beverage industry.

This study aimed to evaluate the presence, diversity and persistence of *L. monocytogenes* in an industry dedicated to produce RTE foods with meat ingredients.

Over 16 months, 462 samples from two RTE food production lines (burritos and migas) were analysed, including raw materials, final products, pre-operational and operational contact surfaces (CS) and non-contact surfaces (NCS) common to both lines. *L. monocytogenes* detection involved impedanciometry and isolation on two chromogenic agars, and subsequent characterisation of the isolates was performed by multiplex PCR (serotyping) and PFGE (pulsetype).

The pathogen was isolated in the operational beef mincer of the burritos line (17%) and common pre-operational stainless-steel trays (1,4%). Regarding NCS, *L. monocytogenes* was detected in the blast chiller (17%) and drains (33%). The pathogen was not detected in final products.

Two serotypes were identified, 1/2a (92%) and 1/2c (8%), with 1/2a being the most prevalent in the meat industry and the second most associated with listeriosis cases.

Furthermore, five strains were identified, implying low genetic diversity in the industry, with three predominant strains: strain S1, detected on surfaces at risk of direct contamination as well as repeatedly in drains; and strains S3 and S4, isolated only from raw materials.

Results suggest that meat raw materials are an important route of entry of *L. monocytogenes* into the processing environment. Moreover, the persistence in the drains highlights the importance of regular analysis of the drains to know the contamination status in the industry.

In addition, the predominance of three strains and the low genetic diversity in the industry underline the adaptability of some strains to the processing environment and the need for specific control strategies to ensure food safety.

P-045

WHOLE-GENOME COMPARATIVE ANALYSIS OF THE GENETIC DIVERSITY OF *CAMPYLOBACTER* SPP. FROM SPAIN

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Whole-Genome Sequencing has proven to be essential for surveillance and control of foodborne diseases like campylobacteriosis, one of the most widespread infectious diseases worldwide.

In this study, 145 *Campylobacter* spp. randomly selected from our collection of isolates were genomically characterized to analyze the genetic diversity of the pathogen in Spain. The strain dataset comprised 135 *Campylobacter jejuni*, 7 *Campylobacter coli* and 3 *Campylobacter lari* isolated from different stages of the poultry supply chain (farm, slaughterhouse, poultry processing plant and retail) in northern and central Spain, and from human clinical samples of the region of Burgos.

Isolates were sequenced and genomes were assembled using the in-house pipeline CamPype (https://github.com/

JoseBarbero/CamPype). Multilocus sequence typing (MLST) was performed on draft genomes using mlst v2.17.6 and assigned to Clonal Complexes (CCs). Phylogenetic comparison of the *Campylobacter* genomes was performed using a neighbour-joining (NJ) tree based on the core genome MLST determined by chewBBACA v2.8.5 and cgmlst-dists v0.4.0 that was subsequently constructed with MEGA X v10.2.5.

The phylogeny showed a wide genetic diversity between locations, with a total of 126 isolates (86.9%) grouped into 14 defined CCs and 30 known Sequence Types (STs). Larger diversity was found in hospital (CC21/48/52/206/257/353/354/443/464/607/677/828), followed by retail (CC21/48/353/354/443/464/658/677/828), farm (CC21/206/353/354/464), slaughterhouse (CC21/443/460/607) and poultry processing plant (CC21/52/460/464), and most lineages (CC21/48/52/206/353/354/443/460/464/607/677/828) were detected in at least two of these stages, except CC658 that was represented by 2 clinical isolates. Such diversity of lineages was predominant in *C. jejuni*, whose most frequent CC was CC21 (69 isolates, 51.1%) that was the only one found in all sampling sites, followed by CC607, which was significantly linked to slaughterhouse ($p < 0.05$), and CC464 with 10 isolates each (7.4%). All *C. coli* isolates found in retail and hospital were grouped in CC828, while the *C. lari* isolates from retail were represented by ST-21 (*C. lari* MLST scheme). To the best of our knowledge, the present study is the largest genomic survey of *Campylobacter* species from Spain and paves the way for future studies focused on the control of campylobacteriosis cases in this country.

P-046

PRODIGIOSIN PRODUCTION IN *SERRATIA MARCESCENS* ISOLATED FROM COOKED CHICKEN MEAT IN ITALY

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Introduction

Serratia marcescens, a ubiquitous opportunistic pathogen, is responsible of nosocomial, urinary tract, and bloodstream infections in immunocompromised individuals. In food, it can be found in a wide range of products, such as raw and processed meats, dairy, and fresh products, causing food spoilage, discoloration, and loss of flavour, but also foodborne illness. *S. marcescens* ability to adapt and survive in different environments can be related to the presence of numerous genetic factors. *S. marcescens* is also known for its characteristic red pigment, prodigiosin, associated with its virulence and which plays a fundamental role in its ability to cause disease, cytotoxicity, and antibiotic resistance.

The aim of this study was to investigate the presence of virulence factors, including the prodigiosin synthesis gene cluster, and antibiotic resistance genes in a *S. marcescens* strain isolated from cooked chicken meat with a red colour.

Methods

During 2023, a cooked poultry meat sample with chromatic alteration was sent for microbiological investigation that led to the isolation of *S. marcescens* with the selective medium Cetrimide Agar. Then, the strain genome was analyzed with WGS performed on the Illumina MiSeq to identify *in silico* virulence and antibiotic resistance genes.

Results

The virulence results showed the presence of genes involved in quorum sensing, in particular, the *luxR* gene has an essential role encoding LuxR, a regulatory protein implicated in biofilm formation, synthesis of virulence factors, and

antibiotic resistance. *pga* and *wzc* genes, involved in biofilm formation, help the biofilm stability maintenance and the surface polysaccharide production respectively. Then, the analysis allowed the identification of the prodigiosin synthesis gene cluster consisting of 14 genes, from *pigA* to *pigN*. In conclusion, the strain was resistant to different classes of antibiotics including β-lactams, tetracyclines, macrolides, aminoglycosides, and fluoroquinolones.

Significance

The present work is one of the first report of *S. marcescens* associated with a red colour defect in a cooked chicken meat. In addition, *S. marcescens* biofilm formation ability can lead to a higher risk of foodborne disease and food contamination.

P-047

PATHOGEN ANALYSIS IN VALENCIAN MUSSELS: SHELLFISH SAFETY THROUGH COMPREHENSIVE EXAMINATION

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Introduction

Water is a fundamental resource for human life but may contain a variety of contaminants, including bacteria, viruses, protozoa, and emerging pollutants, such as microplastics (MPs), antibiotic resistant genes (ARGs) and pharmaceuticals. Depending on the performance of wastewater treatment plants (WWTPs) high amounts of pathogens can be released into rivers, lakes and oceans, and can be introduced into the food chain through these water bodies. Mussels are animals that feed through filtration, and as a result, they bioaccumulate contaminants and pathogens. Therefore, shellfish aquaculture is highly impacted by water quality. In this study the presence of human enteric viruses and viral faecal indicators as crAssphage and somatic coliphages were analysed in mussels and surrounding seawater.

Methods

Mussels batches (n=10) were sampled before and after the depuration treatment between March and August 2022. Mussel seawater was also collected in each sampling. Mussels were analysed according to ISO 15216-1:2017 for enteric viruses detection (Human Norovirus GI and GII (HuNoVGI and HuNovGII), Hepatitis A and E Viruses (HAV and HEV), Astrovirus, and Rotavirus). Somatic coliphages were quantified by culture method using a commercial kit (Bluephage, Spain). Seawater (5 L) was concentrated by dead-end hollow fiber ultrafiltration (DEUF). Nucleic acid extraction was performed by using the Maxwell® RSC Instrument (Promega, Spain) with the Maxwell RSC Pure Food GMO and authentication kit (Promega). Viral detection and quantification was performed by RT-qPCR and qPCR.

Results

Enteric virus levels in mussels and seawater were higher for HuNoVGI, with mean values of 4.81 ± 0.39 and 4.52 ± 0.55 Log_{10} genomic copies (GC)/g in non-depurated and depurated, respectively, and 5.19 Log_{10} GC/L in seawater, being found in most of the mussels and seawater samples. The rest of the analysed viruses were found only in few samples, however were detected in some depurated mussels. Additionally, viral indicators were found in half of the seawater and mussel (depurated and no-depurated) samples.

Conclusion

Mussels are products that are typically consumed lightly cooked; thus, the presence of enteric viruses in mussels, even after depuration processes, poses a risk to human health.

P-048

EFFECT OF SPORULATION TEMPERATURE AND HEAT ACTIVATION ON GERMINATION OF *BACILLUS WEIHENSTEPHANENSIS* SPORES

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Spores of *B. weihenstephanensis* are important food spoilage agents of thermal-processed refrigerated foods, and some strains may cause foodborne illnesses. In these products, bacterial spores are controlled by a combination of different hurdles that inhibit or delay germination and/or growth, and therefore accurate prediction of germination kinetics is essential to ensure food safety and stability. Sporulation temperature exerts a considerable influence on germination, but data reported on the magnitude and direction of its effect are inconsistent.

In this study, we compared germination kinetics of spores of three *B. weihenstephanensis* strains (WSBC 10204, WSBC 10202 and SC) produced at optimal temperature (T_{opt}) and minimum (T_{min}) and maximum (T_{max}) boundary temperatures for sporulation in response to different nutrients (inosine, L-alanine, L-glycine, L-glutamine and their combinations) with and without previous heat activation.

There was a high variability in germination kinetics among non-activated spores produced at T_{opt} from different strains and nutrient germinant. The combination of inosine and L-alanine was the most efficient stimuli in the three strains. In general, non-activated spores of WSBC 10202 and SC obtained at T_{max} showed higher germination rate and/or efficiency than those produced at T_{opt} especially in inosine and its combinations, while populations produced at T_{min} were the least fit spores in most nutrients. However, sporulation temperature changes did not affect germination of WSBC 10204 spores. Heat activation of T_{opt} spores increased germination rate and/or efficiency only in the strains WSBC 10204 and WSBC 10202, especially when induced with inosine combined with L-glycine or L-glutamine. In the case of WSBC 10202, heat activation had a greater effect in spores produced at T_{opt} than those from T_{max}, so that germination kinetics of both populations were virtually equal.

Our results demonstrate that the effect of sporulation temperature on germination of *B. weihenstephanensis* spores depends on prior heat activation, the strain and the nutrient germinant.

P-049

PREVALENCE AND CHARACTERISTICS OF FOODBORNE PATHOGENS IN BULK TANK RAW MILK IN 2019-2023

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Pathogens like *Salmonella*, *Listeria monocytogenes*, verotoxigenic *Escherichia* 0157, *Campylobacter* spp., *Salmonella* spp. and *Staphylococcus aureus* are often part of the animal intestinal microbiota, they can be isolated from the udder of healthy dairy animals or from operators' hands, and thus, they can easily contaminate the milk during the milking process.

The aim of the study was to assess the occurrence and hazard characteristics associated with the presence of *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter* spp. and verotoxigenic *Escherichia coli* in raw milk.

The project established cooperation with the Veterinary Inspection for the collection and transport of samples for testing and rules for the sampling of raw milk. Eight hundred and fifty samples of bulk tank cow's, goat's and sheep's raw milk from dairy farms located in 17 voivodeships were analyzed during 2019-2023. The study was carried out on the basis of the screening immunoenzymatic method with the use of mini Vidas analyser, and positive samples were confirmed with reference methods. Pheno- and genotypic characterization of the isolates was performed.

The presence of *L. monocytogenes*, *Campylobacter* spp, *Salmonella* spp. and *Escherichia coli* 0157 in raw milk from dairy farms was found. The percentage of the positive samples was 7,18% *for Listeria monocytogenes*, 2,59% for *Campylobacter* spp., 0,47% for *E. coli* 0157 and 0,12% for *Salmonella* spp. Raw milk samples contaminated with *Listeria monocytogenes*, *Campylobacter* spp., *Salmonella*

spp. and *Escherichia coli* O157 came from 14, 10, 2 and 1 voivodeships, respectively. All three pathogens *Listeria monocytogenes*, *Campylobacter* spp. and *Escherichia coli* O157 were present in milk in two voivodeships and all four pathogens in one voivodeship. *L. monocytogenes* and *Campylobacter* spp. were present in both cow, goat and sheep milk. *E. coli* O157 was detected in cow and goat milk. *Salmonella* spp. was found only in one goat milk sample.The results indicate the presence of pathogenic bacteria, such as *L. monocytogenes*, *Campylobacter spp*, *Salmonella* spp. and *Escherichia coli* O157 in raw milk in Poland.

P-050

PRELIMINARY STUDY ON THE EFFECTIVENESS OF DETERGENT AND SANITISER PRODUCTS ON *ARCOBACTER BUTZLERI* STRAINS

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Arcobacter butzleri is a zoonotic pathogen mainly isolated from poultry industry. Despite the use of disinfection procedures, numerous studies have documented the prolonged presence of *A. butzleri* in food processing environments.
This study investigates the susceptibility of *A. butzleri* isolated from poultry carcasses and slaughter equipment to the most used commercial disinfectants and detergents in food processing plants for surface disinfection and organic matter removal. Thirty-one strains of *A. butzleri* isolated from a chicken slaughterhouse together with the reference strains *A. butzleri* LMG10828T, *Escherichia coli* ATCC35150 and *Staphylococcus aureus* ATCC25923 were evaluated for the susceptibility test. The detergents used are neutral, slightly alkaline, and alkaline. Disinfectants used include peracetic acid, peracetic acid combined with hydrogen peroxide, quaternary ammonium salts, active chlorine, and finally amine-based disinfectants. The analysis was carried out using the agar disk diffusion method, the measurement was made in millimeters from the inhibition front of the commercial products to the edge of the disk.
Considering the *A. butzleri* strains, 3% peracetic acid and 0.5% peracetic acid with hydrogen peroxide showed the greatest inhibition (14.7 and 12.6 mm respectively). In comparison, *A. butzleri* strains with 0.6% quaternary ammonium compound, 1.25% amine and lastly 9.5% active chlorine disinfectant produced an inhibition zone of approximately 3 mm. Regarding alkaline and neutral detergents, no susceptibility was observed in most cases, except *S. aureus* for which 5.5% neutral detergent produced an inhibition zone (6.3 mm).
Statistically significant findings have been identified about the disinfectants employed, without the emergence of generally more or less resistant strains from the totality tested. This study highlighted the variable efficacy of different formulations against *A. butzleri*. Based on these initial results, it appears that the microorganism is more susceptible to peracetic acid than to other disinfectants. To better understand the mechanisms of resistance, future RNA sequencing analyses will be performed to understand the genes and pathways involved. Given the persistence of *A. butzleri*, it is necessary to establish appropriate disinfecting procedures to reduce the presence of the pathogen in food-processing plants.

P-051

UNDERSTANDING CROSS-CONTAMINATION DYNAMICS OF *LISTERIA MONOCYTOGENES* IN POLYMICROBIAL BIOFILMS ON READY-TO-EAT FOODS

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Listeriosis is linked to the consumption of foods contaminated with *Listeria monocytogenes* cells, often resulting from cross-contamination during the food processing stages. *L. monocytogenes* presence has been consistently reported in food processing environments (FPE), where it persists

by forming polymicrobial biofilms. Despite this, quantitative microbiological risk assessment (QMRA) analyses typically overlook biofilm cells. This omission may lead to an incomplete understanding of the actual risk posed by *L. monocytogenes* in FPE.

This study aims to quantify and compare cross-contamination events of *L. monocytogenes* cells originating from both single-species and polymicrobial biofilms, using strains and genres recovered from FPE. Contamination points of *L. monocytogenes* and the accompanying microbiota, previously characterized, were selected for the study. Three polymicrobial biofilms, foci F96, F107, and F168 and L96 single-specie *L. monocytogenes* biofilm were grown on stainless steel coupons under starvation conditions in diluted tryptone soy broth. After 72 hours of static culture, the attached biofilm cells were transferred by contact to smoked salmon slices. Each coupon underwent twenty-five consecutive contacts, with a total of twenty replicas each contact. The transfer rate was determined from the CFU/g transferred, and each *foci* data was fitted to a Weibull distribution.

Results demonstrated that all three foci showed a similar model dynamic, regardless of the associated microbiota differences. With respect to single-species *versus* polymicrobial effect, interestingly, the transfer rate of F96 *foci* was significantly higher than the corresponding L96 single-species biofilm. Modeling *L. monocytogenes* transfer enables the identification of critical points in biofilm cross-contamination, emphasizing biofilms as a crucial phenomenon for *L. monocytogenes* survival within the food chain. Additionally, differences in transfer rates between single-species and foci transfer events underscore the importance of research studies focusing on the dynamics of polymicrobial biofilms.

This study offers an accurate and comprehensive insight into cross-contamination events, contributing to the reinforcement of QMRA modeling. It provides a realistic assessment of the microbiological risks associated with *L. monocytogenes* in FPE. The outcomes of this research hold broader applicability beyond *L. monocytogenes*, extending to other foodborne pathogens.

P-052

DECONTAMINATION OF *LISTERIA MONOCYTOGENES* IN ATLANTIC SALMON PRODUCTION TARGETING PROCESS WATER AND SALMON PRODUCTS

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Listeria monocytogenes (*Lm*) is a major food safety challenge in which the Atlantic salmon industry invest a substantial effort to control. *Lm* are shown to survive and multiply in processing environments and can thus represent a continuous source of contamination of salmon during processing. Several recent multi-national outbreaks of listeriosis were linked to lightly conserved and ready-to-eat (RTE) salmon products with prolonged shelf-life, which draws attention to the contamination of *Lm* in the fish both before and during processing to RTE products. The persistence and possible routes of transmission of *Lm* in processing facilities have been extensively studied, but the industry still seeks knowledge on effective measures to obtain increased control with this pathogen.

Here, we present the strategy and preliminary results of the DEKOLAKS project, aiming to apply hurdle technology for decontamination and growth control of *Lm* in salmon products throughout the value chain. Our hypothesis is that indirect decontamination by treatment of process water combined with direct decontamination of salmon can be applied to reset the contamination level to obtain increased *Lm* control in various stages of processing.

More specifically, we present results from process water treatment during bleeding by physical removal of blood combined with physiochemical decontamination using either H_2O_2 or ozone. Furthermore, the effect of sub-chilling conditions (0-2 °C) in the rinsing tank after gutting, combined with decontamination using H_2O_2/ozone will be presented. These treatments are also applied as atomized substances directly on fillet surfaces. Such measures are to a little extent

tested regarding *Lm*-reducing effects in salmon production. Eventually, a risk model targeting survival and re-growth of *Lm* after decontamination will be built to evaluate the impact of contamination, decontamination, and recontamination in each processing step and furthermore to generate performance objectives to reduce the risk of disease following consumption of salmon products.

P-053

IN THE QUEST FOR RELIABLE TOOLS TO EXPLORE THE VIRULENCE POTENTIAL OF *LISTERIA MONOCYTOGENES* CLONAL COMPLEXES

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Listeria monocytogenes is the etiological agent of human listeriosis, which manifest itself in both invasive or non-invasive forms. The former is most common in immunosuppressed patients and entails crossing the placental and blood-brain barriers. The impact of *L. monocytogenes* extends beyond health concerns to economic implications since detection of contaminated food results in production interruptions and product recalls. Despite its well-known pathogenicity, *L. monocytogenes* displays prominent diversity, and its strains can be grouped into clonal complexes (CCs) with varying virulence levels, defined as hyper- or hypovirulent CCs. The former are associated with severe clinical cases, whereas hypovirulence is mainly linked to food and food processing environments. Considering the persistence of *L. monocytogenes* in the food industry, there is an urgent need to define reliable biomarkers and to determine whether CC characterization is robust enough to discriminate high-risk strains.

Whereas *L. monocytogenes* has a typical β-hemolytic profile, which is outlined by crucial virulence factors (*hly* and *prfA* genes), and as strains exhibiting non-hemolytic or attenuated hemolysis have been reported, it was hypothesized that these phenotypes could be associated with hypovirulent CCs. Therefore, the hemolytic activity of 16 *L. monocytogenes* isolates was investigated qualitatively and quantitatively using blood agar plates and a microplate technique, where OD values were measured. Additionally, the expression of selected virulence genes was investigated.

Results showed that on blood agar plates there was a clear variation in hemolytic profiles, with strains exhibiting strong to weak hemolysis. In the microplate, the differences were not so evident, but a strain from a hypovirulent CC, exhibited the highest hemolysis. Gene expression analysis also showed an up-regulation of *hly* gene in this strain. These results suggest that: i) there was no association with increased hemolysis and hypervirulence, despite the relevance of *hly* gene to the infection cycle; ii) there may be an effect associated with the overexpression of some virulence genes in the pathogenic profiles of strains, reducing infection periods and iii) measuring hemolysis does not seem to be a reliable tool to study either hyper- or hypovirulent strains.

P-054

LISTERIA MONOCYTOGENES DIVERSITY IN THE UNDERSTUDIED SEAFOOD INDUSTRY IN THE WESTERN CAPE, SOUTH AFRICA AND COMPARISON WITH HUMAN LISTERIOSIS ISOLATES

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Several outbreaks have been reported worldwide linked to seafood products including smoked trout, smoked salmon, smoked mussels, raw fish and molluscan shellfish. *Listeria monocytogenes* is a problem in fish and the fish food processing environment (FPE). However, there is limited information in South Africa regarding *L. monocytogenes* from these products.

The aim of this study was to investigate the genetic diversity of *L. monocytogenes* isolated from seafood varieties and its FPE and compare it to isolates from clinical origin in Western Cape, South Africa.

Forty-six (n = 46) *L. monocytogenes* isolates from seafood (n = 38) (salmon, smoked trout, fresh hake, oysters) and clinical *L. monocytogenes* isolates (n = 8) were included. *L. monocytogenes* was isolated using culture-based

methods, screened for the housekeeping *hly* gene by PCR, and categorised into lineage groups using PCR-RFLP. All 46 isolates were screened for quaternary ammonium compound (sanitiser) resistance genes, *bcr*ABC and *emr*C. Based on these results, a subset of twenty-four isolates (n = 16 seafood varieties –six factories– and n = 8 clinical) were processed by WGS using Illumina technology.

Seafood isolates belonged to two lineage groups and three serogroups. Lineage I (n = 7) contained serogroups 1/2b (sequence type –ST– 3, ST5, ST87) and 4b (ST54 and ST515). Lineage II (n = 9) consisted of serogroup 1/2a (ST121, ST204 and ST155). Clinical isolates belonged to serogroup 4b with ST1 (n = 1) and ST876 (n = 7). Of the 46 isolates, 43% were positive for the *bcr*ABC gene. WGS revealed the presence of *listeria* pathogenicity islands (LIPI) 1-4 across the seafood subset (LIPI-3 and LIPI-4 are reported to contribute to hypervirulence). Further, additional sanitiser resistance genes (food and clinical isolates) and stress survival islets (food isolates), which may confer survival advantages where stress is encountered in the processing environment, were observed.

All ST's in this study, have been implicated in clinical cases around the world, highlighting the risk, especially to the immune-compromised. The results are concerning as many of these seafood products are ready-to-eat, and with no or improper heat treatment and the ability of *L. monocytogenes* to survive and grow in refrigeration temperatures, it can pose a significant risk to the health of consumers.

P-055

SALMONELLA CROSS-CONTAMINATION RISKS BETWEEN TOMATOES AND BRUSH ROLLERS DURING POSTHARVEST ACTIVITIES

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Tomatoes have been associated with salmonellosis outbreaks.The purpose of this study was to evaluate *Salmonella*'s transfer potential from contaminated tomatoes to adjacent tomatoes, brush rollers, and wastewater during washing on a brush bed under a spray bar using different concentrations of chlorine (pH 6.5) and peracetic acid (PAA). To replicate a commercial brush washing process, a bench top brush system was used. The brush washing system included two roller brushes beneath overhead sprayers. Tomatoes were spot inoculated with a Rifampicin resistant *Salmonella* cocktail (10^5) and dried for 1h in a biosafety cabinet. Inoculated tomatoes were placed on the brushes with an uninoculated tomato on each side. Wash water was mixed with chlorine (pH 6.5) or peracetic acid (PAA) to concentrations of 20-100 ppm and 20-80 ppm, respectively. Tomatoes were washed for 30 s. Wastewater was collected into sterile containers. After each run, the length of each brush was swabbed with a sterile sponge that was rehydrated with 50 mL of Dey-Engley neutralizing broth. *Salmonella* populations were enumerated from each swab, 25 mL of wastewater, and inoculated and uninoculated tomatoes; when counts fell below the limit of detection (2.30 log CFU/mL) enrichment occurred following a modified FDA BAM method. There were 6 biological and 3 technical replications (n = 18). Results were reported as % positives and the Chi-Squared test was used to determine statistical significance.

A significant ($p \leq 0.05$) decrease in the number of positive enrichments for each enumerated sample was observed as chlorine concentrations increased; at 40, 50, and 70 ppm the total number of samples positive was 60, 18 and 0%, respectively. A significant ($p \leq 0.05$) decrease of the % positive samples was observed as PAA concentrations increased at 50, 70 and 80ppm, the total number of samples positive was 46, 14, and 0%, respectively. There was no observable transfer of *Salmonella* to uninoculated tomatoes, brushes, or wastewater in the presence of at least 70ppm of chlorine (pH 6.5) or 80ppm of PAA.

The concentration of chlorine (pH 6.5) and PAA needed to minimize the potential transfer of *Salmonella* does not exceed the recommended concentration of either sanitizer in postharvest processes.

P-056

ENSURING SAFETY IN DRY-CURED FISH PRODUCTS: DESCRIBING THE BEHAVIOUR OF *LISTERIA MONOCYTOGENES* IN ARTISANAL READY TO EAT SALMON, SWORDFISH AND TUNA

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Introduction

Dry-cured fish is a Ready-to-Eat (RTE) fish product preparation with an increasing popularity. During production, it is first covered with a salt brine and then left to dry for several days. However, the association of *Listeria monocytogenes* contamination with RTE seafood is a significant concern due to its potential health risks.

Purpose

This work was aimed at describing the behaviour of *L. monocytogenes* in artisanal dry-cured salmon (*Salmo salar*), swordfish (*Xiphias gladius*) and tuna (*Thunnus albacares*), produced in the Stagionello Fish Curing Device®, during shelf-life.

Methods

Challenge tests were conducted on three batches of vacuum-packed dry-cured salmon swordfish and tuna following the EURL Lm Technical Guidance Document on challenge tests for *L. monocytogenes*. The effect of dynamic storage temperature (7-10 °C) for up to 60 days of storage on *L. monocytogenes* was evaluated on the products inoculated with a three-strain cocktail of the pathogen (3 log cfu/g). Physico-chemical parameters (water activity, pH) and microbial counts including aerobic mesophilic bacteria (AMB), lactic acid bacteria (LAB), *Enterobacteriaceae*, were measured.

Results

In both salmon and swordfish, *L. monocytogenes* was able to grow to a maximum population level of approximately 7 log cfu/g by the end of the experiment, while in tuna slowly decreased to approximately 2 log cfu/g. In salmon, swordfish, and tuna respectively, water activity had a mean value of 0.961 (± 0.012), 0.941 (± 0.006), and 0.906 (± 0.010), while pH was 5.81 (± 0.29), 5.83 (± 0.11), and 5.86 (± 0.04). AMB consistently varied, from the start to the end of the experiment, from 2.36 (± 0.20) to 9.09 (± 0.09) log cfu/g in salmon, from 3.85 (± 0.07) to 3.06 (± 0.02) log cfu/g in tuna, and from 4.00 (± 0.16) to 5.88 (± 0.12) log cfu/g in swordfish. LAB were below the enumeration limit (< 1 cfu/g) in all the products except for swordfish where they ranged from 2.82 (± 0.23) to over 5.5 log cfu/g. Similarly, *Enterobacteriaceae* (< 1 cfu/g) were detected only in tuna, ranging from 3.53 (± 0.12) to 2.49 (± 0.20) log cfu/g.

Conclusions

This work provides insights into the microbial behaviour of both background microbiota and *L. monocytogenes* in these artisanal RTE fish products.

P-057

OPTIMIZATION OF YIELD AND PURITY OF RECOMBINANTLY EXPRESSED *SHIGA* TOXIN 2A A-SUBUNIT

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Shiga toxins are the major pathogenicity factors of entero-hemorrhagic *Escherichia coli* (EHEC). Stx are AB_5 toxins, the A-subunit of which inhibits eukaryotic protein biosynthesis. The B-pentamer is responsible for binding to the receptor globotriaosylceramide. Since the A-subunit alone was already shown to be functional without its corresponding B-subunit, investigation and analysis of the mechanisms are of interest. Since mg amounts of toxin are needed for detailed analysis, current protocols of protein purification had to be optimized.

The aim of the current study was to increase the yield of the pure A-subunit to a final yield of 200-300 µg $StxA_{2a}$ per litre expression culture.
For the expression of $StxA_{2a}$, the *stxA2a* gene was cloned into the vector pET45b(+) under the control of a T7 promotor, and transformed into *E. coli* BL21 (DE3) C43. In a first step the yield of $StxA_{2a}$ was optimized under different expression conditions (expression medium, expression time, expression temperature, IPTG concentrations). In a second step, $StxA_{2a}$ was purified with a single step purification using ion exchange chromatography.
The optimal expression of $StxA_{2a}$ was achieved in terrific broth after an incubation of 24 h at 30 °C. The expression of $StxA_{2a}$ was induced with 1 mM IPTG. Since the His-tag present on the $StxA_{2a}$ was not accessible for purification on a metal-ion affinity column, ion exchange chromatography was used. Following this one step purification protocol, a yield of 567 µg pure $StxA_{2a}$ per litre expression culture was achieved. The optimization of the expression and purification of $StxA_{2a}$ leaded to a 5.5 times higher yield of pure $StxA_{2a}$ to former protocols. The purified $StxA_{2a}$ will be used for further studies on the cytotoxicity, the cellular uptake and the intracellular transport of $StxA_{2a}$.

P-058

LAST-MILE FOOD DELIVERY: SURVEY ON DELIVERY PRACTICES, QUALITY AND FOOD SAFETY ASPECTS

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Last-mile food delivery from restaurants or catering facilities is increasingly popular, driven by rapid urbanization, population growth, innovation in e-commerce, and changing consumer behaviour. The covid-19 pandemic boosted this increase with high demand for quick and reliable contact-free food delivery. The contribution of last-mile practices to the emergence of food safety hazards or the occurrence of foodborne illness is little investigated. This research explores the last-mile concept from a microbiological food safety perspective, focussing on doorstep delivered, ready-to-eat multi-ingredient bowl salads, provided through third-party e-commerce platforms.
A market survey was conducted to identify restaurants and catering facilities that supply these products in the city of Ghent (Belgium). A selection of products containing (A) vegetables or (B) carbohydrates, supplemented with a protein component (fish, chicken or alternative) were sampled seasonally and analysed for several microbiological parameters; including (i) generic parameters (total count and Enterobacteriaceae), (ii) relevant foodborne pathogens (*L. monocytogenes*, *Salmonella* spp. and *B. cereus*) and (iii) *E. coli* as hygiene indicator. Challenge tests simulating last-mile delivery were performed to assess the growth potential of *L. monocytogenes* and *B. cereus*, also considering different consumer handling practices.
The relevance and occurrence of foodborne pathogens depends on the ingredients used in the bowl salad, being highly variable. The same accounts for the food temperature at delivery, which should be < 7 °C when the food is send out for delivery. This delivery temperature depends on the ingredients, related practices, and outdoor temperature. However, the time of delivery was observed to be short (< 1 h) in an urban setting. Therefore, (i) the implementation of good practices during meal preparation, with a special emphasis on the cold chain, rapid cooling of cook-chilled ingredients, and proper food packaging, and (ii) consumer food handling practices, preferably direct consumption, are key to ensure microbiological food safety.

P-059

MICROBIAL FOOD SAFETY ISSUES ASSOCIATED WITH PLANT-BASED MEAT ANALOGUES

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The consumption of plant-based meat analogues (PBMA) has grown in response to health and environmental concerns. The aim of the present study was to perform an extensive literature search on the microbiological hazards occurring in PBMA, to benchmark antimicrobial ingredient and additive

trends and to evaluate the behaviour of *L. monocytogenes* through challenge testing in different PBMA and tofu from the market. Web of Science Core collection database was used searching for terms related to meat analogue/substitute/alternative and microbial hazards. Notifications on the European Commission RASFF database were collected.
The Mintel-Global New Products Database was used to gather information from the labels of commercial products. The growth of *L. monocytogenes* was characterized by challenge testing and growth kinetic parameters were estimated to determine the impact of temperature on the safe shelf-life. From 251 articles, 25 were selected as relevant and data about spoilage and pathogenic microorganisms' levels, their behaviour and physicochemical parameters of the food products were extracted. *L. monocytogenes* was the only pathogen showing growth during storage of the analogues. In fact, RASFF notifications for meat analogues were only found for *L. monocytogenes*.
Among commercial products, only one-quarter were formulated with antimicrobials and organic acids salts, being lactate and especially acetate/vinegar the most frequently used. According to challenge test results *L. monocytogenes* was able to grow in plant-based frankfurters and tofu formulated without antimicrobials and with high a_w (0.99) and pH (>5.9). Instead, no growth was observed in analogues containing lactate and/or vinegar, high levels of lactic acid bacteria and/or MAP packaging with CO_2.
Storage temperature greatly affected the growth rate of *L. monocytogenes* and consequently the safe shelf-life of plant-based analogues supporting its growth. *L. monocytogenes* is a relevant hazard in PBMA. A safety-by-design approach taking into account the effect of antimicrobials in the formulation and LAB, physicochemical characteristics, packaging atmosphere, postlethality treatment and shelf-life duration will ensure and enhance PBMA safety.

P-060

HUMAN TOLL-LIKE RECEPTORS ACTIVATION BY PATHOGENIC *ARCOBACTER* SPECIES

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The increase of *Arcobacter* spp. infection cases in humans, coupled with varying symptomatology, highlights the need to study the virulence mechanisms of this foodborne pathogen, which are largely unknown. *Arcobacter butzleri* can induce the release of several proinflammatory cytokines in human monocytic-derived macrophages, but the mechanism used to achieve this is still unclear.
Therefore, we aimed to investigate the innate immune response triggered by *Arcobacter* spp. associated with human infection, through the activation of the human TLR1/2, TLR4, TLR5, and TLR2/6. *Arcobacter skirrowii* was the only species that showed the ability to activate all the TLRs, being the only one that activated TLR5. *Arcobacter cryaerophilus* demonstrated to be able to activate TLR1/2, TLR4, and TLR2/6. *A. butzleri* hardly activated the TLRs, only TLR2/6 and TLR1/2 to a small extent, the latter of which was only activated by some strains. To investigate why only *A. skirrowii* activated TLR5, motility assays, electron microscopy, and alignment data were generated.
While all *Arcobacter* spp tested possess flagella, the protein sequence of the flagellin (A) of *A. skirrowii* shares more crucial amino acids for TLR5 recognition with the FLiC of *Salmonella* than the other *Arcobacter* spp flagellins, explanating why A. *skirrowii* is activating TLR5. Our results demonstrated that activation of the different TLRs is *Arcobacter* species dependent and there might be a correlation between activation of the TLRs and pathogenicity of the *Arcobacter* species.

P-061

INVESTIGATING THE ANTIMICROBIAL EFFECT OF CARROT JUICE ON *LISTERIA MONOCYTOGENES*

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Listeria monocytogenes is a highly relevant foodborne pathogen, given its unique ability to adapt to challenging environmental conditions. Due to the high case fatality rate of listeriosis, implementing strategies to prevent contamination is crucial for the food industry. Furthermore, given consumers' preference for natural products over synthetic preservatives, identifying new, natural antimicrobial agents is a promising approach for improved food safety.

According to the literature and our preliminary experiments, carrot juice may exhibit an antibacterial effect on *L. monocytogenes.* However, the mechanism behind this antimicrobial property is unknown. This study aimed (i) to understand whether all carrot varieties we have exhibit antilisterial properties and (ii) to elucidate the transcriptional response of *L. monocytogenes* upon exposure to carrot juice. Different carrot cultivars, including commercial cultivars, ancient landraces, and wild forms, provided by the German Julius Kühn Federal Research Centre for Cultivated Plants (Bundesforschungsinstitut für Nutzpflanzen), as part of a collaboration, were tested for their antilisterial effects. These specific carrots were selected based on their varying concentrations of several potentially antimicrobial substances.

The antilisterial effect of carrots is highly variable between carrot cultivars. Gene expression profiles of *L. monocytogenes* in response to the stress posed by different carrots will be presented as a first approach towards the molecular mechanisms behind these interactions.

P-062

CHARACTERIZATION OF *LISTERIA MONOCYTOGENES* ISOLATED FROM MEAT PRODUCTS IN KOREA

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Listeria monocytogenes, a foodborne pathogen causing human listeriosis, is commonly found in meat products. Over the past few years, foodborne listeriosis outbreaks linked to meat products have been reported worldwide, including the USA, European union, and other countries.

In this study, we analyzed the virulence genes, serogroups, and antimicrobial susceptibility of *L. monocytogenes* isolated from meat products in Korea. Additionally, Whole Genome Multi Locus Sequence Typing (wgMLST) and Single Nucleotide Polymorphism (SNP) analysis were used to classify and compare genetic diversity among isolates. A total of 140 L. monocytogenes strains were isolated from 34 beef, 79 pork, and 27 chicken meat products. The serotypes of the isolated strains were classified in the following order: 1/2c (42.0%), 1/2a (37.0%), 1/2b (20.3%) and 4b (0.7%).

Genetic analysis showed that, except for clpE(87.9%) and inl(64.3%), all isolates were positive for six virulence genes (prfA, iap, fbpA, plcA, plcB, and hlygenes). Antimicrobial susceptibility testing showed that over 40% of the isolates were resistant to oxacillin and tetracycline, while over 95% were susceptible to 13 antibiotic agents. A total of 140 isolates were grouped into 15 different sequence types (STs) by wgMLST analysis. The most prevalent ST was ST9 (53.4%), followed by ST155 (8.7%), ST87 (7.8%), and ST3 (6.8%).

Phylogenetic relationships showed that the isolates were divided into 12 clusters, with these clusters of isolates sharing the same sequence type. However, the isolates classified as the same serogroup could be differentiated into different STs. These results could be used as valuable information for epidemiological and public health research on *L. monocytogenes*.

P-063

TOLERANCE TO STRESS CONDITIONS ASSOCIATED TO FOOD SAFETY AND GENOMIC CHARACTERIZATION OF *CAMPYLOBACTER COLI* STRAINS ISOLATED FROM DIFFERENT SOURCES IN BRAZIL

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Although in Brazil campylobacteriosis has been a neglected disease, some *Campylobacter* species are important causative agents of human diarrheal diseases in many countries. The aims of this study were to analyze the effect of freeze-thaw and rapid pasteurization process in the survivability of 80 *Campylobacter coli* strains isolated from humans (12), animals (34), the environment (20) and chicken meat (14) between 1995-2019 in Brazil. In addition, the genetic similarity and the frequency of several virulence genes in theses strains were searched.

The strains were analyzed for their tolerance to incubation at –20 °C during 3 and 7 days (freeze-thaw) and their tolerance to a process mimicking the rapid pasteurization by using a thermocycler at 75 °C for 15s and 4 °C for 15 s. The phylogenetic analysis was performed by wgMLST using a Web service called PGAdb-builder and the presence of virulence genes were evaluated *in silico* in Virulence Factors Database using the whole genome sequencing data of the strains studied. Five out of the 80 strains showed a survival percentage of 87.5%, 68 strains a survival of 75.0% and 7 strains a survival of 62.5% after an incubation at –20 °C for 3 days.

After 7 days at –20 °C, 64 strains showed a survival percentage of 75.0% and 16 strains a survival of 62.5%. Likewise, after the assay mimicking the rapid pasteurization process 18 out of the 80 strains remained 100% viable, 24 strains showed a survival of 87.5%, 23 strains a survival of 75.0%, 10 a survival of 62.5%, four a survival of 50% and one a survival of 37.5%. The wgMLST analysis grouped the strains studied in six distinct clusters and 107 virulence genes related to motility, adherence, immune modulation, chemotaxis, invasion, biofilm and biosynthesis were detected. In conclusion, the results obtained showed a genomic diversity among the strains studied and reinforced their pathogenic potential by the presence of important virulence genes. Furthermore, once freeze-thaw and rapid pasteurization process are methods widely used to control bacterial growth, the survival rates obtained may suggest that more rigorous control measures may be needed given the importance of contaminated food as vehicles of *C. coli.*

P-064

PERSISTENCE OF HUMAN *NOROVIRUS* IN BERRY SMOOTHIES

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Human Noroviruses (HuNoVs), members of the Caliciviridae family, commonly cause viral foodborne outbreaks. The main ingredient of smoothies is berries which can be a source of HuNoVs. Smoothies are well-liked due to their delicious and nourishing features.

Our study aimed to determine the presence of murine norovirus in berry smoothies with or without milk. Since HuNoV is difficult to cultivate in a cell culture lab we have used Murine Norovirus (MuNoV). We have used raspberry and blueberry smoothies in our experiment on different time points and temperatures (0 h-4 °C, 1 h-4 °C, 1 h-RT, 1 h-37 °C, 24 h-4 °C, 24 h-RT, and 24 h-37 °C). MuNoVs (MNV-1) and RAW 264.7 cells were used in the experiments. The smoothie was made in a blender using blueberries or raspberries, semi-skimmed milk, and a quarter of a banana. In one experiment, PBS was used in place of milk. Samples were prepared in 10-fold dilutions and added to the cells. TCID50 assay was used to assess the virus load and the cytopathic effect of the cells was examined using microscopy. There was a decrease in the raspberry smoothie in the MuNoVs infection at 37 °C as compared to 4 and 21 °C. TCID50/mL values stayed mostly consistent about ~ 6 $\log_{10}$ at 4 and 21 °C but reduced to ~ 5.5 $\log_{10}$ at 37 °C. A faster decline in MuNoVs was also observed when

PBS was substituted for milk in the blueberry smoothie. If NoV-contaminated berries are used in the smoothies and the ingredients are not heat-treated, they could result in foodborne illnesses. As a first finding, we found that milk might shield MuNoVs.

P-065

QUANTITATIVE COMPARISON OF THERMOTOLERANT *CAMPYLOBACTER* SPP. GROWTH IN BOLTON AND PRESTON BROTH WITH AND WITHOUT GROWTH SUPPLEMENT (FBP)

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Thermotolerant *Campylobacter* spp., the leading cause of foodborne bacterial diarrhoea in Europe, are fastidious *in vitro*. Thus, a cultural detection can be challenging. ISO 10272-1:2017/Amd 1:2023 recommends direct streaking or enrichment with either Bolton or Preston broth supplemented with growth supplement (FBP), depending on the expected number of *C.* spp. and microbiota.

Here, we compared the growth performance of *C. jejuni*, *C. coli* and *C. lari* field isolates using Bolton broth, Preston broth and Preston broth with FBP. Media performance testing was investigated using 123 *C.* spp. isolates from food and animal matrices according to ISO 11133:2018. The presence of FBP supplement in Preston broth reduced false negative results in 100% of the *C. jejuni,* 60% of the *C. coli,* and 84% of the *C. lari* isolates on mCCDA. In comparison, 98% Bolton enrichments supported *C.* spp. detection. Qualitative growth was quantitatively confirmed by Real-time PCR (qPCR).

In addition, chicken meat, chicken neck skin, frozen spinach and egg shells were pre-inoculated with representative *C.* spp., either in exponential growth phase ("fresh bacteria") or after stress exposure upon incubation with matrix at cooling conditions before enrichment. Except for chicken neck skin, addition of matrix enhanced growth of "fresh" *C.* spp. in Preston for up to ~5.5$\log_{10}$ *C.* spp./mL and further addition of FBP had either no effect or a marginal extra growth enhancement of up to 1.5$\log_{10}$ *C.* spp./mL. Enrichment in Bolton (24 or 48 h incubation) always showed highest detection ~8-9$\log_{10}$ *C.* spp./mL.

Using naturally stressed *C.* spp. cells pre-incubated with matrix at cooling conditions, addition of FBP to Preston broth drastically enhanced growth of stressed *C.* spp. in raw milk from a level of <1$\log_{10}$ *C.* spp./mL to ~6$\log_{10}$ *C.* spp./mL. Stressed *C.* spp. in chicken meat, egg shell or iceberg lettuce reached comparable growth levels in Preston, Preston + FBP and Bolton after 24 h, while maximal growth levels of ~8$\log_{10}$ *C.* spp./mL were observed in Bolton after 48h incubation.

Our data suggest that testing naturally stressed *Campylobacter* spp. in different matrices is important for performance evaluation of enrichment media. Whenever low matrix microbiota is expected, Bolton broth might be superior to Preston broth, which can partially be improved by addition of FBP supplement.

P-066

GENOME DESIGN AND MORPHOLOGICAL CHARACTERIZATION OF *LISTERIA MONOCYTOGENES* BACTERIOPHAGES FROM ENVIRONMENTAL MATRICES

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Listeria monocytogenes is a pathogen of interest to human health, representing a huge challenge for food quality. Methods based on bacteriophages have re-emerged, aiming at the natural inactivation of *L. monocytogenes* in products and the food industry. In this sense, knowing and prospecting

phages capable of infecting *L. monocytogenes* becomes an important target for food technology, both for biological control purposes and to contribute to the understanding advancement of resistance genes and microbial stability mediated by phages.

This study aimed to bioprospect new *Listeria* phages and characterize them morphologically and molecularly. One hundred and eight (108) phages were isolated from different environmental matrices based on the double-agar technique and eighteen (18) were morphologically and molecularly characterized. Molecular characterizations were done using paired-end sequencing through the MiSeq - Illumina platform, considering one million reads of coverage, while the morphological characterizations were done using transmission electron microscopic (TEM).

The characterizations revealed one phage belonging to the *Inoviridae* family, three phages belonging to *Peduoviridae*, and three phages belonging to the *Casjensviridae* family. Besides, other phages categorized within the class *Caudoviricetes*, which could not be classified at the species level. In addition, with molecular studies, it was possible to evaluate the presence of antibiotic-resistance genes associated with the isolated phages. The results confirmed the presence of genes related to beta-lactam resistance in SMLM9, PLLM1, and SMLM7 phages belonging to the *Caudoviricetes* class, *Inoviridae* and *Peduoviridae* families, respectively.

These findings provide valuable insights into the genetic and phenotypic of *Listeria* phage characteristics, expanding our knowledge about their diversity and highlighting the potential process of the dissemination of antibiotic resistance characteristics among microorganisms through horizontal genetic transfer mediated by phages. Horizontal genetic transfer is a phenomenon that can result in the dispersal of mobile genetic elements among groups of bacteria. Among these mobile genetic elements, resistant genes can be found, which are interpreted as a threat to the health, environmental safety, food chain, and society.

P-067

MOLECULAR DETECTION OF *SARCOCYSTIS SUIHOMINIS* IN WILD BOARS HUNTED FOR HUMAN CONSUMPTION IN POLAND

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Sarcocystis spp. are intracellular protozoa parasites with an obligatory prey-predator life cycle. Domestic and wild pigs may act as intermediate hosts for two species of *Sarcocystis*, *S. miescheriana* and the zoonotic *S. suihominis*. Humans can get infected by the consumption of raw or insufficiently heat-treated pork.

In this study, 125 diaphragmatic muscle samples were collected from wild boars hunted in different regions of Poland to evaluate the presence of *Sarcocystis* spp. in game meat. Samples were molecularly tested for the presence of *S. miescheriana* and *S. suihominis* by a multiplex-PCR protocol targeting the mtDNA *cox1* gene. Samples resulted positive for the presence of the zoonotic *S. suihominis* were sequenced to confirm species identification and further processed by homogenization and direct microscopy to collect single *Sarcocystis* spp. cysts. Single-cyst DNA was analysed by PCR targeting the *cox1* mtDNA and the complete 18S rRNA gene and sequencing.

Out of 125 wild boars, 117 resulted positive for the presence of *S. miescheriana* DNA (93.6%), six of which were also positive for *S. suihominis* (4.8%). Sanger sequencing of the six amplicons resulted in 374-377 bp fragments showed a percentage of identity of 97.3-98.1% with *S suihominis* GenBank entries. A single *S. suihominis* cyst and 12 *S. miescheriana* cysts were individually collected; amplification and sequencing of the *cox1* mtDNA gene yielded a 1049 bp sequence with a percentage of identity of 98.7% with *S. suihominis* and twelve 1015-1055 bp sequences with a percentage of identity of 94.1-99.9% with *S. miescheriana* GenBank entries, respectively. The amplification and sequencing of the complete 18S rRNA gene of the *S. suihominis* cyst

yielded a 1840 bp sequence with a percentage of identity of 98.9-100% with *S. suihominis* GenBank entries.
This is the first study investigating the prevalence of *Sarcocystis* spp. in wild boars intended for human consumption in Poland; as around 5% of wild boars tested positive for the presence of *S. suihominis*, the risk for the consumer of raw or undercooked wild boar meat cannot be excluded.

P-068

APPLICATION OF PHAGES TO CONTROL *LISTERIA MONOCYTOGENES* ON FOOD FACILITIES SURFACES

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Listeria monocytogenes pose a significant challenge to both food safety and human health, with its resilience in food products and within food industry settings. This pathogen demonstrates notable resistance to factors such as salinity, pH variations, low temperatures, and the formation of biofilms. Additionally, *L. monocytogenes* may exhibit resistance to antimicrobial and biocidal agents, complicating the elimination process during cleaning and disinfection procedures. In response to these challenges, methods utilizing bacteriophages have gained prominence in recent years, aiming to naturally deactivate *L. monocytogenes* in both food products and food industry facilities.
This study focuses on applying novel phages for the biocontrol of *L. monocytogenes* on stainless steel surfaces. The approach involves inducing bacteriophage infections *in vitro* using environmental samples sourced from diverse matrices. In experiments on metal surfaces (stainless steel) measuring 2 × 1 cm^2, cleaned and sterilized at 121 °C for 15 minutes. An artificial contamination with *L. monocytogenes* (ATCC 19111) was conducted using 1 × 107 UFC/mL of *L. monocytogenes*.
The evaluation focused on the bacterial control capacity in the presence of bacteriophages applied through dripping on the contaminated area. Bovine serum albumin (BSA) served as a natural contaminant mimicking organic food surfaces, following the EN 13697:2015 protocol for assessing chemical disinfectants, using microbial suspension (107 CFU mL-1) and phage suspension (107 PFU mL-1). All inoculated surfaces were kept at room temperature for 12 h, with samples collected at 3, 6, 9, and 12 h into the experiment.
The results encourage the scaling of tests on an expanded scale in food processing plants, considering the high applicability of this tool for bacterial biological control and to prevent bacterial regrowth and/or resilient bacteria. Then, treatment with bacteriophages in samples considered contaminated with organic matter (3 mg/mL bovine serum protein) achieved reductions until 1.2 logs CFU/mL of *L. monocytogenes*, compared to the control group not treated with phages.
Based on the results obtained, this study highlights the potential of phages as biological control agents. Furthermore, it demonstrates the importance of reducing the possibility of bacterial cell residues that may remain after the standard sanitization protocol.

P-069

THE QUORUM QUENCHING ACTIVITIES OF PROBIOTIC *LACTICASEIBACILLUS RHAMNOSUS* AND *BIFIDOBACTERIUM* SPECIES. POTENTIAL FOR INHIBITION OF *LISTERIA MONOCYTOGENES* GASTROINTESTINAL TRACT INFECTIONS

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In recent years, the role of probiotics in promoting gut health and preventing gastrointestinal infections has garnered significant attention.

This study investigates the potential of probiotic lactic acid bacteria (LAB) in mitigating infections caused by *Listeria monocytogenes*, a notorious foodborne pathogen. The research focuses on revealing the quorum quenching (QQ) abilities of LAB, particularly *Lacticaseibacillus rhamnosus* and *Bifidobacterium species*, against *L. monocytogenes*. The QQ activity of culture-free extracts of probiotics is determined via a *Chromobacterium violaceum* assay, while the effects of these extracts on *L. monocytogenes* biofilm formation and virulence expression is evaluated to determine their potential in preventing gastrointestinal tract infections.
The project investigates the survivability and QQ activity of probiotics in yogurt following simulated gastrointestinal transit to enhance our understanding of their real life efficiency. This research contributes to the development of novel strategies for improving food safety and public health.

P-070

COMPARISON OF *SALMONELLA* SPP. GROWTH IN RAW AND PASTEURIZED MILK

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The aim of the study was to evaluate the behavior of *Salmonella* spp. in raw and pasteurized milk during storage at 5, 10 and 15 °C. In the study, the pathogen was introduced into raw and pasteurized milk to assess its storage behavior, simulating insufficient heat treatment of milk or cases of cross-contamination during production. Growth of the pathogen was assessed using the plate method. The total microbial count and pH of raw and pasteurized milk were also determined. Using the DMFit application, first-order models based on the Baranyi and Roberts equation were obtained to determine the growth rate of Salmonella ssp.
Based on the study, it was shown that the growth or survival of microorganisms in milk depends on the application of heat treatment. It was also found that temperature has a significant effect on the growth of *Salmonella* spp. In raw milk, the growth of *Salmonella* spp. was slower compared to pasteurized milk. It was also observed that in milk stored at 15 °C, the growth of *Salmonella* spp. occurred the fastest, compared to the other temperatures. The use of the DMFit application is an effective tool for estimating the microbiological risk to consumers associated with the presence of *Salmonella* spp. in raw and pasteurized milk.
The data obtained provides an opportunity to better understand the risks that may be caused by *Salmonella* spp. infection, making both dairy producers aware of the importance of hygiene and care during the production process and consumers aware of how the presence of a large number of bacteria, carries potential risks.

P-071

COMPARISON OF THE GROWTH OF *LISTERIA MONOCYTOGENES* IN RAW AND PASTEURISED MILK

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Milk is one of the perishable products unless it is properly processed, packaged, distributed and stored. It can be potentially dangerous due to the growth of pathogenic microorganisms. Among foodborne pathogens, *Listeria monocytogenes* poses a serious safety risk to the dairy industry, as several listeriosis outbreaks in recent years have been linked to the consumption of dairy products including pasteurized milk. Control of *L. monocytogenes* in pasteurized milk involves, among other things, application of HACCP system. It is mainly the selection of raw milk and its control of its processing, packaging, distribution and storage conditions. Although the pathogen does not survive pasteurization, its presence in the finished product is possible due to reinfection from sources within the plant.
The purpose of present study was to compare the growth of *Listeria monocytogenes* in raw and pasteurized milk during storage at temperatures of: 5, 10 and 15 °C. The subjects of the study were raw milk and pasteurized milk. Raw milk was pasteurized at 85 °C for 20 seconds. The milk samples were then inoculated with a culture *of Listeria monocytogenes*, so that the final number of the pathogen in the samples tested

was at a level of about 103 cfu/mL of milk. The samples were placed in incubators at 5, 10 and 15 °C. To determine the number of colony-forming units (CFU), the classical plate method was used with surface culture on microbiological media. The plates were then incubated under aerobic conditions at 37 °C for 48 hours. The results obtained were modeled to determine the growth rate coefficient in ComBase DMFit software.

The results showed that the growth of *L. monocytogenes* in raw and pasteurized milk samples was affected by storage temperature. More intense growth of *L. monocytogenes* was found in pasteurized milk than in raw milk. The highest growth rate was observed in pasteurized milk stored at 15 °C (2.17 ± 0.11 log cfu/mL/day), while the lowest rate was observed in raw milk (0.13 ± 0.02 log cfu/mL/day) and pasteurized milk (0.32 ± 0.02 log cfu/mL/day) stored at 5 °C. The low storage temperature inhibited the growth of *L. monocytogenes*.

P-072

EVALUATION OF THE HEAT RESISTANCE OF *ACINETOBACTER BAUMANNII* ISOLATED FROM RAW MEAT AND MEAT PRODUCTS

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Acinetobacter baumannii is a pathogenic bacterium frequently involved in nosocomial infections. Food, such as raw meat and meat products, can serve as a reservoir and therefore a significant source of transmission to consumers. High temperatures during cooking of meat could in principle inactivate this pathogen and avoid its dissemination, however, there are few studies on *A. baumannii* heat resistance.

The aim of this study was to evaluate the thermal resistance at different temperatures of *A. baumannii* isolates obtained from turkey, pork, beef, chicken, mixed pork-beef and mixed turkey-chicken meat products.

A total of 33 *A. baumannii* strains were tested at 57 °C using the thermoresistometer Mastia. An inoculum of each strain was injected into the heat treatment medium (400 mL of sterile BHI) stabilized at the treatment temperature. Then, samples of 1 mL were taken at different times during the heat treatment, plated on BHI agar and incubated at 35 °C for 48 hours. After incubation, survivors were counted and D-values (min) calculated. D_{57} values obtained ranged from 0.66 to 5.65 min. Afterwards, five strains with variable heat resistance at 57 °C were selected and their thermoresistance was also tested at 54 and 60 °C using the same method. For this subset of strains, D_{54} values ranged from 1.39 to 7.88 min, D_{57} values from 0.73 to 4.20 min, and D_{60} values from 0.39 to 1.25 min. A mean Z-value of 10.38 ± 2.13 °C was calculated.

Overall, this study shows that the heat resistance of *Acinetobacter baumannii* is relatively high, similar or slightly higher than that described in the literature for other non-sporulated pathogenic species of relevance in the food chain, such as *Salmonella typhimurium* and *L. monocytogenes*. Therefore, the thermal treatment applied during meat cooking may not be effective to eliminate this pathogen, depending on the time-temperature combinations used and the temperature reached in the centre of the food.

P-073

INHIBITORY EFFECT OF PROBIOTIC BACTERIA ISOLATED FROM TRADITIONAL IRANIAN CHEESES (*LACTOBACILLUS PLANTARUM*) AGAINST *ASPERGILLUS FLAVUS, FUSARIUM SPOROTRICHIOIDES* AND *ASPERGILLUS NIGER* GROWTH AND AFLATOXIN PRODUCTION

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Introduction

One of the new methods to eliminate mycotoxins are biological methods that can reduce the risks of toxins by inhibiting the growth of fungi, binding to mycotoxins, and the

structural changes. Probiotics, including *L. plantarum*, may have advantages in reducing mycotoxin production and fungal growth. The aim of this study was to assess the inhibitory potential of probiotic bacteria isolated from traditional Iranian cheeses (*Lactobacillus plantarum*) on *Aspergillus flavus*, *Aspergillus niger*, *Fusarium sporotrichoides* growth and in vitro production of aflatoxin.

Methods

Three strains of *L. plantarum* were cultured on Mann-Rogosa Sharpe (MRS) medium under anaerobic condition.The antifungal activity was tested using the agar overlay method. HPLC method was performed to determine mycotoxin production.

Result

All three strains of *L. plantarum* B2, C2, and D2 showed appropriate antifungal activity by using the overlaymethod. The highest percentage of growth reduction belonged to *Lactobacillus plantarum* D2 with 40.33% inhibitory ability against the growth of *Aspergillus flavus*, *Lactobacillus plantarum* B2 with 48.0% inhibitory ability against *Aspergillus niger,* while all three strains of *Lactobacillus plantarum* B2, C2, and D2 had 100% inhibition against *Fusarium sporotracheoides*. Also, anti-mycotoxigenic results indicated a reduction rate of AFB1 from 2.66 ± 0.0 to less than 0.36 µg kg−1 by both *L. plantarum* B2 and D2 and 2.66 ± 0.0 to 0.46 ± 0.0 µg kg−1 by *L. plantarum* C2. The amount of ochratoxin and deoxynivalenol in positive and negative controls was lower than the detection limit.

Conclusion

These results suggest that *L. plantarum* strains isolated from traditional Iranian cheeses have the ability to prevent the growth of pathogenic and mycotoxigenic fungi specially AFB1 mycotoxin and could be suggested as a biological agent.

P-074

ISOLATION OF *LACTOBACILLUS SALIVARIUS* FROM CHICKEN MEAT AND EVALUATION OF ANTIMICROBIAL ACTIVITY IN CELL-FREE SUPERNATANTS AGAINST FOODBORNE PATHOGENS

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Objective

Meat, including chicken, provides an ideal environment for microbial pathogens and spoilage bacteria, especially in developing countries with a higher risk of foodborne illnesses. Our study aimed to explore a natural antibacterial agent sourced from chicken meat by isolating bacteriocin-producing lactic acid bacteria (LAB) and evaluating their antimicrobial properties against foodborne pathogens. Bacteriocins, which are antimicrobial metabolites produced by LAB, offer a natural alternative to chemical additives that may have adverse health effects.

Methods

Ten chicken meat samples were collected from various supermarkets in Mashhad, Iran, and LAB were isolated and screened for bacteriocin production. The antimicrobial activity of cell-free supernatants (CFS) containing bacteriocins was assessed using the well-diffusion method and optical density (OD) measurements via a plate reader. Biochemical tests were conducted to identify the genus of the isolated LAB, including temperature sensitivity, growth in the presence of high salt concentrations, and fermentation capabilities with various sugars. Genetic characterization was performed using PCR and genetic sequencing.

Results

The CFS of five LAB strains isolated from chicken meat samples exhibited antimicrobial activity against foodborne pathogens, including *Salmonella typhimurium*, *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* in the well diffusion test. The strains exhibited stability

at temperatures of 15 °C for 72 hours and 45 °C for 48 hours, indicating their ability to survive under different temperature conditions. They also showed tolerance to high salt concentrations and the ability to utilize various sugars as energy sources. Based on biochemical tests, the isolated lactic acid bacteria were identified as *Lactobacillus*. Further genetic characterization confirmed that all five LAB isolates were *L. salivarius*. OD measurements using a plate reader indicated a range of 1 to 3 log reduction in the tested pathogens after 24 hours when exposed to the each CFS at 4 and 37 °C.

Conclusion

In conclusion, this study highlights the potential of bacteriocin-producing lactic acid bacteria isolated from chicken meat as natural antimicrobial agents against foodborne pathogens. The findings contribute to the development of safer and more sustainable approaches for meat preservation in the food industry.

P-075

WHAT IS THE FATE OF *LISTERIA MONOCYTOGENES* CONTAMINATING BABY FOOD PUREES?

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Listeria monocytogenes is a foodborne pathogen widely present in foods and industrial environments due to its ubiquitous presence in water, soil, vegetation and animals. Its ability to multiply at refrigeration temperature is responsible for most listeriosis cases associated with consumption of contaminated food products. Baby food such as fruit purees undergo several heat treatments to ensure safety and stability during several months at room temperature. In these products, *L. monocytogenes* is unlikely to survive but accidental contamination of opened pouches not immediately fully consumed should not be overlooked.

In this study we explored the survival of *L. monocytogenes* in fruit purees whether contamination took place in the kitchen at room temperature or in the refrigerator.

L. monocytogenes strain 10403S previously grown until late exponential phase at 10, 25 and 37 °C at pH 7.2 and 5.5, was used to inoculate blueberry fruit puree (pH 3.59) at 7 $\log_{10}$CFU.g–1, and monitor its survival at 25 °C ("kitchen" storage), 10 °C (storage in "refrigerator") and 37 °C (control condition). The Weibull model was used to estimate the storage duration associated with three-log reduction of *L. monocytogenes* (t3d). Analysis of variance performed on t3d identified storage temperature as the most significant explanatory variable ahead of growth temperature ($p < 0.0001$). Indeed, t3d in fruit puree was shown to increase inversely with storage temperature, being highest at 10 °C (26.7 ± 1.1 h) and lowest at 37 °C (0.481 ± 1.1 h), suggesting a protective effect of the "refrigerator" condition. At 37 °C, *L. monocytogenes* became rapidly not detectable from 2 hours' storage, while a 2-log concentration could be enumerated after 24 h at 25 °C, particularly after growth at 37 °C. t3d was the highest when growth occurred at 37 °C with 18.3 ± 1.1 h, compared to previous growth at 10 and 25 °C (7.09 ± 1.1 h and 4.60 ± 1.1 h respectively). Surprisingly, previous growth at pH 5.5 did not produce any acid protection during storage, pH being a not-significant variable ($p > 0.05$).

This study highlights the importance of *L. monocytogenes* preadaptative conditions for its further survival to acidic stress. It may also invalidate the conventional wisdom that it is always preferable to store opened products in the refrigerator.

P-076

DEVELOPMENT OF GELATIN-PULLULAN FILMS FOR PHAGE-BASED CONTROL OF *SALMONELLA* CONTAMINATION IN CHICKEN MEAT

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Salmonella enterica is a major pathogen causing economic losses and human health concerns. *Salmonella* Typhimurium and *Salmonella* Enteritidis are particularly prevalent in foodborne illnesses, and the emergence of antibiotic-resistant strains poses a serious threat. Bacteriophages (phages) offer a potential solution for preventing foodborne infections. The environmental impact of conventional packaging materials also calls for alternative options.

Selecting a suitable phage and polymer for film preparation and storage is crucial. SALMONELEXTM, an FDA-approved phage cocktail, has demonstrated efficacy against various Salmonella strains. However, its stability in active packaging systems remains understudied.

In our study, a film composed of 20% gelatin, 80% Pullulan, and 5% cellulose nanofibers was selected for phage biocontrol experiments. This film exhibited superior mechanical properties and phage stability compared to other tested films. The stock phage was incorporated into the film at a final concentration of 5×1010 plaque-forming units (PFU)/mL.

The resulting films were evaluated for antibacterial properties against *Salmonella typhimurium* and *Salmonella enteritidis* SE 13 resistant to streptomycin. Incorporating phages into the films improved elongation at break, opacity, and UV-barrier properties compared to control films. However, it reduced the thermal stability of the films.

When the phage-containing films were applied to chicken breast contaminated with Salmonella strains (at a dose of 103 CFU/g), significant reductions in bacterial counts were observed at 4 and 10 °C. Reductions of approximately 0.3 log and 0.5 log were observed after 24 hours at 4 and 12 °C, respectively. These reductions continued until the ninth day of incubation, reaching reductions of 1.12 log and 1.41 log for *Salmonella enteritidis* and *Salmonella typhimurium* at 4 °C. At 12 °C, reductions of 2.9 log and 3.13 log were observed for *Salmonella enteritidis* and *Salmonella typhimurium*, respectively, on the ninth day. Similar reductions were observed with a higher bacterial dose (104 CFU/g).

Overall, the gelatin-pullulan film containing SALMONELEXTM phage demonstrated the potential to reduce *Salmonella* contamination in chicken meat, even at high levels of contamination. These films offer promise for mitigating *Salmonella* contamination during storage for over a week.

Development of gelatin-pullulan films for phage-based control of *Salmonella* contamination in chicken meat.

P-077

MONITORING THE PRESENCE OF HEPATITIS E VIRUS IN PORK AND WILD BOAR SAUSAGES

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Hepatitis E Virus (HEV) is a positive-sense single-stranded RNA virus. Hepatitis E is considered an emerging disease in Europe. There are two exclusively human genotypes, whose transmission is waterborne in underdeveloped and developing countries, while the transmission in developed countries is associated with the consumption of contaminated food, in which genotypes 3 and 4 are involved. HEV genotypes 3 and 4 of have several animal hosts, being the domestic pig *(sus crofa domesticus*) one of the largest ones. Reservoirs also exist among wild animals, such as the wild boar (*sus scrofa*).

In previous works by our research group, the presence of HEV in pig organs, tissues and fluids at the time of slaughter was studied, detecting a high prevalence of HEV RNA. In this case, the presence of HEV RNA was studied in

samples of raw cured pork and wild boar sausages, as well as in a typical local product made with pig blood, Burgos blood sausage.
Samples of sausages from local producers were collected monthly: chorizo, salchichón and blood sausage. RNA extraction was performed using 3 methods to compare them. RT-qPCR was used to study the presence of virus RNA. Murine Norovirus was used as a surrogate to control the process. To obtain the complementary DNA of the samples that were positive, a reverse transcriptase reaction was performed, and the resulting cDNA was stored frozen for subsequent typing and sequencing.
This study is a part of the European FoodSafeR research project.

P-078

TRANSFER OF DEOXYNIVALENOL FROM MAIZE FLOUR TO MAIZE/WHEAT-BASED BREAD

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Deoxynivalenol (DON) is one of the mycotoxins resulting from the secondary metabolism of *Fusarium* spp., being one of the most abundant mycotoxins in nature. DON intake and bioaccumulation causes serious problems for human and animal health.
Maize (*Zea mays*) is one of the most consumed cereals worldwide and the main host of *Fusarium,* which is why DON represents a food safety problem and has a great impact in raw materials management for maize-based products elaboration.
The aim of this study was to determine the effect of the breadmaking process on the concentration of DON during the production of maize-based bread (40% maize/60% wheat).
Bread loaves were made using naturally contaminated maize flour mixed with uncontaminated wheat flour to obtain a contamination of either 800 or 1200 µg DON/kg. Dough fermentation was carried out using either industrial sourdough + yeast, artisan sourdough + yeast or just yeast; in all cases fermentation was carried out for 2 and 12 h. All loaves were baked in tins at 200 °C for 35 min. Extraction of DON was performed in the flour, after fermentation and after baking, using immunoaffinity chromatography columns and analyzed by high-performance liquid chromatography with diode array detection (HPLC-DAD). Additionally, sourdough microbiota was characterized by identifying the yeasts and lactic acid bacteria present. Yeasts isolated from the sourdough were always *Saccharomyces cerevisiae*, while lactic acid bacteria showed great variability, being *Lactobacillus plantarum* the only one found in both sourdoughs.
DON concentration did not show significant changes from kneading to fermentation, except in the loaves containing 1200 µg DON/kg, prepared using industrial sourdough and 12 h fermentation period, where DON concentration increased by 30%. This was attributed to DON released from the protein and polysaccharide matrix due to enzymatic activity. During baking, DON reduction was observed under all conditions, showing a reduction range between 6 and 20%.
These results show that the reduction of DON during breadmaking may not be as high as expected and required to meet the regulatory levels, and thus ensure the food safety of the product.
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P-079

ESTERCIN A, A NOVEL BACTERIOCIN FROM *CLOSTRIDIUM ESTERTHETICUM* COMPLEX, DISPLAYS POTENT ANTIMICROBIAL ACTIVITY AGAINST FOODBORNE AND CLINICALLY RELEVANT PATHOGENS

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Foodborne pathogens continue to be of great public health concern due to their contribution to global disease burden. There is therefore a need for food preservatives that control the growth of the pathogens in foods. This coupled with consumers' demand for natural food preservatives has allowed the application of bacteriocins, which are small antimicrobial peptides, during food processing. However, the most widely used bacteriocin, nisin, has limitations including proteolytic degradation and solubility.

Recently, application of genome mining led to the identification a novel bacteriocin estercin A, in the rarely studied psychrophilic *Clostridium estertheticum* complex. However, estercin A has so far not been further characterized. In this study, we heterologously expressed estercin A in *Escherichia coli* and characterized its antimicrobial activity and physiochemical properties. Estercin A comprises of 27 amino acids and features three overlapping (methyl-) lanthionine rings, with a shorter C-terminal part compared to most reported class II lanthipeptides. It exhibits potent antimicrobial properties against foodborne and clinically relevant pathogens.

The mode of action of estercin A was determined as binding to lipid II on the cell membrane. Additionally, estercin A exhibited remarkable stability across a range of pH values and temperatures and showed resistance against degradation by the protease trypsin. In summary, our findings highlight estercin A as a novel and stable bacteriocin.

P-080

PERFORMANCE COMPARISON OF CHEMICAL AND PHYSICAL TERTIARY TREATMENT SYSTEMS FOR MICROBIAL PATHOGEN REMOVAL

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Water reuse has emerged as a sustainable solution to preserve freshwater resources and reduce environmental pressure. Furthermore, efficient sewage treatment is among our key defenses against the dissemination of infectious diseases. The present study aims to estimate the efficacy of different water treatments (i.e. chlorination and aerobic membranes) in reducing bacterial and viruses present in urban wastewater.

The two treatment systems were operated at the same wastewater treatment plant (WWTP) and their performances in reducing viruses, the fecal indicator *E. coli* and the pathogenic bacteria *Salmonella* spp. were monitored under the same conditions. For viruses, initial attempts were performed to improve the limit of detection of the current by comparing aluminium flocculation and the Innovaprep concentration pipette. The Innovaprep pipette was the method of choice due to better performance in terms of volume of water analyzed and reduced overall turnaround time. Thereafter, the occurrence of several human enteric viruses (human norovirus genogroup I, NoV GI, NoV GII, and rotavirus) was monitored by real time polymerase chain reaction (RT-qPCR) along with the level of somatic coliphages by plate count methods.

To assess the potential viral infectivity, a capsid integrity propidium monoazide (PMAxx) in combination with RT-qPCR method was performed on positive reclaimed water samples. In the case of bacteria, the presence of culturable bacteria was determined by plate count in selective media, while viable but non cultivable (VBNC) cells were assessed using quantitative PCR (qPCR) combined with different DNA dyes

such as PMAxx plus and ethidium monoazide (EMA). The two treatments were effective in reducing the presence of culturable *E. coli*. However, VBNC cells of *E. coli* were found in the reclaimed water use as agricultural water regardless of the treatment used, with a higher level remaining after chlorination. On the other hand, no *Salmonella* cells were found in reclaimed water.

This study highlights the risks linked to the presence of VBNC cells. More studies are needed to understand the role of VBNC cells in fresh produce irrigated with reclaimed water.

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P-081

PARTICIPATION IN *TRICHINAE LARVAE* DETECTION PROFICIENCY TEST AS A TOOL FOR IMPROVEMENT IN SLAUGHTERHOUSE LABORATORIES

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Trichinella larvae detection assays performed by slaughterhouses should be conducted employing ISO 18743. Although ISO 17025 accreditation is not required, these laboratories must be supervised by an accredited laboratory and periodically participate in PT.

In the present communication we present the results obtained by 47 slaughterhouse laboratories in 3 consecutive participations, reviewing the most important difficulties encountered and the improvements produced throughout this period and other advantages that this type of controls entail in the reliability of the results.

The advantages obtained have been:

- Ensuring a continuous competence control of the personnel (slaughterhouse and competent authority).
- Identification of systematic problems for improvement.
- Alignment of resources by gathering information and establishing measures.
- Impartiality of the evaluation, by an independent body.
- Tool that complements the audits of the Public Health Inspection Management System.
- Discussion of performance improvements found after 3 years (1/3 reduction in% of unsatisfactory results).

IMPACT OF CLIMATE CHANGE ON FOOD SAFETY AND SPOILAGE

P-082

THE ABILITY OF LACTIC ACID BACTERIA TO REDUCE THE ALLERGENICITY OF ARGININE KINASE IDENTIFIED IN BLACK SOLDIER FLY

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Predictions on population growth and climate change are driving research toward alternative protein sources to conventional ones. In this context, the larvae of black soldier fly (*Hermetia illucens*, BSF) have great potential as a sustainable source of nutrients for human nutrition thanks to a high content of proteins of high nutritional quality. However, numerous studies highlight the potential risks deriving from the consumption of insects or their components due to the presence of allergenic molecules. Among the allergenic proteins identified in arthropods and mollusks, tropomyosin (TPM) and arginine kinase (AK) are considered the main causes of allergic reactions in the population.

Lactic acid bacteria (LAB) fermentation emerges as a promising solution to mitigate allergenic risk. Through a proteolytic system composed of cell envelope proteinases (CEP), LAB can degrade proteins into oligopeptides, inactivating IgE-epitopes. This approach could help make the use of insects as a protein source safer and more acceptable for human consumption.

The purpose of this work is to investigate the proteolytic activity, aimed at the degradation of insect allergens, of 5 LAB strains with known genomes belonging to the University of Parma Culture Collection (UPCC) (*Lacticaseibacillus rhamnosus* 1019 and 1473, *Lacticaseibacillus paracasei* 2306 and 2333, *Lacticaseibacillus casei* 4186). First, through an *in silico* analysis, CEPs (PrtP; PrtR) capable of degrading the predicted epitopes of insect proteins were searched within the complete genome of LAB strains. *In vitro* assays to evaluate the proteolytic activity of the selected LAB strains were conducted on a recombinant AK protein identified in BSF. Different growth conditions (time, temperature and medium) were tested in the presence of AK and the allergen degradation was verified by using SDS-PAGE. AK immunoreactivity was then studied by performing IgE and IgG-immunoblotting assay (Dot-blot; Western-Blot). From the preliminary results it was observed that, in different conditions, some LAB strains can reduce the IgE cross-reactivity of AK, showing how the use of fermentation could represent a strategy against food allergies.

P-083

SPONTANEOUS FERMENTATION OF AGRICULTURAL BY-PRODUCTS AS A SOURCE OF LACTIC ACID BACTERIA WITH HEMICELLULOLYTIC POTENTIAL

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Wastes from cereals and some plants of economic importance in EU are hemicellulose-rich materials, and may represent an interesting source of (arabino) xylooligosaccharides (A(XOS)), compounds associated to appealing bioactive properties, including prebiotics for human use. However, economic and environmentally viable valorization strategies for hemicellulose-rich materials through (A)XOS obtainment have not yet been thoroughly explored. The objective for this work was the exploration of spontaneous microbial fermentation processes of two agricultural by-products, brewer's spent grain (BSG) and artichoke by-products, as a source of microorganisms with potential to release oligosaccharides with prebiotic potential

from these by-products.
Samples of each by-product were allowed to ferment at 25 °C, collecting samples at different times throughout 8 days. In parallel, a second batch of samples from BSG was fermented under similar conditions but simultaneously treated enzymatically with Viscozyme ® Barley. pH and bacterial colony counts were determined at each sampling point and isolates were collected and preserved for subsequent analyses including: RAPD-PCR genotyping; identification through partial 16S rRNA sequencing; and determination of their capacity to utilize hemicellulose rich materials. Further, carbohydrates composition determined by GC-FID and shotgun metagenomics profiling was determined at 0, 48 h and 8 days of fermentation.
A total of 142 and 112 isolates were recovered from BSG and artichoke by-products fermentations, respectively, with a dominant presence of lactic acid bacteria, including *Pediococcus, Leuconostoc, Lactococcus, Lactobacillus* and *Weissella* species, in consistency with the metagenomics sequencing data. Genomic and phenotypical analyses confirmed the ability of some species to metabolize hemicellulose-derived carbohydrates. Besides, an increase of the soluble fraction of oligosaccharides together with a concomitant reduction of glucose was found in all spontaneously fermented samples. However, some dissimilarities were observed depending on the nature of the by-product. Thus, the soluble fraction of the fermented artichoke was enriched in compounds with xylose, arabinose, galactose and galacturonic acid, while in the fermented BSG, new glucose-based di- and trisaccharides likely derived from the fermentation of residual starch present in the starting BSG were determined. The microorganisms herein investigated could open new avenues to develop bioconversion strategies to obtain (A)XOS from hemicellulose-rich materials.

P-084

MYOPATHIES SHORTEN SHELF-LIFE OF CHICKEN BREAST MEAT

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Myopathies in poultry significantly impact meat quality, and Wooden Breast (WB), characterized by a hardened texture, and Spaghetti Meat (SM), characterized by a disaggregation of fibre bundles, are the most concerning.
This study aimed to assess the effects of WB and SM on the visual appearance, physicochemical and microbiological parameters of chicken breasts during refrigerated storage. Seventy-five chicken carcasses, categorized as WB, SM, and control (25 each) selected in a commercial slaughterhouse were deboned, packaged in MAP (30% O2, 40% CO2, 30% N2) and stored at 4 °C to simulate supermarket shelve conditions.
Fifteen breasts (5 per group) were evaluated after packaging and on days 4, 7, 11 and 14 of storage, focusing on willingness to purchase, exudate percentage (measuring weight before package and on sampling day), instrumental colour (L*a*b*), pH (with a penetration probe), water activity (aw, using grounded meat) and microbiological parameters (Total Viable Counts (TVC), *Enterobacteriaceae* (Ent), *E. coli*, Lactic Acid Bacteria (LAB), *Brochothrix* spp., and *Pseudomonas* spp.). ANOVA and pairwise t-tests in R software tested fixed effects (myopathy presence, time, and their interaction).
Myopathies significantly affected willingness to purchase, exudate and pH, but temporal change was not different from control. WB exhibited lower willingness to purchase than control (1.73 points on a scale from 5, "would definitely buy", to 1, "would definitely not buy"), higher exudate (7.95% more), and a 0.21 increase on pH. SM scored lower on willingness to purchase (0.94 points) and had more exudate (3.54%). Instrumental colour and aw were not affected by myopathy. Initial microbial loads were similar in all breasts. However, except for *E. coli* and LAB, myopathy presence influenced microbial growth, with WB and SM showing significantly higher counts on day 11, reaching approximately

2 and 1 log more than control on all studied microbial groups, by day 14.
These findings indicate that myopathies diminish the visual appearance of chicken breasts and shorten shelf-life, entailing economic losses for producers and contributing to food waste.

P-085

SOURDOUGH: A MODEL SYSTEM TO STUDY ECO-EVOLUTIONARY DYNAMICS OF MICROBIAL COMMUNITIES IN FOOD

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The field of food microbiology experienced a technological transformation through the advent of DNA sequencing methods and microbiome research in general over the last decade. Using these novel technologies, researchers around the globe catalogued microbial communities of various foods and their production environment at an unprecedented depth. While this led to an increased understanding of the taxonomic and functional diversity of microorganisms along the food production chain, the complex microbial interactions that shape community assembly are still poorly understood. One of the core issues is the empirical nature of microbiome studies. Thus, converting the correlations observed in OMICs data into an actual mechanistic understanding of what exactly is happening under specific circumstances remains challenging. In modern biology, model organisms are studied in controlled experiments to investigate specific biological processes. However, they are studied in pure cultures and consequently, are not suitable to investigate ecological and evolutionary theories on the formation and dynamics of entire communities.
Therefore, we need to establish model microbial communities that we can control and manipulate for food microbiome research purposes. As such, fermented foods, such as sourdough, offer great potential and have been proposed as model systems before. Sourdoughs are populated by microorganisms, especially yeasts and lactic acid bacteria, which are responsible for its characteristic properties. Notably, the microbial community of sourdoughs is less complex than communities from other environments and they develop in a short manner of time, within days to months. This rapid development facilitates predictable access to many replicated samples over relatively short time periods, enabling the exploration of various experimental questions.
In a pilot trial, we established stable sourdough microbial communities and then challenged them with different types and amounts of flour to investigate whether the communities remain stable or to what extent the invasive microorganisms change the whole community structure. Investigating these different outcomes of community coalescence can have ramifications for the use of microbial communities in biotechnology, food production, and medicine. In addition to this, we will showcase other microbial ecology and evolution experiments that we are currently performing with sourdough in our lab.

P-086

MICROBIAL DYNAMICS IN EQUILIBRIUM MODIFIED-ATMOSPHERE PACKAGED FRESH-CUT ROMAINE LETTUCE. A CULTUROMICS APPROACH

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Introduction

Fresh-cut vegetables house a diverse and complex microbial community, influenced by a wide range of pre- and post-harvest factors, posing challenges in predictability.

Purpose

This study wants to capture the composition, diversity, and dynamics of the microbiota of equilibrium modified atmosphere packaged (EMAP) fresh-cut Romaine lettuce. Additionally, seasonal variations in the microbial community and the impact of an industrial chlorine washing process were assessed.

Methods

Whole heads of Romaine lettuce sourced from Murcia, Spain were sampled in triplicate in January, June, and October 2022. Following manual cutting, the vegetables were treated by washing in potable water and a 200 ppm chlorine solution, respectively. Treated and untreated vegetables were subsequently stored under EMAP conditions (3% O_2, 10% CO_2 and 87% N_2) at 7 °C for a maximum of 7 days. Sampling and analysis were conducted on the day of processing, and after 7 days of storage. Employing a culture-dependent approach, quantitative data were obtained through plating on selective and non-selective culture media, followed by bacterial isolation and taxonomic identification using MALDI-TOF MS.

Results

Plate counts revealed a highly variable psychrotrophic aerobic count depending on the season, dominated primarily by *Pseudomonas* spp.. Enterobacteriaceae initially exhibited up to 2 log CFU/g lower counts but displayed a higher growth potential during storage compared to *Pseudomonas* spp.. Chlorine washing induced a significant reduction up to 2 log CFU/g in total count, which was, however, not observable at the end of storage. In total, 808 isolates were identified using MALDI-TOF MS, of which 396 isolates (49%) were identified as *Pseudomonas* species, and 199 isolates (25%) as *Pantoea agglomerans*. Products harvested in June exhibited greater variability in Enterobacteriaceae isolates, while those harvested in October demonstrated a higher variability in *Pseudomonas* isolates.

Significance

Understanding the dynamics of the food microbiota is crucial, having both a direct (presence of opportunistic pathogens) and indirect (interaction with foodborne pathogens) impact on food safety.

P-087

DISTRIBUTION RATE OF *LISTERIA MONOCYTOGENES* IN READY-TO-EAT PROCESSED SEAFOOD PRODUCTS IN JAPAN AND A POTENTIAL RISK ASSESSMENT

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Listeria monocytogenes is the causative agent of human listeriosis, causing serious symptoms such as sepsis and meningitis, mainly in immunocompromised people, such as older adults and pregnant women. *L. monocytogenes* can grow at low temperatures, such as 0-10 °C, and in ready-to-eat (RTE) processed foods, often causing food poisoning. In Japan, there are many opportunities to eat processed RTE seafood, but there is insufficient information about the distribution rate of *L. monocytogenes*, and the risks for human health. The purpose of this study was to investigate the distribution rate of *L. monocytogenes* in RTE processed seafood products and to evaluate its potential risks based on the results of genetic analysis.

The distribution rate of *L. monocytogenes* was investigated in five types of RTE processed seafood products: cod roe, seasoned cod roe, salmon roe, minced tuna, and smoked salmon. Five hundred samples, with 100 samples of each food, were purchased from a retail store and stored at 4 °C until the expiration date. Qualitative testing of *L. monocytogenes* was performed according to the ISO method (ISO11290-1: 1996), and a typical suspected colony on selective medium was picked. The 16S rRNA gene region was sequenced, and the bacterial species was identified using a Blast search. Food samples that were positive for *L. monocytogenes* were also quantitatively tested using the most probable number (MPN) method, and the number of *L. monocytogenes* in the food was calculated.

The positivity rate of *L. monocytogenes* in RTE processed seafood products was 28.0% for minced tuna, 14.0% for salmon roe, 7.0% for cod roe, 12.0% for seasoned cod roe, and 9.0% for smoked salmon. The highest MPN values were found in minced tuna, smoked salmon, and salmon roe, at 46 MPN/g. The MPN values of other samples were extremely low, ranging from < 0.3 to 0.9 MPN/g. Genetic analysis was

performed on the *L. monocytogenes* isolates to determine their genotype and the presence of pathogenic genes, and the results will be presented at the venue.

P-088

EXPLORING COLD-SMOKED SALMON SPOILAGE THROUGH THE SIGNATURE MICROBIOME OF THREE DIFFERENT PROCESSING PLANTS

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Cold-smoked salmon (CSS) products are very perishable and during their refrigerated storage the development of microorganisms leads to spoilage.
CSS core microbiota is mainly composed of bacteria with an important taxonomic and phenotypic diversity that evolve all along the storage. It results from an association of bacteria originating from fish and its marine environment but also from bacterial implantation occurring during the food chain processing. Its microbiota is also influenced by abiotic parameters like the process undergone by the product (i.e. smoking, salting), the packaging (vacuum) and the storage temperature. At the end of the process, CSS microbiota presents specific bacterial signature related to plant processing environment. The specific endogenous microbiome composition as well as the bacterial interaction occurring during storage are important biotic parameters known to influence the variability of off-odours and off flavours production in the product.
The current work focuses on the characterisation of the CSS signature microbiota and its influence on the organoleptic changes for three different processing plants during storage. An integrated approach combining multiple techniques like omics analysis (metabarcoding, volatilome analysis), classic microbiology and sensory analysis was used. A statistical approach was performed to draw links between these different groups of data and highlight the impact of these different bacterial signatures on the CSS quality.
In addition to the decryption of the bacterial communities, the microbiome functionality of CSS is studied. Using shotgun metagenomics, 15 MAGs (Metagenomic Assembled Genomes) were obtained and mined to identify functional responses linked to spoilage (i.e spoilage metabolites biosynthesis pathways).

P-089

ANTIBIOTIC RESISTANCE AND BIOGENIC AMINES IN RIPENED CHEESE PRODUCTION. A CRITICAL EXAMINATION IN THE POCTEFA REGION TO IMPROVE FOOD SAFETY AND QUALITY STANDARDS

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Background

The intake of cheese produced with raw milk might convey antimicrobial resistances (AMR) and biogenic amines (BAs) that might cause severe health disorders in consumers. The World Health Organization classifies AMR as one of the main threats to humanity. These antimicrobial resistances mainly originate from the extensive use of antibiotics in human and veterinary medicine, and food chain is recognized as a transmission pathway. Furthermore, research indicates that certain commercial cheeses exhibit detectable concentrations of toxic BAs, due to the metabolism of spoilage microorganisms, exceeding the limits established in the UE for other products.

Objective

Enhancing the safety and quality of cheese production by conducting a comprehensive analysis of the main health risks (antibiotics, antibiotic resistance genes, biogenic amines) associated with the consumption of ripened cheeses and designing innovative solutions for their detection and control.

Methods

A sample bank will be established with ripened cheeses produced with cow, goat, and sheep raw milk, and marketed

in the POCTEFA area (from cities such as Zaragoza, Logroño, Perpignan, and Toulouse). First, the presence of antibiotic residues and BAs will be investigated using immunochemical tests, and positive samples will undergo chromatographic analysis to confirm and quantify the presence of antibiotics and BAs. In addition, the presence of antibiotic-resistant microbiota, antibiotic-resistant genes and BAs-producing microbiota in cheeses will be assessed through culture-dependent and -independent (metagenome sequencing) techniques. Finally, innovative strategies based on new biocides formulations will be developed to control antibiotic-resistant and histamine-producing microorganisms in cheeses, farms, and industries of the POCTEFA area.

Results and impact on society

This project will validate detection techniques and transfer sustainable and marketable cleaning and disinfection measures to reduce the presence of antibiotic-resistant and BAs-producing microorganisms in ripened cheeses of the POCTEFA area. This initiative will establish a network of interregional agri-food excellence, fostering collaboration among universities, public entities, and agri-food companies from Spain (Universidad de Zaragoza, Universidad de La Rioja, Zeulab S.L., OX-CTA S.L) and France (INRAE, Université de Perpignan Via Domitia).

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P-090

INVESTIGATING CO-OCCURRENCES TO DECODING THE INTERPLAY BETWEEN BACTERIAL COMMUNITIES AND THE FOODBORNE PATHOGEN *CAMPYLOBACTER* IN BROILER MEAT

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Campylobacter continues to be the primary source of zoonotic infections in Europe, persisting throughout the food chain, within the broiler meat microbiota. The microbial communities associated to food products are crucial indicators of food quality and safety, potentially impacting the proliferation or inhibition of foodborne pathogens such as *Campylobacter*.

The objective is to identify bacterial species positively or negatively correlated with *Campylobacter*, potentially influencing *Campylobacter* behavior by restricting or, conversely, promoting its survival on broiler carcasses.

480 carcasses were collected after chilling over 10 slaughter days over 3 months in a French slaughterhouse. *Campylobacter* was enumerated by plate counts on CASA agar and bacterial communities were identified using 16S rRNA gene metabarcoding, from carcass rinses before (day 0, D0) and after seven days of refrigerated storage (D7). CoNet software was used to perform network analyses employing the spearman correlation coefficient adjusted to 0.3 and Bonferroni correction.

184 clusters were identified before storage, with the dominant genus being *Lactobacillus* (25%), *Limosilacobacillus* (9%) and *Bacillus* (8.9%). After storage, 96 clusters were identified, mostly belonging to *Pseudomonadaceae* (37.5%), *Moraxellaceae* (32.7%) and *Shewanellaceae* (18.9%). *Campylobacter* survived during the 7 days of refrigerated storage at ambient atmosphere, decreasing slightly from an average concentration of 1.38±0.95 log CFU/mL of carcass rinses to 1.25±0.95 log CFU/mL at day 7 (Wilcoxon test, $p = 0.0499$). At D0, *Helicobacter pullorum*, *Bifidobacterium pullorum* and *Barnesiella visceriola* were shown as co-occurring with *Campylobacter* (interaction weight = 0.435,

0.437, 0.379, respectively). These species, mainly found in the poultry digestive tract, suggest a co-contamination of the carcasses with the intestinal content during the first steps of the slaughter process. At D7, *Campylobacter* is co-occurrent with *Lactococcus raffinolactis* and *Pseudomonas sp.*; a prevalent genus in refrigerated poultry meat. Additionally, psychrotrophic spoilage bacteria like *Shewanella baltica* and *Shewanella frigidimarina*, were excluded in presence of *Campylobacter*.

Refrigerated storage favors the growth of anaerobic and psychrophilic bacteria, but the foodborne pathogen *Campylobacter* can also survive. The identification of bacterial species associated with the presence or absence of *Campylobacter* on carcasses may help understanding the biological mechanisms underlying its survival on poultry meat and be used in biocontrol strategies.

P-091

ISOLATION, IDENTIFICATION AND SPOILAGE CHARACTERIZATION OF *PSEUDOMONAS* SPP. FROM SPANISH MILK AND DAIRY PRODUCTS

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The widespread use of refrigeration along the dairy chain has improved the quality of dairy products by limiting the growth of spoilage and pathogenic microorganisms. However, an extended cold storage of raw milk favours psychrotrophic bacteria growth, predominantly *Pseudomonas* spp., well recognized as dairy spoilage organisms. *Pseudomonas*-related spoilage in milk usually results in flavour, odour, texture (due to production of bacterial enzymes) and colour defects. Although pasteurization eliminates these psychrotrophs, their heat-resistant proteases and lipases remain active in pasteurized and even UHT milk. Furthermore, *Pseudomonas* are commonly associated with post-pasteurization contamination of milk and dairy products due to their ability to form biofilms on the surfaces of dairy equipment. As contaminants of fresh cheeses, *Pseudomonas* can induce serious defects, such as anomalous colour, slime production, texture defects, and unpleasant flavour. In terms of quality, psychrotrophic *Pseudomonas* have become a major concern for the dairy industry as leading cause of spoilage and significant economic losses.

In the present work, we have isolated and characterized spoiler *Pseudomonas* spp. from Spanish milk (raw and pasteurized) and dairy products (whey, cream, cheese). Additionally, a tofu sample with anomalous pigmentation was also analysed. A total of 248 presumptive *Pseudomonas* colonies were isolated from *Pseudomonas* Agar Base with CFC selective supplement. Preliminary characterization of the isolates using a genus-specific region of 16S rDNA gene, revealed that 84% (n = 208) of the isolates were *Pseudomonas* spp.

These isolates were typed by PFGE using the *Xba*I restriction enzyme, and were grouped into 108 pulsotypes. PFGE analysis revealed a high genetic diversity among *Pseudomonas* strains with a *D* index of 0.989. *Pseudomonas* pulsotypes were classified into 22 putative species by *ileS* gene sequence analysis, being *P. fluorescens* (26%), *P. fragi* (21%), *P. lundensis* (9%) and *P. shahriarae* (7%) the main isolated species. Most pulsotypes (70%) showed proteolytic activity on Milk Plate Count Agar. However, only 35% and 15% of the pulsotypes showed lipolytic activity on tributyrin and Tween 80 agar, respectively. On the other hand, 89% of the pulsotypes were able to produce some colour defect on fresh cheese at refrigeration temperatures.

P-092

THE INFLUENCE OF DAIRY ENVIRONMENT AND PRACTICES ON THE MICROBIOME OF RAW EWE MILK-DERIVED PROTECTED DESIGNATION OF ORIGIN CHEESES: A SHOTGUN SEQUENCING APPROACH

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Cheese microbiota is of great importance since it contributes to numerous biochemical processes that determine cheese quality, such aroma, and safety, including toxicity. However, differences not only among types of cheeses but also among producers of the same type of cheese have been reported. Sources of bacteria in raw milk and cheese are diverse and complex, which may be related to flock management and feeding, as well as materials and ingredients employed during milking or in the dairy environment. However, no study has comprehensively analysed all the potential factors that could affect, evaluating the contribution of each one.

Thus, the presented work was conducted within the Protected Designation of Origin Idiazabal cheese, a semi-hard or hard cheese manufactured from raw ewe milk in the Basque Country (Southwestern Europe). Several samples were collected from two closely located artisanal dairies to avoid geographical factors, including all potential microbial sources, namely feed (straw, grass, home-made feed and commercial feed), teat skin surfaces, raw milk, food contact and non-food contact surfaces of dairies, rennet, whey, brine, and ripened cheeses (60-days-ripened). DNA was then extracted from fresh samples, 150 bp paired-end libraries were prepared (Nextera XT kit), and shotgun sequencing was conducted (Illumina NextSeq 500 platform). Bioinformatic analysis included taxonomic classification against the Kraken2 database and SourceTracker analysis to identify potential microbial sources.

A total of 414,367,387 high-quality paired-end reads were obtained among all samples, with a percentage of microbial reads of 82.1±31.3%. Reads differed among sample type ($P \leq 0.05$), which were confirmed by α-/β-diversity and taxonomic analyses. Over 1300 bacterial genera and 3400 species were identified among all samples, with 53 genera and 58 species predominating (> 1%). SourceTracker analysis identified commercial feed and teat skin surfaces as the primary bacterial sources for raw milk (34.0 ± 14.5% and 33.6 ± 21.5%, respectively), sources of *Lactococcus* and, *Jeotgalicoccus* and *Psychrobacter*, respectively. After the cheese manufacturing, the rennet was reported as the main source of the cheese microbiota (37.8 ± 8.46%), mainly *Lactobacillus_H, Lactobacillus, Lactobacillus_G, Prevotella* or *Streptococcus*; together with the commercial feed (15.8 ± 1.68%) and teat skin surfaces (10.5 ± 1.86%).

P-093

IDENTIFICATION OF BREAD SPOILAGE ORGANISMS ISOLATED FROM PAR-BAKED BREAD PRODUCED IN EUROPE

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The shelflife of food products with high water activity ($0,94 \leq a_w \leq 0,99$) is mostly limited by microbiological spoilage. Being a high moisture product, bread is easily spoiled by yeast, bacteria and molds, resulting in a high economic loss. Moreover, these spoilage organisms can negatively affect consumer's health. As the species causing spoilage is dependent on the storage temperature and bread type, we were interested in identifying the dominant spoilage organisms of par-baked bread from different countries in Europe.

A total of 35 samples of par-baked bread were obtained from different production sites located in Europe. All samples were packaged under modified atmosphere. To

allow microbiological growth, we opened the packaging in a laminar flow cabinet, resulting in exposure to sterile oxygen. Upon spoilage, the spoilage organisms were isolated on PDA plates and by implementing DNA barcoding, we identified 101 isolated organisms. Sequencing of the ITS region allowed identification of the genus of each isolated organism, however, sequencing of additional genes was necessary for identification on the species level.

Most strains could be identified by using one additional primer pair. The majority, or 34,65%, was identified by sequencing the b-tubulin gene using the universal primer pair Bt2a and Bt2b, while 30,69% of the strains were identified using the primer pair Btub2Fd and Btub4Rd. Sequencing of the RNA polymerase II second largest subunit resulted in identification of 14,85% of our collection. The primer pair CMD5 and CMD6, used for amplifying and sequencing of the calmodulin gene, resulted in species identification of 4,95%. The remaining 14,85% of our collection was identified by combining sequencing data of the b-tubulin gene, RNA polymerase II second largest subunit and/or the calmodulin gene. In correspondence with what is reported in literature, most of our collection belonged to the *Penicillium* species (97,03%), while only few *Aspergillus* species were isolated (2,97%). The most dominant species were *Penicillium palitans*, *Penicillium chrysogenum* and *Penicillium bialowiezense*.

Based on this data, an approach for fast and easy identification of (bread) spoilage organisms could be developed. This could have an impact on the development of optimized strategies, thereby targeting the specific spoilage organism and preventing food waste.

P-094

INSIGHTS INTO TERRITORIAL CHARACTERISTICS OF *NEBBIOLO* VINEYARDS AND EFFECT ON ALCOHOLIC FERMENTATION BY IN-DEPTH UNDERSTANDING OF MICROBIAL COMMUNITIES

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Wine production is a process in which different factors from vineyard to winery could affect the final product. Microbial activity is an integral part of wine making, as it can affect the health of the grapevine, the fermentation process, as well as the quality of the wine. Despite the observed correlations between vineyard and climatic conditions, the degree to which these microbial patterns are associated with the chemical composition of wine from specific wine regions is poorly investigated.

The aim of this study was to explore the regional distribution patterns of fungi and bacteria and their association with the chemical composition of wine. Thirty-eight Nebbiolo vineyards from seven different geographical zones, divided into two macro areas in Piedmont and one in Lombardy, were selected for this study. Grape samples from each vineyard were collected at harvest time, to examine the geographical dispersion trends of fungi and bacteria and explore their correlation with the chemical composition of wines.

For this purpose, harvested grapes were processed at the experimental winery of the University of Turin (Bonafous, Turin, Italy), and samples were taken for analysis at different time points of the alcoholic fermentation. Fungal and bacterial communities were investigated by high throughput sequencing on bacterial 16S rRNA and fungal 26S rRNA genes. Chemical analyses of the main oenological parameters were carried out to determine the correlations between regional distribution patterns of microbial communities and their association with wine chemical composition.

The importance of this study is to understand the possible use of microbiota as an indicator of origin and, in the meantime, as a predictor of the chemical composition of wine and, therefore, of wine quality.

P-095

INFLUENCE OF ENVIRONMENTAL FACTORS ON THE MICROBIAL ECOLOGY OF THE PRODUCTION PROCESS IN A MEAT PRODUCTION PLANT

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Microbial ecology is specific of each type of food processing plant, but can by influenced by environmental factors. For this reason, the aim of this study was monitoring the microbial populations of a complete production procedure, from raw materials to final products at different seasons of a meat production plant.
For this purpose, 3 samplings (summer, winter and spring) were done in farms, hatcheries, slaughterhouse, cutting rooms, feeds, faeces and final products. Samples were analysed by traditional microbiology and 16S metagenomic analysis. Antibiotic resistance was also identified by inhibition halos and antibiotic resistance genes (ARGs) analysis.
The results showed that the microbial plate counts were higher in summer and lower in winter, with intermediate results in spring, confirming the effect of environmental conditions such as temperature in the microbial populations. The 16S analysis obtained 25.725 Amplicon Sequence Variants (ASVs) and identified *Proteobacteria* in feed and final products, *Firmicutes* and *Actinobacteria* in faeces as the major phyla. Regarding production areas, in farms and slaughterhouses the most important phyla were *Proteobacteria, Firmicutes* and *Actinobacteria,* very similar to the living animals microbiome. In contrast, in cutting rooms a reduction of *Firmicutes* and an increase of *Bacteroidetes* were observed. Focusing on the genus, the most abundant ones were the following: *Acinetobacter, Lactobacillus, Corynebacterium, Pseudomonas, Staphylococcus, Photobacterium, Escherichia-Shigella* and *Clostridium*. In addition, some pathogenic and biofilm-forming microorganisms were identified such as the genus *Escherichia* in farms, seasonal *Campylobacter* in slaughterhouses and cutting rooms, *Clostridium* in farms and hatcheries and *Pseudomonas* throughout the whole production process. Analysis of antibiotic resistance identified lincomycin as the antibiotic against which bacteria have developed the most resistance and also ERM-B (Erythromycin resistance related gen) were detected in all areas of all samplings.
In conclusion, environmental factors have a direct influence on the specific microbial ecology of a meat production plant and, consequently, on productivity, quality and food safety.

P-096

ISOLATION AND SCREENING OF PROBIOTIC POTENTIAL LACTIC ACID BACTERIA FROM FERMENTED PLANT-BASED PRODUCTS AND THEIR GABA PRODUCTION ABILITIES

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Lactic acid bacteria (LAB) are widely used probiotic microorganisms, but the screening and examination of novel alternative isolates is still of great interest from industrial and scientific point of view. In the current study, eight lactic acid bacteria from *Enterococcus, Pediococcus, Leuconostoc, Lactiplantibacillus* and *Lacticaseibacillus* genera isolated from different fermented plant sources were identified based on 16S rRNA gene based identification method.
These isolates were evaluated for their probiotic potential using a variety of analysis: Hydrophobicity, auto-aggregation, co-aggregation, antimicrobial activity, and antibiotic susceptibility. While two of the isolates, *Leuconostoc mesenteroides* DD_NB_B49 and *L. mesenteroides* DD_NF_B59, exhibited limited probiotic potential, other isolates showed more remarkable results. Gamma aminobutyric acid (GABA) is a type of non-protein amino acid produced by microorganisms and plants, and there is an increasing interest in production of new and health benefited food products enriched by GABA. Therefore, LAB isolates with probiotic potential were evaluated for their ability to produce glutamate decarboxylase (GAD) enzyme, and all of them showed high activity.
Since it was shown that addition of monosodium glutamate (MSG), which is the substrate of GAD enzyme, had a significant impact on the synthesis of GABA, fermentation

medium for LAB was supplemented with 2% MSG. In light of the findings, isolates were incubated for 72 h, and the GABA contents in the fermented medium were found between 27.46-39.25 mg/mL which is a considerable amount. On the other hand, an increase of 2.88-4.06 log CFU/mL was observed in viability.
Consequently, new isolates with probiotic potential are presented to the literature and shed light on the production of naturally GABA-enriched fermented products. In the present study, new probiotic potential isolates have been presented to the literature for production of GABA-enriched fermented products by natural means.

P-097

DECODING THE METAGENOMIC DIVERSITY IN ANIMAL-DERIVED MEAT AND PLANT-BASED MEAT ALTERNATIVES

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Plant-based meat alternatives offer an environmentally sustainable option as a substitute for animal-derived meat. These products aim to mimic the taste, texture, and nutritional profile of animal-derived meat. However, consumers are unsure of the food-safety and shelf-life implications of consuming plant-based products compared to traditional meat equivalents. Study aim is to compare metagenomic diversity in animal-derived meat and plant-based meat alternatives and how this impacts the food-safety and spoilage characteristics.
Fresh beef, pork, and poultry, as well as cooked pork and poultry products, were sourced along with 65 Ready-to-Cook (RTC) plant-based meat alternative products. Samples were stored at 5°C before analysis. Meta-data pH, water activity (Aw), moisture, salt, including manufacturer, protein source, and any preservatives used, was established. 16S and ITS metagenomics sequencing for bacterial and fungal communities respectively, was performed for population analysis. Relative abundance plots were created in R, using OTUs (operational taxonomic units) classified to the genus level. Median values of abundance were calculated for each meat sample per timepoint across the replicates. Any OTU's present at <1% were collapsed into a category termed "Other". Beta diversity analysis was done using the R package “PhyloSeq” v1.34.0.
Initial microbial diversity was relatively high in fresh meat products. Most abundant bacteria included *Carnobacterium*, *Pseudomonas*, *Photobacterium*, *Latilactobacillus,* and *Lactococcus*. A dramatic decrease in bacterial diversity was observed during storage with facultative anaerobic Lactic Acid Bacteria (LAB), *Leuconostoc* and *Latilactobacillus* dominating. In cooked products, *Carnobacterium, Leuconostoc* and *Latilactobacillus*, represented the prevailing Genera. Overall, diversity in plant-based meat products varied significantly, with some population structures very homogeneous, while others were quite diverse. Microflora and ultimately shelf-life, were highly influenced by product characteristics and manufacturing site. LAB were dominant in high Aw products, while yeasts like *Debaryomyces* and *Candida* were dominant with low Aw. Interestingly, *Sphingomonas* and several Gram+ve spore formers, were detected in abundance across plant-based meat alternatives of soya, wheat, vegetable and mycoprotein.
This work demonstrates the diversity of microbial populations found in traditional meat and plant-based meat alternative products. This knowledge will be critical to optimise preservation strategies to ensure food-safety and enhance the shelf-life.

P-098

STUDY ON THE MICROBIAL COMMUNITIES OF THE *TAGGIASCA* OLIVE TREE AND FERMENTED OLIVES

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The olive tree (*Olea europaea L.)* represents one of the most important crops in the Mediterranean regions from an economic and agronomic standpoint. In Liguria (North-West Italy), the Taggiasca cv. is grown in a peculiar landscape characterized by steep hills and terraced slopes and both Taggiasca extra-virgin olive oil and fermented olives are produced and considered high-quality products. In this study, the bacterial and fungal communities of above- and below-ground plant compartments were evaluated with an amplicon sequencing approach. The microbial ecology of the fermented fruits was also analyzed through culture-dependent and independent methods coupled with volatilome analysis via GC-MS.

The plant-related ecosystems of 24 trees considered were rhizosphere, soil, bark, leaves and raw olives. Sampled trees were distributed in different areas of the orchard according to a slope gradient to monitor the variability provided by altitude. For fermented fruits, two batches were considered, and both brines and olives samples were analyzed. Culture-dependent analysis showed that the most abundant species overall were *Candida diddiensae, Wickerhamomyces anomalus, Pichia membranifaciens* and *Aureobasidium pullulans*, with differences in species distribution between batch 1 and 2.

Amplicon-based sequencing confirmed the presence and dynamics of *W. anomalus* in batch 1 in brines and olives throughout the fermentation, while *Cyteromyces nyonsensis* and *Aureobasidium* spp. dominated the fermentation of brines and olives in batch 2, respectively. Volatilome results showed a prevalence of hydrocarbons, alcohols and esters, with main differences in volatiles distributions as a function of the fermentation time.

This study represents a first effort towards the understanding of the complex olive tree related microbiomes, as well as an insight into the microbiological and biochemical quality of the fermented fruits. It will also set the basis for future connections between the plant ecology and the fermented olives' one.

P-099

FERMENTED SAUSAGE MICROBIOME: INVESTIGATION, STORAGE AND EXPLOITATION

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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. Indeed, based on literature survey and integrating the expertise of the SUS-MIRRI.IT partners, 12 SOPs were created. Only food, soil and water were subsequent tested in intra-laboratory validation trails.

For the characterization of community structure and diversity, 46 spontaneously fermented sausages from different regions of Italy were collected and, following the created SOPs for food sampling, were subjected to microbiological and metabolomic analysis. The study of the bacterial diversity of

the different fermented sausages showed a high variability between samples. Besides, basing on sensory analysis, 3 samples were chosen for the optimization of conditions and protocols for long-term preservation and propagation of microbiomes.
The microbiome study showed a variability among different preserved forms and propagation media.

P-100

METAGENOMIC ANALYSIS OF VINEYARD MICROBIOTA: SPATIAL AND TEMPORAL DYNAMICS

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Microorganisms colonizing the vineyard possess broad functional capabilities that influence vine health, growth, and productivity. Furthermore, this microbiota is transferred to grape must and affects quality, chemical composition, and volatile profile of the resulting wine. Recent studies highlighted strong correlations between the plant and soil microbiome, environmental conditions, as well as geographic area. However, the vineyard microbiota community composition, functions, and evolution across various seasons throughout the year remains poorly investigated.
In this study we characterized the spatial and temporal dynamics of microbial populations of *Vitis Vinifera* cv. Barbera grapevines by focusing on microbiome tracking using shotgun sequencing. Bacterial and fungal populations were followed on samples of rhizosphere soil, leaves, and grapes collected at different times from spring until the harvesting.
In general, α-diversity was higher in fungal than bacterial communities. Regarding fungal population, it was constant in root soils and generally higher than other samples, while grapes and leaves showed an opposite behaviour during time. The PERMANOVA analysis demonstrated that fungal and bacterial β-diversity was significantly affected by sample origin (p value < 0.001) and not by time.
Linear discriminant analysis (LDA) effect size (LEfSe)analysis on fungal population identified 164 significantly discriminant taxa considering the samples origin, while only two evaluating the sampling time; namely *Mucor* (summer) and *Tilletaria* (spring).
At level 2, KEGG database genes associated with aminoacids metabolism were the most abundant in all samples, followed by carbohydrates metabolism in grapes and soils. Divergently, in leaves samples, genes most abundant were associated to oxidative phosphorylation and purine metabolism.
The findings of this study provide new insights into the evolutional and functional characteristics of the vineyard microbiota and contribute in our understanding of how it affects wine quality.

P-101

UNVEILING THE CULPRIT: HALOTOLERANT *TETRAGENOCOCCUS* IN MULTI-AMPLICON MICROBIOME ANALYSIS OF DEFECTIVE DRY CURED HAMS AND PANGENOMIC INSIGHTS

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The production of dry-cured ham Struggles with ongoing technological challenges, leading to defects and financial setbacks. Traditionally, the identification of microbial defects relies on the "probe and sniff technique". These potential defects, broadly, classified into deep spoilage and surface defects, pose a significant hurdle for the ham industry.
In this study, we sampled and analyzed three defective dry-cured hams, tested from 36 months onwards, exhibiting noticeable defects, and three normal hams from a producer in Andalusia, Spain. The objective was to scrutinize the microbiome of these samples to understand whether the observed defects were associated with shifts in the microbial population.

16s multi-amplicon sequencing was performed using the Ion 16S Metagenomics Kit and Ion Torrent Sequencing Platform. Raw reads were then analysed using QIIME2 for data processing. Alpha-diversity and beta-diversity indexes, ANCOM test, Linear Discriminant Analysis (LEfSe) and co-occurrence network analysis were computed and visualized using the "microeco" R package.

A pangenomic and functional enrichment analysis of genomes available in the NCBI database was conducted.

The analysis unveiled a distinctive association between *Tetragenococcus*, a genus recognized for its prevalence in fermented fish and vegetable products, as well as in cheese microbiota, and defective dry-cured hams. Both the LEfSe and ANCOM tests concurred in highlighting this taxon as a potential culprit.

Subsequent scrutiny of *Tetragenococcus* genomes highlighted disparities between the two species *T. koreensis* and *T. halophilus* in terms of metabolic activity. Notably, *T. koreensis* manifested a distinctive set of genes encoding enzymes for carbohydrate metabolism.

This study not only provided the opportunity to address the producer's inquiry regarding the potential role of a specific microbial population in causing the defect, but also indicated the likelihood of a halotolerant microorganism being the culprit.

While previous research has linked halotolerant microorganisms to defects in dry-cured meat, this represents the first time *Tetragenococcus* has been linked to such cases. This result sheds light on *Tetragenococcus'* involvement in defected dry-cured hams.

Ongoing investigations will focus on identifying the specific *Tetragenococcus* strains responsible for these defects, helping to refine our knowledge of their impact on dry-cured ham production.

P-102

ASSOCIATIONS BETWEEN MICROBIOTA AND GEOGRAPHICAL ORIGIN OF SPONTANEOUSLY FERMENTED FOOD MATRICES: THE CASE OF GREEN COFFEE BEANS AND COCOA BEANS

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For thousands of years, people have enjoyed the distinctive flavors, extended shelf life, and nutritional benefits provided by microorganisms in fermented foods and beverages. In recent years, consumers have become increasingly attentive to the quality of these products, particularly in relation to their geographical origin, and have noticed that the same fermented products from different geographical origins have different flavor profiles and therefore differences in the quality of the final product. The production process of coffee and chocolate is long and consists of several stages that affect the microbiota and therefore the quality of the final product. This is due to agricultural and farm practices, weather conditions, and available infrastructure, as well as post-harvest processing methods. The microbial ecology evolves during the different stages of processing and each microbial group in green coffee beans and cocoa beans can be associated with different functionalities and the type of microbiota present could be used as an indicator of origin.

This study aimed to examine the microbial ecology of green coffee beans and cocoa beans, respectively, to investigate a possible correlation between the microbiota and the geographical origin of these fermented foods and to understand how the microbial communities can be used as indicators of origin. Samples were collected from thirteen different regions for both cocoa and green coffee beans at the end of the fermentation process to study the patterns of geographic distribution of fungi and bacteria and their correlation with final product quality. For this intent, fungal and bacterial communities were investigated by high throughput sequencing on bacterial 16S rRNA and fungal 26S rRNA genes.

The importance of this study is to understand the possible use of microbiota as an indicator of origin and, therefore, of the quality of the final product.

P-103

IMPACT OF STORAGE TEMPERATURE ON THE MICROBIOLOGY, SENSORY QUALITY, AND METABOLOMICS OF INDUSTRIALLY PACKAGED BROILER

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Research into microbial metabolism is essential to understand spoilage. In this study, the quality of modified atmosphere packaged (40% CO_2: 60% N2) broiler was investigated during storage at 4 and 6 °C. The products were assessed daily by sensory evaluation and the study was terminated on the day after the sensory panel rated the samples to be spoiled. In addition to sensory evaluation, bacterial counts were determined daily using selective/specific media for the major bacterial groups, and samples were taken for nontargeted metabolomics analysis (ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry, UPLC-QTOF-MS).
Spoilage was detected by sensory evaluation on day 8 at 6 °C and three days later (day 11) at 4 °C. The odor of the spoiled broiler was described as a sour. Also sulphur odor was observed in broiler stored at 6 °C. Total bacterial levels reached log6 on days 7 and 5 and the log8 on days 12 and 7 at storage temperatures of 4 and 6 °C, respectively. Lactic acid bacteria (LAB) dominating at both storage temperatures, while enterobacteria were present at notable levels (> log 4) only in the broiler stored at the higher storage temperature. A total of 31712 metabolites were identified, mostly small peptides, amino acids, and polyamines. Over half of the observed metabolite differences (Spearman correlation, $q < 0.05$) were related to sensory evaluation (18438) and bacterial growth (15616). Most of these features (14653) were common to both variables. A total of 10930 metabolites varied with time, while only 29 differences were related to temperature.
In both temperature groups, the concentration of inosine-5'-monophosphate (IMP) decreased with time, reaching a very low level in samples stored at 6 °C. This suggests microbial utilization of ribose, which is a common feature for meat LAB. Since storage temperature significantly affected the microbiota, we also expected a greater impact on the metabolite profile.
Particularly interesting are the changes in amines. Among biogenic amines, the amount of tyramine increased, especially at 6 °C. Spermidine was only formed at 6 °C, and further research will investigate its relationship with bacterial growth. The volatile profile of the samples will be examined in future studies.

P-104

FEASIBILITY STUDY OF A MICROFLUIDICS SYSTEM FOR THE EVALUATION OF CLEAN-IN-PLACE TREATMENTS: APPLICATION TO *GEOBACILLUS STEAROTHERMOPHILUS* BIOFILM

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The dairy powder industry faces contaminations by thermophilic spore-forming bacteria (mainly *Geobacillus stearothermophilus*) able to settle in biofilms in production plants and to contaminate the final powders thanks to suited growth and sporulation capacities. Therefore, cleaning in place (CIP) operations play a key role in industrial facilities to maintain the microbiological quality of these food products. It is then important to be able to evaluate the efficiency of the industrial cleaning treatments. Pilot installations reproducing, on a smaller scale, the fluid circuits encountered in industry do exist. However, these installations use large volumes of solutions and energy supply for heating. Thus testing CIP protocol with a pilot remains expensive and time-consuming. Recently, microfluidics tools have been developed and allow to circumvent many various issues on a very small scale.
We therefore performed a first study of a microfluidics system to evaluate the feasibility and the effectiveness of CIP treatments. We applied it to a *G. stearothermophilus*

industrial strain biofilm and compared it to a pilot CIP rig.
The system is based on a pressure driving flow and a distribution valve which allows the different fluids to be precisely injected into a microchannel. The fluid injection can be adjusted to mimic the CIP conditions. The *G.stearothermophilus* biofilm is obtained by circulation of culture medium in the microchannel for 70 h leading to a reproducible biofilm, which is then subjected to CIP treatments. The amount of biofilm is evaluated by image analysis before and after CIP, and the regrowth of the biofilm was used as a test of the biocidal efficiency of the treatments.
In parallel, this same strain was used in a CIP test rig using coupons covered with biofilms and subjected to the similar CIP treatments. Surviving cells were assessed by plate counting.
Similar results were obtained in the effectiveness of the treatments evaluated with both systems, even if these results must be confirmed by more systematic trials. This microfluidic system could therefore complement the CIP test rigs for the evaluation of treatments efficiency using CIP standard sequences and/or disinfectants.

P-105

VAGOCOCCI ARE ACTIVE IN MODIFIED ATMOSPHERE PACKAGED BROILER STORED AT 4 AND 6 °C

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RNA Seq and culturing were used to study the longitudinal activity of specific spoilage bacteria in modified atmosphere packaged (MAP) broiler products at 4 and 6 °C. After the end of shelf life when the product was spoiled, *Vagococcus proximus* was the dominating species detected. Notably, at 6 °C, 40% of the microbial transcriptomic activity at was mapped to *V. proximus* based on NCBI database, whereas total vagococci had activity of 20%.
Concurrently, *Carnobacterium divergens* and *Carnobacterium maltaromaticum* had activities of 17 and 2%, respectively. In contrast when the experiment was carried out at 4 °C, the same species were dominating but with reversed proportions: carnobacterial activity accounted for 40%, while *V. proximus* activity was only 13%. Additionally, vagococcal activity constituted only 13% of the total microbial transcriptome.
These findings show, that the newly described species *V. proximus* grows actively in poultry packaged under 30% CO_2 and 70% N_2. This species is an H_2S producer and thus, may cause sensory defects in meat, characterized by the odor of rotten eggs. *V. proximus* grows poorly in the presence of oxygen and is thus likely to grow only in meat and poultry packaged under anoxic atmospheres.

P-106

MICROBIOLOGICAL QUALITY AND RISK FACTORS IN CULINARY HERBS

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Herbs have been used mainly for their flavouring and aroma properties. Their microbiological quality reflects the hygienic situation of the production environment. Because they are grown, harvested and processed in warm, humid climates, using simple production methods, they may contain a diverse indigenous microflora. Occasionally, herbs contain pathogens which may pose a risk to public health, especially when added to meals that do not undergo further preservation treatments. This study aimed to determine the microbiological quality and safety of four types of herbs commercially available on the market. The 205 packages of herbs were tested (3 batches of every herb): basil (60), oregano (67), rosemary (30) and thyme (48). They were produced by 7 different manufacturers.
The viable count was in the range of 0-5.79 log cfu/g. TVC less than 10 cfu/g was in 10.8% of samples. Moreover, 37% of samples contained more than 4 log CFU/g. Yeasts and moulds were determined in 24.1% of culinary spices in the range of 0-5 log CFU/g, including 16.9% containing more

than 3 log CFU/g/. Bacteria of the genus *Enterobacteriaceae* were determined in 27.5% of samples in the range of 0-5.68 log CFU/g, including 7.6% of samples containing more than 4 log CFU/g. *E. coli* was found in 12.3% of samples as well as *Bacillus cereus*. At the same time, no *Salmonella* was found in the tested herbs.

The contamination was primarily related to the herb manufacturer. However, among the types tested, the largest number of microorganisms was determined in basil samples.

All tested culinary herbs showed different microbiological quality but met the hygiene requirements for this type of product i.e. 68% of samples had TVC number < 104 cfu/g which means acceptable quality according to ICMSF specifications. On the other hand, the study identified samples that contained microorganisms potentially hazardous to health (*E. coli* and *B. cereus*). In addition, high numbers of *Enterobacteriaceae* may also pose a threat to some consumers, e.g. those with reduced immunity.

P-107

ISOLATION AND IDENTIFICATION OF NATIVE MICROORGANISMS FROM MARE'S MILK: PRELIMINARY RESULTS

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Mare's milk is frequently consumed in Russia and some Asian countries, mainly as a fermented product, which increases its probiotic properties. But there is no tradition of consumption of this milk or its derived products in Spain, where its use is anecdotal. However, consumption of mare's milk may increase in the future because its physicochemical composition is more similar to human milk than that from other mammals, including domestic ruminants. In this context, the objectives of this work were to assess the microbiological profiles of samples of milk obtained from healthy Pura Raza Española (PRE) mares and to find it they may contain species with the potential to be used as probiotics for animal and human applications.

Samples were collected from the milking of three PRE mares over several months of lactation and were frozen at –30 °C until further analysis. They were inoculated on three different growth media: De Man, Rogosa and Sharpe (MRS), Columbia (CNA), and MacConkey (MCK) agar plates. A total of 243 isolates were obtained and screened for morphology and, among them, 79 isolates were selected for subsequent identification by PCR amplification and sequencing of a fragment of the 16S rRNA gene.

The 79 isolates identified were distributed in 18 genera and 28 species. The genera with the highest frequency of isolation were *Enterococcus*, *Staphylococcus*, *Agromyces* and *Lacticaseibacillus*. Many of the species found may be related to the microbiota of the maternal mucosal membranes, such as *Enterococcus gallinarum*, *Staphylococcus saccharolyticus* or *Ligilactobacillus salivarius*. Genera related to the environment, water and soil (such as *Stenotrophomonas* spp., *Advenella* spp. or *Streptomyces* spp.) were also observed, which suggests that they may originate from bedding and soil from the farm ambient.

Regarding those species with a potential probiotic use, 10 of the isolates belonged to species included within the lactic acid bacteria group, including 7 isolates of *Lacticaseibacillus paracasei*, 1 isolate of *Ligilactobacillus salivarius* and 2 of *Pediococcus pentosaceus*. Therefore, this work is a starting point for the characterization of probiotic candidates derived from mare milk to be used in the future for maintaining or improving equine and human health.

P-108

MICROBIAL BIODIVERSITY OF POWDERED INFANT FORMULA AND MILK POWDERS MARKETED IN ALGERIA. FIRST STEPS OF RISK ASSESSMENT WITH SPORFORMERS TOXIGENIC STRAINS

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The aim of this paper is to determine the biodiversity of spore formers present in milk powders and powdered infant formula (PIF) marketed in Algeria. A characterisation of the mesophilic aerobic spore formers isolated from those products was carried out. An analysis of milk powder isolates based on RAPD-M13 and 16S rRNA sequencing was performed. Phylogenetic groups were distinguished by sequencing of the panC gene. On the other hand, aerobic sporeformers isolated from PIF were identified by MALDI-TOF mass spectrometry and evaluated for their ability to produce spoilage enzymes and haemolytic activity. and evaluation of bceT, entFM, nhe (A, B, C) and hbl (A, B, C) toxin genes.

Fifty-four (n=54) samples of PIF of three brands and 21 samples of milk powder from 10 different origins were analyzed. The main species isolated from PIF were *B. licheniformis* (54%), followed by *B. cereus* (33%) and *B. subtilis* (5%). The ASB counts ranged from 1 to 4 log CFU/g, and the mean was 2.9 log CFU/g for mesophilic aerobic sporeforming bacteria (MSC). Among the ASB isolates, 97% had protease, 84% hydrolyzed starch, 66% had hemolytic activity, and 61% had lecithinase activity. A total of 44 out of 120 isolates harbored at least one toxin gene; 56% for *B. cereus*, 34% for *B. licheniformis*, and less than 5%for *B. subtilis*, *B pumilus*, and *L. sphaericus*. All *B. cereus* isolates harbored the *nhe* gene, 60% *entFM*, 44% *cytK*, 32% *bceT*, and 28% *hbl* genes. Besides, 17% of *B. licheniformis* harbored *nhe*. A small proportion of *B. subtilis*, *B. pumilus*, and *L. sphaericus* carried toxin genes. The *rpoB* based phylogenetic tree provided high resolution among *Bacillus* species. The findings of this study provide insights into the phenotypic and genotypic biodiversity of *Bacillus* present in PIF.

Molecular analysis of species isolated from mik powder revealed 17 groups. Bacillus cereus groups II, III and IV were the main phylogenetic groups. Bacillus cereus was present in all analysed powders. The *Bacillus cereus* and *Bacillus subtilis* groups demonstrated high proteolytic activity.

P-109

UNVEILING THE EPIPHYTIC MICROORGANISMS OF ROSEHIP AND ROWANBERRY AND THEIR VOLATILE ORGANIC COMPOUNDS PROFILES

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Rosehip (*Rosa canina* L.) and rowanberry (*Sorbus aucuparia* L.) are two forest berries known for their nutritional content and historical use in traditional medicine. These berries are not only valued for their diverse bioactive compounds, including antioxidants, phenolic compounds, organic acids, minerals, vitamins, fatty acids, and dietary fibers, but they also hold a distinct importance in the culinary and pharmaceutical industries. Therefore, it is important to characterize the berries-associated epiphytic microbial communities and uncover beneficial constituents. Various microorganisms, especially yeasts, act as biological control agents by producing toxins, volatile organic compounds (VOCs), which could influence the quality of plant-based food and human health, environmental protection, and industrial biotechnology.

During our study, the fungal and bacterial microbiota of rowanberries and rosehips were investigated. Following DNA

isolation, DNA fragments of the ITS2 rRNA gene region and the V3-V4 region of the 16S rRNA for each sample were individually amplified and subjected to high-throughput Next Generation Sequencing. Bioinformatics data indicate that the dominant fungal microorganisms on rowanberries are *Dothiora, Aureobasidium*, *Filobasidium*, *Vishniacozyma*, and *Cladosporium*, while on rosehips prevailed *Dothiora*, *Aureobasidium*, *Cladosporium*, and *Taphrina.* Bacterial species from *Sphingomonas*, *Hymenobacter*, *Methylobacterium*, and *Pseudomonas* genus dominated on rowanberries, whereas *Pseudomonas* and *Sphingomonas* were found on rosehips.

Various yeasts, including *Hanseniaspora* sp., *Saccharomyces* sp., *Torulaspora* sp., *Pichia* sp., and *Metschnikowia* sp., were isolated through culture-dependent techniques, followed by molecular methods-based identification. Functional analysis of identified yeast was performed. The ability of yeasts to produce toxins was tested using a well-assay method against control strains. Subsequently, four isolated yeast species (*Hanseniaspora uvarum, Metschnikowia pulcherrima, Saccharomyces cerevisiae,* and *S. paradoxus*) were tested to assess their production of VOCs. The GS-MS analysis revealed that the predominant volatiles produced by these yeasts were alcohols and esters.

The dominance of specific fungal genera as well as bacterial genera highlights the unique microbial signatures associated with each berry type. Additionally, the production of VOCs by these yeasts underscores their potential role in influencing the quality of plant-based food, environmental protection, and industrial biotechnology.

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P-110

HOW INGREDIENTS AND COMPOSITION CAN SHAPE THE ENDOGENOUS MICROBIOTA OF CHILLED BURGER ANALOGUE

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Givaudan France Naturals, Taste and Wellbeing, Science & Technology, Explore

The undeniable impact of climate change on the sustainability of the food chain is evident. As an emerging category of food products, meat analogues offer a wide variety of products on the market. Plant-based meat products focus on the organoleptic properties of the products to mimic meat in color, texture and taste. Little is known about the microbial communities associated with these products even though this information is crucial for assessing the food safety and shelf-life of plant-based meat products. Many strategies have been developed in order to manage the microbial growth for food preservation such as physical techniques (extrusion or packaging) as well as biological processing (fermentation). Ingredients may also impact the microbial populations and their evolution during the shelf life.

In the present study, assessment of the microbial communities during the shelf life of chilled beef burger analogue has been performed. Combining both traditional cultural methods and high throughput sequencing approach, spoilage microorganisms were identified. Dominant at the end of the shelf life, various species of lactic acid bacteria were identified. This can explain the production of lactic acid increasing during the shelf life which acidifies the food products and affects the taste and texture during the storage. In the present study, natural extracts were combined with existing ingredients in meat analogue burger formulations. Such ingredients can prevent microbial outgrowth (up to 5 log CFU/g difference at the end of the shelf life). In addition, the metabarcoding approach has shown that microbial diversity is preserved over the shelf life.

The results of this study have to be correlated with a whole sensory dataset, such as color degradation, taste and potential off-notes in order to extend the shelf life of meat analogue and provide the best food experience for consumers.

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INTERSPECIES INTERACTIONS IN CHEESE MICROBIOTA: METABOLIC SYNERGIES AMONG LACTIC ACID BACTERIA SPECIES

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Microbial interactions play a crucial role on the intricate dynamics of food ecosystems, encompassing a diverse array of microorganisms such as bacteria, fungi, and viruses, which interact with each other and their environment, profoundly impacting the growth, survival, and metabolic activities of individual organisms and the broader community. Despite advances in high-throughput sequencing, knowledge regarding the intricate network of biochemical interactions within the microbiota of food products remains limited. Moreover, conventional methods for studying microbial physiology often involve pure cultures, constraining the development of microbial solutions and impeding the exploration of their typical interactions with the surrounding microbiome.

Based on these considerations, this project aims to establish a food model to investigate the metabolic interactions among species of Lactobacillus helveticus, Lactobacillus delbrueckii, and the Lacticaseibacillus genus, characteristic of the microbiota in long-aged cheeses.

Eight strains from the mentioned genera and species underwent genotypic characterization via Amplified Fragment Length Polymorphism (AFLP), offering resolution at the subspecies level. Their metabolic proficiency was assessed across over 70 substrates using GENIII plates from Biolog, spanning sugars, amino acids, carboxylic acids, among others. Subsequently, strains were screened for metabolic properties across an array of nitrogen sources and peptides provided by Phenotype Microarray plates (Biolog), with tailored culturing media devised for each assay. Model cheeses were then produced, with microbial growth monitored over four weeks, and volatile compound production analyzed using SPME/GC-MS.

Results highlight discernible metabolic variations among strains, even within the same species, when cultured individually. Conversely, within the model cheese system, diverse binary and ternary strain combinations exhibit analogous growth patterns but diverge in volatile compound production. This understanding elucidates the determinants of species assembly within intricate cheese microbiota, shaping the organoleptic and sensory attributes of the final product. A thorough characterization of the metabolic attributes of individual strains and their combinations offers valuable insights for selecting starter and supplementing cultures in the dairy industry.

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NEXT GENERATION SEQUENCING AND TRADITIONAL MICROBIOLOGY TO IMPROVE FOOD SAFETY: PRACTICE FOR IDENTIFICATION OF BACTERIA DYNAMICS IN TRADITIONAL PRODUCTS

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Introduction

Foodborne pathogens may occur in meat products and their survival may be affected by ingredients, process technologies, or bacteria interactions. 16S metabarcoding allows the description of bacterial communities and their dynamic evolution.

Purpose

The focus of this study was to evaluate the microbial dynamics during the processing and aging of Spianata, a traditional Italian Salami, combining the NGS with traditional methods, as a potential tool to gain insight on microbial dynamics.

Methods

Three multi-strain cocktails of *Salmonella* spp. were used to contaminate a total of 80 kg of Salami batter. The control batter (80 kg) was inoculated with a sterile solution to evaluate the microbial and physical-chemical changes during the process. Samples were analyzed during the aging

(45 days), and microbial survival was estimated by plate count method. Relative bacterial abundances were assessed with Illumina MiSeq amplicon sequencing of V4-V5 region of the 16S rRNA gene for the in-depth study of microbial populations.

Results

The meat fermentation caused rapid acidification, with a decrease in pH from 5.7 to 4.7 within 2 days of the process. Significant changes were also observed for *aw* values, with a decrease from 0.97 to 0.90 after 45 days of ripening. *Salmonella* spp. counts decreased during the process, to achieve a total reduction of about 5.8 log CFU/g. Metabarcoding 16S analysis showed the presence of several bacterial populations (*Brochothrix* spp., *Staphylococcus* spp., *Pediococcus* spp., and *Enterobacteriaceae*) and their dynamics during the Salami ripening, but not the *Salmonella* spp. genus underling the usefulness of the combined DNA-sequencing/culture methods approach.

Significance

This study may help to solve the gaps in the knowledge of the microbial communities in traditional food and to promote the application of NGS technologies in complex environments to drive future studies and increase food safety.

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MICROBIAL PROFILE OF GREEK PROTECTED DESIGNATION OF ORIGIN ANEVATO CHEESE IDENTIFIED BY CULTUROMICS, METATAXONOMICS AND SHOTGUN METAGENOMICS

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Anevato is a traditional Greek white soft cheese produced from sheep or goat milk or mixtures of them. It is produced in the Grevena region in Western Macedonia, Greece and has been recently categorized as protected designation of origin (PDO). The objective of the present study was the identification of the microbial ecosystem of the Anevato cheese. The microbiome of the cheese was characterized by culturomics, metataxonomics (16S rDNA and ITS amplicon sequencing) and shotgun metagenomics.

Samples from three different Anevato cheese producers, four from each producer, were analyzed. The main microbial populations were lactic acid bacteria (LAB) and yeasts, but in lower abundance coliforms, *Enterobacteriaceae, Staphylococcus* spp., *Escherichia coli* and *Pseudomonas* spp. were also identified. The shotgun metagenomics as well as MALDI-TOF MS microbial identification allowed us to analyze the microbiome of the Anevato cheese at the species level. Thus, the main LAB species were, *Lactococcus lactis, Streptococcus thermophilus, Lactococcus raffinolactis, Lactobacillus helveticus, Lactiplantibacillus plantarum, Streptococcus parauberis*, among others. At lower abundances, yeast species were also identified as *Kluyveromyces lactis* and *Saccharomyces cerevisiae*. Furthermore, several metagenome-assembled genomes (MAGs) were identified.

Further investigation of the isolates may provide new starter cultures or adjuncts that could be used to standardize/improve the production of this cheese type.

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MICROBIAL DIVERSITY OF DIFFERENT GREEK TABLE OLIVE VARIETIES INVESTIGATED THROUGH SHOTGUN METAGENOMICS AND MOLECULAR TECHNIQUES

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Over the last few years, knowledge about the microbial diversity of different table olive varieties has been on the rise. Nevertheless, the microbiota of different Greek table olive varieties requires further investigation.

In this study, nine brine samples from three different olive cultivars, namely cv. Kalamata, cv. Konservolia, and cv. Halkidiki were subjected to physicochemical and microbiological analysis. In all samples, lactic acid bacteria (LAB) and yeasts were randomly selected and isolated, followed by rep-PCR and RAPD-PCR analysis, respectively. Moreover, total DNA was extracted directly from the olive brines and was subjected to shotgun metagenomics for the identification of the representative microorganisms at the species level.

Shotgun metagenomics revealed *Lactiplantibacillus pentosus* as the predominant species in natural black olives of both cv. Kalamata and cv. Konservolia, whereas green olives of cv. Halkidiki were dominated by yeasts and exhibited minimal detection of LAB. Microbial isolates were identified by MALDI-TOF, confirming the prevalence of *Lactiplantibacillus pentosus* in both natural black olive samples (cv. Kalamata & cv. Konservolia) and the limited detection of LAB in cv. Halkidiki green olives.

These findings suggest that both natural black table olives share a similar microbiota, possibly due to the same production method, in contrast to the green table olives.

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THE MICROBIOME OF NEMEA AND MANTINEIA PROTECTED DESIGNATION OF ORIGIN WINES: A SHOTGUN METAGENOMICS AND A CULTUROMICS APPROACH

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Wine is one of the most consumed alcoholic beverages around the globe. Nemea PDO wine is produced solely from the "Agiorgitiko" variety which is one of the most important Greek red varieties. In addition, the Mantineia PDO wine is mainly produced from the "Moschofilero" variety which is an equally important Greek white variety. These wines are considered two of the finest and most renowned Greek varieties and they are both produced in the Peloponnese region.

The purpose of this study was to investigate the microbial complexity as well as the metabolome of these two varieties. In order to examine the microbial ecosystem, we employed shotgun metagenomics analysis and a culturomics approach based on the identification of a high number of isolates with MALDI-TOF/MS.

The results of the shotgun metagenomic analysis showed that, among all samples, *Saccharomyces sp.* was the most abundant (over 45% in every sample) even though a species-level identification was not possible. *Saccharomyces cerevisiae* was the second most abundant species (over 30% in every sample).

This is in accordance with the MALDI-TOF/MS results, where the majority of the total isolates were identified as *Saccharomyces cerevisiae*. Additional species identified with abundance higher than 1% in most cases were *Metschnikowia* aff. *pulcherrima* (in Moschofilero) and *Lachancea thermotolerans* in both wines. In certain samples of Moschofilero shotgun metagenomics revealed the

presence of *Apilactobacillus kunkeei*. Furthermore, alpha- and beta-diversity analysis showed *that* the samples of each producer were distinct and diverse.
Our study reveals for the first time the microbiome of these two important PDO wines using both metagenomics and culturomics.
This research has been co-financed by the European Regional Development Fund (ERDF) and Greek National Funds through the Operational Program "Peloponnese 2021-2027" (MIS: 6001408).

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ENVIRONMENTAL MONITORING OF A MEAT PRODUCTS MANUFACTURING FACILITY TO DETECTION OF HARBORAGE SITES FOR *LISTERIA MONOCYTOGENES*

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Contamination with *Listeria monocytogenes* is particulary problematic in foods ready to eat such as dry fermented meats due to this pathogen can grow under refrigeration temperatures and under high salt concentrations. When *Listeria monocytogenes* is introduced in the facilities can persist in harborage points that, usually, are not adequately addressed during the cleaning and sanitation process, leading to cross-contamination of final products.
In this sense, this study aimed to evaluate the level of contamination of different clean surfaces present in two areas of a dry-cured meat processing facility by means of the counts of mesophilic bacteria and the presence of *Listeria* spp. and *Listeria monocytogenes*. The purpose was to verify the effectiveness of cleaning and desinfecting process, identify hotspots of contamination and the risk of cross-contamination between zones and areas.
The two areas sampled were: an area in which raw materials are handled (sausage production room) and an area where final meat products are handled (slicing and packaging room). In each area was selected 14 different points which included four zones: Zone 1, in direct contact with food; Zone 2, not in direct contact with food but close to food; Zone 3, remote to food or food contact surfaces inside food processing area; and Zone 4, not in direct contact with food, outside food processing area.
All surfaces were evaluated through implementing an sensor-based sampling system. Sensors were collected from the surfaces once a week along one month up to a total of 112 samples (56 samples per area). The results indicated that counts of mesophilic bacteria varied according to the zone. The samples evaluated in the zones 3 and 4 had higher mean counts than those counts found in the zones 1 and 2 in both areas as it was expected.
Regarding *Listeria* spp. and *Listeria monocytogenes*, the result obtained in the 80% of samples analized was "not detected" and in around of 18% of samples the result was detected but non culturable. Also, data indicated that *Listeria* spp. and *Listeria monocytogenes* both remained at some sites in both areas even after cleaning which could imply risk of cross-contamination.

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UNDERSTANDING MICROBIAL SPOILAGE OF VACUUM-PACKED RED MEAT THROUGH ANALYSIS OF MICROBIAL COMMUNITY AND METABOLOME PROFILES

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Introduction
The shelf-life of vacuum-packed (VP) lamb is significantly shorter compared to that of beef. Research to date has been extensively conducted to understand changes in the microbial communities over the course of meat shelf-life. However, the basis of the difference in shelf-life remains unclear. This indicates a need for a holistic approach to investigate spoilage through integration of microbial and metabolome profiles as they relate to spoilage development.

Purpose
This study aimed to provide insight into the potential mechanisms underpinning the differences in the shelf-life

of VP lamb and beef through an integrated amplicon-based sequencing and metabolomic approach.

Methods

VP lamb rump and beef ribeye, previously shown to have different shelf-lives (37 vs. 45 d) at 2 °C, were used in this study. Three replicates of each meat type (n = 39) during storage were taken periodically for microbial community analysis using 16S rRNA gene amplicon sequencing (V1-V3 sub-region) and non-targeted metabolomic analysis using HPLC-MS/MS. Microbial communities' (ASVs = 741) and compounds' (n = 2,804) matrices were analysed using linear regression models to determine how these were affected by meat type and storage time.

Results

Similar microbes and metabolites were found in VP lamb and beef, however, their compositions throughout storage showed distinct differences between the meat types. Specifically, the relative abundance of *Carnobacterium, Hafnia,* and *Pseudomonas* species started to increase much earlier (~5 d) in VP lamb compared to beef. Lamb also had faster rate of accumulation of certain metabolites, such as acetophenone, butane-1,3-diol, isobutyraldehyde, and proteolysis products like amino acids and small peptides. These differences through time may explain the shorter shelf-life observed for lamb.

Significance

Through a holistic approach, this study significantly advances our understanding of the microbiology of red meat spoilage. This provides opportunities for the development of more targeted approaches to guarantee and extend the quality shelf-life of VP meat, ensuring food security.

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GLUCOSE, A KEY FACTOR TO MANIPULATE MICROBIAL SPOILAGE OF VACUUM-PACKED LAMB FOR SHELF-LIFE EXTENSION

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Australian vacuum-packed (VP) lamb has a reputation for being high-quality with an exceptional shelf-life of 90 days under export conditions (–1 to 0 °C). However, access to some markets may require a longer shelf-life. This is due to poor temperature control during shipment and the need for more time to sell product after arrival. Here, we exploited the roles of natural glucose/glycogen in meat and assessed its effects on the shelf-life of VP lamb.

Trials in both laboratory and industrial settings were conducted to determine the quality shelf-life of various VP lamb cuts (*i.e.,* boneless and bone-in shoulder, and bone-in foreshank) previously surface-treated with and without glucose at different concentrations (from 0% to 10% *w/v*) during storage at low temperatures (from –0.5 to 4 °C). Their shelf-life was determined by microbial and organoleptic assessments. It was evident that meat with elevated glucose (up to 0.8 g/kg) had a longer shelf-life, ranging from 8% to > 76% increase relative to the control.

However, there were no differences in the growth rates of bacterial indicators (total viable counts and lactic acid bacteria) between the control and meat treated with glucose. This was despite the observations from subsequent studies using meat slurry in which only the 5 and 10% glucose treatments resulted in a reduction of meat pH by up to 0.4 units. Furthermore, analysis of the microbial community data for the most effective treatment (at 5%; using 16S rRNA gene amplicon sequencing) revealed that both *Vagococcus* OTUs and *Serratia proteamaculans* had a longer lag duration and lower maximum population density on glucose-treated when compared to the control.

These indicate that elevated glucose may alter the microbial community composition by shifting substrate utilisation and the accumulation of acidic end-products (through glucose fermentation), leading to the improved shelf-life.

The results of this study confirm the important roles of glucose in VP lamb spoilage and highlight its underlying mechanisms for shelf-life extension. Such knowledge would aid the development of practical approaches that maximise glucose level in meat for shelf-life extension, enabling the industry to increase access to further markets, while minimising product wastage.

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EVALUATION OF THE MICROBIAL DIVERSITY OF STAINLESS STEEL, POLYPROPYLENE AND POLYURETHANE SURFACES AFTER PRE-OPERATIONAL CLEANING IN BRAZILIAN POULTRY AND FISH INDUSTRIES

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Pathogenic and spoilage bacteria can contaminate food processing environments, compromising products' hygienic and sanitary quality. Therefore, to evaluate the contamination of abiotic surfaces of stainless steel, polypropylene, and polyurethane of different equipment and utensils used in the preparation of meat products, this study aimed to verify the microbial diversity present on these surfaces of poultry and fish facilities after the pre-operational cleaning procedures.
Mesophilic isolates were obtained using the standard ISO 4833-1:2013 method and the cultures were subjected to MALDI-TOF analysis. A total of 219 isolates from poultry slaughterhouses and 150 from fish slaughterhouses were submitted for analysis, of which 169 and 102 isolates were characterized to species level by MALDI-TOF technique. In poultry slaughterhouses, 27 genera were identified, of which *Serratia* (27/169; 15.98%), *Staphylococcus* (25/169; 14.79%), *Kocuria* (25/169; 14.79%), *Aeromonas* (12/169; 7.1%) and *Lelliottia* (12/169; 7.1%) were the most common. Among these genera, the most common species were *Serratia liquefaciens* (25/27), *Staphylococcus epidermidis* (11/25), *Kocuria rhizophila* (25/25), *Aeromonas veronii* (10/12) and *Lelliottia amnigena* (12/12). In fish, 21 genera were identified: *Enterobacter* (18/102; 18.36%), *Acinetobacter* (13/102; 13.26%), *Staphylococcus* (12/102; 12.24%), *Rothia* (10/102; 10.2%) and *Klebsiella* (6/102; 6.12%) were the most abundant.
Among these genera, the most abundant species were *Enterobacter asburiae* (10/18), *Staphylococcus warneri* (6/12), *Rothia endophytica* (10/10), and *Klebsiella pneumoniae* (4/6). In contrast, for the genus *Acinetobacter,* most isolates (7/13) were identified only at the genus level. When comparing the microbial diversity between the sectors, 50% (n=16) of the identified genera were shared between the two sectors, 34.4% (n = 11) were identified only in poultry slaughterhouses and 15.6% (n = 5) in fish slaughterhouses. For the species, only 20% (n = 12) were identified in the two industries, 53.3% (n = 32) and 26.7% (n = 16) in poultry and fish respectively.
The diversity of microorganisms identified in this study demonstrates the ability of these microorganisms to resist the pre-operational cleaning and sanitization procedures used in the food industry, and further studies of these isolates are needed to characterize the profiles of resistance to sanitizers and biofilm formation.

MICROBIAL RESISTANCE

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ALTERNATIVES FOR ANTIBIOTIC RESISTANCE REVERSION IN MULTIDRUG RESISTANT *ENTEROCOCCI* ISOLATED FROM SLAUGHTERHOUSE ENVIRONMENT

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Enterococci are a dominant bacterial group in the gut microbiota of humans and animals, and emerged as important nosocomial pathogens over the last three decades with acquired resistance to many antimicrobials such as antibiotics and biocides. Spread of multidrug resistant (MDR) *enterococci* and their antibiotic resistance genes (ARGs) along the food chain and also the environment has become a global threat. Thus, the emergence of *enterococci* with multiple resistance determinants critically shortened the repertoire of disinfections methods usually used in different industry environments such as slaughterhouse.

The present study analysed the effect of alternative methods to revert the resistance mechanisms of MDR enterococci using induction by sub-inhibitory concentrations of antimicrobials such as essential oils. The results obtained showed that MDR *Enterococcus* sp. E9.8 strain, induced by eugenol (selected essential oil derived from clove oil and commonly used in disinfection formulations) exhibited different phenotypic and genotypic profile. Eugenol treatment induced irreversibly a reduction of the minimum inhibitory concentration (MIC) of several antibiotics such as kanamycin, erythromycin and tetracycline.

To corroborate these results, in depth molecular analysis of *Enterococcus* sp. E9.8 straintranscriptome under both conditions (non-induced “control” and induced with eugenol) was done and the results indicated down-regulation of genes coding for proteins involved in antibiotic resistance related to the main transporter families.

In conclusion, these results allow us to propose the use of eugenol among other essential oils as antibiotic resistance reversing agent to be included in disinfectant solutions to limit the spread of MDR enterococci and their ARGs in the food chain and the environment.

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EFFECT OF DIFFERENT DISINFECTION STRATEGIES ON THE SPREAD OF MULTIDRUG RESISTANT BACTERIA AND THEIR RESISTANCE GENES IN SLAUGHTERHOUSE ENVIRONMENT

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Slaughterhouses are important reservoirs of antibiotic-resistant bacteria and their resistance genes (ARGs) due to the extensive metaphylactic and prophylactic antibiotic use in veterinary, clinical therapy and agriculture, in this context new foodborne pathogens often emerge from animal reservoirs with increased resistance.

Several strategies have been developed to face the increasing and multi-faceted problem of antibiotic-resistant pathogens –termed "superbugs"– in the (meat) food chain and thus in the environment. Initially, the use of disinfection strategies was globally extended to reduce pathogens; however, the consequences on ARG transferability and spread were poorly understood until the development of molecular tools. Also, it has now been demonstrated the crossed-resistance of biocidal agents and antibiotics which aggravated more and more the silent pandemic of antibiotic resistance. In the present study, we evaluated different strategies to study the effect of new disinfection strategies (HLE disinfection solution developed by our group / UV treatment) *versus* “business as usual” strategies or their combinations in the elimination/limitation of critical pathogens and the

dissemination of their ARGs in the critical slaughterhouse zones (e.g. sacrifice and cutting rooms).
The results obtained showed some promising combinations such as HLE and "business as usual", which could be used as a key disinfection strategy in the most critical zones in a slaughterhouse. These results were corroborated by resistome determination using metagenomic analysis in hotspot zones with high and diverse microbial community for each disinfection treatment. The comparison of ARG abundances in each disinfection strategy (or combinations) suggested that the combination of HLE disinfection solution with "business as usual" could eradicate both multidrug resistant bacteria and their ARGs.
We can conclude that the new combination of a sustainable disinfection solution HLE and other methods could be a good alternative to limit the spread of antimicrobial resistant bacteria and their resistance genes in the food chain and the environment.

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ANTIMICROBIAL RESISTANCE OF *SALMONELLA ENTERICA* ISOLATED FROM GUINEA FOWLS IN GHANA

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Salmonella enterica is an important bacteria involved in foodborne outbreaks and their resistance to antimicrobials will pose a threat to public health due to treatment difficulties and failures.
This study determines the antimicrobial resistance of *Salmonella enterica* isolated from guinea fowl wet markets in Ghana. *Salmonella enterica* was isolated according to Andrews *et al.* (2018). Antimicrobial resistance was carried out following the procedure of Bauer *et al.* (1996). Whole genome sequencing was done using Illumina DNA Prep (M) Tagmentation kit (Illumina Inc. San Diego, CA 92122, United States) by following the manufacture's procedure. The overall prevalence of *Salmonella enterica* was 6.5% (13/200). *Salmonella enterica* were isolated from water used for washing meat (16%), processing knife (12%), processing floor (8%), meat (8%) and faeces (8%), but not processor's hands, processing table and water from main source.
The *Salmonella enterica* exhibited high antimicrobial resistance >45.0% to amoxicillin, azithromycin and ceftriaxone. Intermediate resistance was relatively high for tetracycline (53.3%), chloramphenicol (40%) and gentamicin (40%). Multidrug resistance (resistance to ≥3 different classes of antibiotics) was found in 46.7% of the isolates. The percentage resistance to two, one and zero antibiotics were 13.3%, 33.3% and 6.7%, respectively. One *Salmonella enterica* each isolated from water used for washing meat (ceftriaxone-azithromycin-gentamicin-tetracycline) and processing floor (azithromycin-chloramphenicol-tetracycline-amoxycillin/clavulanic acid) was resistant to as many as four different antibiotics. All the *Salmonella enterica* isolates were susceptibility (100.0%) to ciprofloxacin (100%) and suphamethoxazole/trimethoprim (100%). Susceptibility was also high for amoxicillin (80%) and amoxycillin/clavulanic acid. Whole genome sequencing of selected isolated identified the presence of antimicrobial genes including *qnrS1, sul3, tet(A), dfrA1, aadA5, qnrS1, qepA1, blaTEM-1B, dfrA17, tet(B), aph(6)-Id, sul2*, and *dfrA17*. Plasmids such as *IncY, IncX1, p0111, IncFIC(FII), IncFIA, IncFIB(pB171)* and *repB(R1701)* were predicted.
This study confirms that some of the guinea fowl samples collected from wet market were contaminated by antimicrobial resistant *Salmonella enterica*. Appropriate handling of guinea fowl meats and it related samples from wet markets in Ghana are recommended to prevent the spread of foodborne infections.

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GENOTYPIC CHARACTERIZATION OF ESBL/AMPC-PRODUCING *E. COLI* FROM THE FOOD CHAIN IN THE GERMAN NATIONAL ZOONOSES MONITORING 2016-2022

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Extended-spectrum beta-lactamases (ESBLs) and AmpC beta-lactamases (AmpCs) hydrolyze 3rd generation cephalosporins. Resistances mediated by ESBL/AmpCs are of great concern and closely monitored in human and veterinary medicine. Here, we present data on the ESBL/AmpC genotypes of 5325 *E. coli* isolates obtained from the national monitoring of antimicrobial resistance (AMR) in the food chain from 2016-2022.

The AMR monitoring was conducted following technical specifications given by Commission Implementing Decision (CID) 2013/652/EU and CID 2020/1729/EU and complemented by additional national programs using the same methods. Isolates were obtained from different matrices from broilers (N = 1650), fattening turkeys (N = 1303), fattening ducks (n=55), fattening pigs (N = 992) and cattle (N = 1206), and at different levels of the food production chain (primary production, slaughterhouse and retail) as well as from wild animals, small ruminants, freshwater fish and plant-based food were included (N = 119). Between 2016 and 2020 presumed ESBL/AmpC-producing isolates were examined using real-time PCR and genotype was determined by PCR sequencing. Since 2021, presumed ESBL/AmpC-producing isolates were characterized by whole genome sequencing.

The variability of ESBL genes is fairly low with 23±3 different detected genes and gene combinations each year. The majority of isolates harbored one ESBL/AmpC gene, only few (< 1%) carried two or more genes. In cattle and pigs, the majority of isolates (75.8%) produce CTX-M-1 or CTX-M-15, whereas in poultry, a more even distribution of CTX-M-1, CTX-M-15, CMY-2, TEM-52 and SHV-12 was observed. In isolates from caecum content of pigs and calves, there were no major variations of genes over the years. In contrast significant changes were observed in isolates from poultry. ESBL/AmpC genes found in isolates from wild animals, small ruminants, freshwater fish and plant-based food were to the same as those found in pigs and cattle.

In conclusion, there are changes in the specific ESBL gene patters observed in some food production chains. It is important to observe them closely to assess their potential role as sources of human exposure. In addition, further research is needed to understand the factors triggering the changes observed in distribution patterns in poultry.

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EVOLUTION OF THE MICROBIOME AND RESISTOME OF PORK LOIN AND TENDERLOIN THROUGHOUT THE PRODUCTION CHAIN AND SHEL-LIFE

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The microbiome of meat can significantly influence its quality and safety. One issue related to this is the potential transmission to humans of antimicrobial resistant microorganisms and antimicrobial resistance genes (ARGs) through meat consumption.

The aim of this study was to characterize the evolution of the microbiome and resistome of pork loin and tenderloin at different stages of processing, from slaughter to the end of shelf-life. For this, 5 animals each from 4 selected farms were monitored through the slaughter line and a cutting plant at different points. Swab samples were taken from carcasses (n=80) and the two pork cuts, at the beginning and end of their shelf-life under modified atmosphere packaging (n=80). Furthermore, 57 environmental samples from contact and non-contact surfaces of the slaughterhouse and cutting plant were also collected.

Extracted DNA was sequenced by Illumina Novaseq platform (150bp PE, 7.5Gb/sample). Reads were quality filtered using trimgalore and taxonomicaly classified by Kaiju using the refseq database. ResFinder database was used to search ARGs by bowtie2 software. Sample *de novo* metagenomic assembly was performed through metaSPAdes and obtained contigs were taxonomically assigned using SqueezeMeta software, which uses Diamond search against GenBank nr database.

Analysis at the read-level revealed differences in microbiome composition among the rooms of the industry and allowed to identify the potential transmission of some taxa, such as *Anoxybacillus flavithermus* and *Psychrobacter* sp., from the environment to the carcass. The analysis of the carcass and products showed a decrease in bacterial richness and diversity as they progress through the production chain.

The resistome analysis revealed a greater number of ARGs at the start of the processing line. In contrast, the number of ARGs was lower in the cutting plant and at the beginning of the product's shelf-life. Read and contig-level analyses showed that the most prevalent ARGs were associated with resistance to tetracyclines, aminoglycosides and beta-lactams. The main genera associated with these ARGs were *Acinetobacter* and *Staphylococcus*.

Overall, our findings provide a detailed account of the changes in the microbiome and resistome that occur in two pork products during processing and commercialization.

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DETECTION AND CHARACTERISATION THROUGH AMPLICON LONG READ SEQUENCING OF INTEGRONS AND THEIR ANTIMICROBIAL RESISTANCE GENES IN MEAT PROCESSING MICROBIOMES

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Integrons are among a variety of genetic elements responsible for the transmission of antimicrobial resistance genes (ARG). However, while antimicrobial resistance spread in meat production and associated environments has been widely studied, there is a shortage of studies focused on the identification and characterisation of integrons within meat processing industries.

The aim of this study was to evaluate the presence of integrons and their associated ARG in microbial communities from different meat processing industries using an optimized PCR amplification and long read sequencing approach. A total of 196 samples from meat, food contact and non-food contact surfaces, were taken from different meat production facilities (producing fermented, cured, aged or fresh meat products). Following DNA isolation, the PCR amplification procedure was optimized on a small subset of samples (n=12), using two pairs of primers (from *attI* to *qacE*, or from *intI* to *attC*). Two different library preparation approaches (use of a short fragment buffer vs a long fragment buffer) were also tested.

We found that the primer set targeting *attI* and *qacE*, combined with Long Fragment Buffer (LFB) library construction resulted in higher number of integron reads (25.6% of all the sequencing reads vs <7.0% for other sequencing approaches) and allowed recovery of up to 77.3 times more ARG. The selected sequencing approach was then used on the 196 samples, with an average integron read length of 1511bp. The vast majority (>97%) of the ARG

found were associated with resistance to aminoglycosides. Minor differences were found in the diversity of ARG among the different sample types or meat producing facilities.
In addition, to evaluate the methodology described in this study, the results obtained were compared with those from direct Illumina whole metagenome sequencing (WMS) in the same samples set, which demonstrated that the integron amplicon sequencing approach allowed the detection of 39 new ARG, undetected through WMS with Illumina.

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MICROBIOME MAPPING IN BEEF PROCESSING REVEALS MICROBIAL DYNAMICS AND ANTIBIOTIC RESISTANCE GENE PATTERNS

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Beef 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 as their transmission routes and mechanisms of action is crucial. The ongoing development of shotgun metagenomic approaches is providing the means to explore AR in food microbiome. For this purpose, reads and metagenome assemblies coming from a beef processing industry were screened by shotgun metagenome sequencing, in order 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 a comprehensive overview of the ARGs reservoir.
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DIVERSITY OF ANTIMICROBIAL RESISTANCE DETERMINANTS IN *CAMPYLOBACTER* SPP. ISOLATES FROM SPAIN

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Campylobacteriosis represents one of the most common bacterial causes of diarrheal illness worldwide with more than 137,107 human reported cases in the EU in 2022. Although antibiotics are only recommended for severe cases, their indiscriminate use has led to the emergence of multidrug resistant strains that threat public health security.
In response to this, we investigated the profile of antimicrobial resistance (AMR) determinants in *Campylobacter* spp. in Spain as a first approximation to tackle the AMR crisis. We selected 86 *Campylobacter* spp. isolates representing different stages of the poultry supply chain (farm, slaughterhouse, poultry processing plant and retail) in northern and central Spain, and 59 *Campylobacter* spp. isolates from clinical origin in Burgos (n = 145), that include altogether the three most prevalent species *Campylobacter jejuni* (135 isolates), *Campylobacter coli* (7 isolates) and *Campylobacter lari* (3 isolates). The isolates were characterized through Whole-Genome Sequencing (WGS), and genomes were assembled and

analysed using CamPype (https://github.com/JoseBarbero/CamPype).
The acquired AMR genes were detected by ABRicate 1.0.1, and point mutations linked to AMR were identified with PointFinder v3.1.1. The analysis of the antimicrobial resistome revealed 12 AMR patterns among the isolates, although β-LAC+TET+(F)QUI was the most prevalent one in each stage. Moreover, hospital and retail, where resistance to aminoglycosides was only found, showed the highest AMR diversity. Furthermore, differences between *Campylobacter* species were observed. High prevalence (> 90%) of bla_{OXA}-type β-lactamase genes and mutations in DNA gyrase gene associated with fluoroquinolones resistance were detected, and at a frequency similar to the *tet(O)* gene in *C. jejuni* (92.6%) and *C. coli* (85.7%), both of which also harboured resistance determinants to aminoglycosides with a higher occurrence rate (42.9%) in *C. coli* (retail isolates), that was the only species in which mutations in the 23S ribosomal subunit conferring resistance to erythromycin (isolates from retail and hospital) were identified (42.9%).
In contrast, *C. lari* only possessed *blaOXA-493*, *CmeA/B/C* and *CmeR* genes. All of this suggests that the effectiveness of drug therapy against this foodborne pathogen in Spain could be endangered, although WGS proves to be essential for effective intervention strategies against *Campylobacter* infection.

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GENOMIC INSIGHTS OF *ENTEROBACTER CLOACAE* COMPLEX STRAINS IN ITALIAN ARTISANAL FOODS OF ANIMAL ORIGIN

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The food production chain has been widely recognized as a route of dissemination of antimicrobial-resistant or pathogenic bacteria posing a heightened risk to public health. The aim of this study was to investigate strains belonging to the *Enterobacter cloacae* complex (ECC), one of the most common *Enterobacteriaceae* groups leading to nosocomial infections from six batches of a soft cheese and of a six-months ripened salami produced in two Italian artisanal facilities. A total of 1170 samples (750 and 420 for cheese and salami respectively) were collected between January 2020 and May 2021, including raw materials, intermediate and final products, as well as processing environment.
Samples were submitted to *Enterobacter* spp. isolation and identification with cultural, biochemical and molecular methods, and positive strains have been whole-genome sequenced by Illumina technology. Sequences were analysed with bioinformatics tools to perform species confirmation, to estimate phylogenies by SNPs calling and to predict genomic traits of interest (antimicrobial resistance and virulence). A total of 26 *Enterobacter* spp. were identified (11 and 15 from cheese and salami respectively), and confirmed by WGS as belonging to *E. hormaechei* (n = 18), *E. kobei* (n = 4), *E. mori* (n = 2) *E. ludwigii* (n = 1) and *Lelliottia amnigena* (n = 1).
Among the three *E. hormaechei* phylogenetic clusters encompassing same sequence types (ST141, ST168 and ST278), the highest genetic proximity was assessed between two ST141 strains collected from 18-weeks ripened salami and the table surface in mixture room (193 SNPs differences), suggesting the absence of a persistent clonal population in the facility. All *E. hormaechei* and *E. kobei* strains carried beta-lactamases (*bla*ACT) and fosfomycin resistance genes (*fos*A), whereas *E. mori* harboured *fos*A and *qnr*E1 gene encoding for quinolone resistance. Main virulence-associated genes were linked to the yersiniabactin iron uptake system (*ybt*, *irp* and *fyu*A), identified in *E. hormaechei* strains from four salami including the final product.
Overall, this study suggest that an enhanced surveillance is recommended towards ECC to mitigate the spread of predicted antimicrobial resistant and pathogenic strains in food-producing systems.

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APPLICATION OF HYBRIDISATION CAPTURE AND TARGET ENRICHMENT OF ANTIBIOTIC RESISTANCE GENES IN AQUACULTURE FOODS

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Aquaculture is a form of intense food production and for some conventional open-system aquaculture high use of antimicrobials is reported. Intensive use of antimicrobials is leading to high rates of antimicrobial resistance (AMR). Spread of pathogens, resistant bacteria as well as drug residues into the environment is easily possible. In contrast, in recirculating aquaculture system (RAS) and aquaponics there is little exchange with the environment and the use of antimicrobial agents is very limited due to biological filter systems. The knowledge on the presence and distribution of antibiotic resistances in these systems is scarce.

Selective isolation of antibiotic resistant bacteria is time and cost intensive and not a feasible approach without clear target genes and/ or bacteria. So, for investigations on AMR in RAS and aquaponics, a broader approach was needed. Two approaches were chosen to compare its applicability in AMR gene detection: isolation of plasmids with subsequent sequencing or bait-based enrichment of metagenomic DNA followed by sequencing. It was shown before that hybridisation capture and target enrichment allows cost efficient, directed and more in-depth sequencing of target genes compared to metagenome sequencing.

For evaluation of the methods, different positive controls were created. First, a mock community was designed, containing *Escherichia coli, Salmonella enterica* and *Staphylococcus hyicus*. Secondly, frozen fish produced in aquaculture were purchased from supermarket, thawed and enriched in buffered peptone water. Plasmid DNA was extracted from enrichments using different plasmid DNA extraction kits and sequenced using Illumina NextSeq 550 System. Further, genomic DNA was extracted using three different DNA extraction kits. AMR genes were enriched using predesigned hybridisation capture kit (Daicel Arbor Biosciences) containing 3565 different AMR gene probes and then sequenced. Results showed, that bait-based enrichment resulted in detection of a larger variety of AMR genes compared to plasmid extraction. Both methods are limited in determination of gene variants differing only in few nucleotides (e.g. blaTEM). Although, further evaluations on run parameters have to be carried out to define the complete workflow, it was shown, that this approach will be useful to collect more precise information on AMR in RAS and aquaponics.

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EVERYTHING HAS A PRICE: SPANC BALANCE IN THE GROWTH FITNESS OF AN *ESCHERICHIA COLI* VARIANT ADAPTED TO GROWTH IN THE PRESENCE OF THYMOL ESSENTIAL OIL

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The growing demand for natural products has driven interest in natural antimicrobial compounds, such as essential oils, to prevent the growth of microorganisms in food. However, some studies have suggested that prolonged exposure to natural antimicrobials may induce bacterial adaptation, and even cross-resistance to other compounds, such as antibiotics. On the other hand, these adaptations are often at the expense of other phenotypes (such as thermal resistance or growth under optimal conditions) through the SPANC balance.

In this research, directed evolution experiments were performed by exposing *Escherichia coli* O157:H7 to 20 successive cycles of a subinhibitory (1.25 mM) and bactericidal (2.5 mM) concentrations of thymol. The adapted variant showed increased resistance not just to this compound, but also cross-resistance to several antibiotics such as ampicillin, cephalexin, chloramphenicol, nalidixin, trimethoprim and norfloxacin. The SPANC balance was

investigated by evaluating its growth fitness at different pH values (7.0, 6.5, 6.0, 5.5, 5.0 and 4.5) and temperatures (25, 30, 35 and 40 °C), with or without the application of previous sublethal heat treatments using the two-fold dilution method. The results showed that the antimicrobial adaptation was at the expense of growth in the absence of these compounds, with the adapted variant having lower growth rate at every pH and temperature values studied. However, the adapted strain had higher growth fitness than the wild strain after the application of a heat treatment, showing a potentially higher ability to recover from sub-lethal damage.
These results are thus of relevance for assessing the possible implications of prolonged exposure to natural antimicrobial compounds on microbial resistance, as well as the possibility for adapted variants to dominate the bacterial population.

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MOLECULAR DETERMINANTS OF ANTIBIOTIC RESISTANCE IN GUAIACOL-PRODUCING *ALICYCLOBACILLUS FASTIDIOSUS* STRAINS

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Alicyclobacillus spp., as a spoiling bacteria, remains a severe problem for the juices and beverages industry. Their phenotypic characteristics, such as spore-forming, resistance to different factors, or production of off-flavor compounds, make them responsible for affecting the taste and aroma of spoiled food and beverages. The possibility of food and beverages being potential vectors for the transfer of antibiotic resistance genes is a growing health concern, and the microorganisms in food and beverages should not be a potential source of drug resistance to consumers. Bacterial resistance to any environmental factor is an adaptive process. Introducing antibiotics into the ecosystem leads to rapid adaptation of a given microbial population. The emergence of antibiotic-resistant (AR) genes is rarely the result of mutations in exposed populations. Most often, the acquisition and spread of "resistance" occurs through mobile genetic elements and their transfer to antibiotic-sensitive microorganisms via horizontal gene transfer (HGT).
Agricultural fields and fertilized orchards may be a place for the duplication of genes that determine drug resistance and their transfer to soil bacteria and further to the food chain. This may be the route by which resistance determinants reach the human body, so revealing potential AR transmission pathway(s) and the critical mechanisms involved in antibiotic resistance are important for scientific understanding.
Our studies provide complete genomes of three *Alicyclobacillus fastidiosus* guaiacol-producing strains, describing most of the functional coding sequences, including those newly revealed, and presenting a global functional analysis highlighting various interesting features. The in-depth whole genome analyses (WGS) focused on identifying metabolic pathways and networks, detailing antibiotic resistance and guaiacol and halophenols production. The WGS and minimal inhibitory concentration (MIC) showed that the strains are susceptible to eight out of nine tested antibiotics (ampicillin, gentamycin, kanamycin, streptomycin, clindamycin, tetracycline, chloramphenicol, vancomycin), and present high resistance to one – erythromycin. Obtaining complete genomes of the studied *Alicyclobacillus fastidiosus* strains can expand the knowledge of the spoiling bacteria and be a resource for the potential involvement of the spoiling bacteria in transmitting resistance to humans through the food chain.

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SEASONAL SYMPHONY: SHOTGUN METAGENOMICS REVEALS A FLOURISH OF MICROBIOMES IN SUMMER MEAT PROCESSING

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This study explores the influence of seasonal variations on microbial communities in meat processing facilities, with implications for food quality and safety. Our primary aim includes characterizing the microbial dynamics in beef processing facilities during summer (current part) and winter (future part) and assessing the impact of seasonality on microbial abundance and diversity, with a special focus on antibiotic resistance genes (ARGs).

Shotgun metagenomics was employed for DNA sequencing of meat samples (n = 400), followed by extensive bioinformatics analyses for microbiome characterization, quantification, and ARGs evaluation. Summer, characterized by open-field, grass-fed livestock, revealed significantly higher alpha diversity indices ($p < 0.05$), indicating increased microbial variety in meat samples with *Moraxella*, *Pseudomonas*, *Brochothrix*, and *Enterococcus* as prime candidates. Similarly, twenty-four unique antimicrobial resistance (AMR) profiles were identified, with 85% of isolates resistant to at least two antimicrobials. Beta diversity analyses confirmed distinct microbiome compositions within each processing step (PERMANOVA, $p < 0.001$).

In conclusion, this study underscores the significant seasonal impact on meat processing microbiomes. Summer, with its pasture-based grass-fed practices fosters a richer and more diverse meat microbiome with significant AMR profiles. Hence, understanding these seasonal dynamics is crucial for enhancing food safety and quality standards within the beef industry, with implications for meat products and consumer health.

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DETECTION OF ANTIBIOTIC-RESISTANT BACTERIA AND GENES FROM COMMERCIAL CHICKEN AND PORK MEAT PRODUCTS

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Antibiotic resistance is a growing public health concern worldwide and a critical issue within the "One Health" approach. It has been suggested that meat can act as a reservoir for Antibiotic Resistance Bacteria (ARB) and Antibiotic Resistance Genes (ARGs). Therefore, this research aimed to study the microbiological quality and the prevalence of ARB and ARGs in chicken and pork meat intended for human consumption.

Fifteen chicken and 15 pork meat samples from different retail establishments in Valencia (Spain) were analyzed. The samples were collected and examined according to standardized methods for total coliform bacteria, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* spp.

In pork meat samples, coliform levels ranged between 101 and 105 cfu/g, while chicken meat showed levels ranging from 100 to 105 cfu/g. Pork meat showed *E. coli* levels below 103 cfu/g, whereas chicken meat presented levels ranging from 103 to 104 cfu/g. None of the samples exceeded the microbiological criteria for *E. coli* cfu/g specified for foodstuffs (Regulation (EC) 2073/2005). A total of 3/15 chicken and 1/15 pork samples were contaminated by *Listeria monocytogenes*. *Salmonella* spp. was detected in 13% of the samples (2/15 chicken samples, 2/15 pork samples).

A total of 127 strains were isolated. Fifty-three Gram-negative and oxidase-negative isolates were selected and identified by biochemical tests or 16S rRNA gene sequencing. Their sensitivity to 12 antibiotics was determined using the disc diffusion technique. Finally, molecular assays were performed to detect main expanded spectrum beta-lactamases (ESBLs), carbapenemase-encoding, and plasmid-mediated quinolone resistance genes.

E. coli (56%) was the most prevalent bacteria isolated, followed by *Salmonella enterica* (13%) and *Proteus mirabilis* (9%). All the isolates were resistant to at least one antibiotic, 66% were multidrug-resistant, and 21% were ESBL producers. The results showed the presence of bla_{TEM} (32%), bla_{SHV} (11%), bla_{CMY-2} (7%), bla_{OXA-48} (4%), bla_{VIM} (2%), bla_{KPC} (13%), *qnrA* (28%), *qnrB* (8%) and *qnrS* (34%) genes.
Thus, fresh meat harboring ARB and ARGs is a potential consumer risk. Further studies must be done to detect ARGs in meat and how they spread in non-medical environments.

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SULPHITE-RESISTANT VARIANTS OBTAINED BY DIRECTED EVOLUTION ASSAYS IN *SALMONELLA TYPHIMURIUM*

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Salmonella typhimurium is one of the pathogenic microorganisms of greatest concern in the food industry. In 2022, it was the second most reported foodborne and the major cause of foodborne outbreaks in the European Union.
Currently, *Salmonella* is a bacterium that can be treated by the employment of effective antibiotics. Nevertheless, the emergence of antimicrobial resistance (AMR) is a serious health issue worldwide. Furthermore, it is unknown whether this phenomenon may also occur in relation to other antimicrobials, such as the case of food preservatives.
Sodium sulphite is a commonly used preservative in meat products to extend their self-life and ensure consumer safety. However, the possibility of *Salmonella* developing resistance to this preservative is still unknown.
The aim of this work was to observe the effect of sodium sulphite on the emergence of resistant variants (RVs) of *Salmonella enterica* serovar *Typhimurium*.
In order to conduct the research, five evolution assays were performed by subjecting *S.* Typhimurium to a subinhibitory concentration of sodium sulphite of 900 mg/L for a period of 20 consecutive days with the intention of obtaining RVs. Once the evolution assay was accomplished, a Minimum Inhibitory Concentration (MIC) test was conducted based on the CLSI method so as to compare the resistance of all the RVs and the original strain.
The results have demonstrated that, after the evolution assay, the resistance of *Salmonella* to sodium sulphite increases in more than 30 times in comparison to the resistance of the original strain. Furthermore, five resistant evolution lines were achieved. Subsequently, a genotypic characterization is going to be performed to understand the nature of the increase in resistance. Moreover, a phenotypic characterization is going to be conducted, which will include the study of cross-resistance of these variants to antibiotics, food preservation and cleaning and disinfection methods.
To conclude, this study has found that a long-term exposure to sodium sulphite can lead to a resistance generation in *S.* Typhimurium. This fact demonstrates that an appropriate use of this preservatives should be made in the food industry with the aim of avoiding potential issues in the food chain.

P-135

SOME DATE SEED POLYPHENOLS EXHIBIT 'BROAD SPECTRUM' ANTIBIOTIC ADJUVANT ACTIVITY WITH MULTIPLE ANTIBIOTICS TO INHIBIT MULTIDRUG-RESISTANT *SALMONELLA TYPHIMURIUM* DT104

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The surge in bacterial antimicrobial resistance (AMR) poses a formidable challenge to successful antibiotic therapy. Innovative approaches and drug combinations are essential to mitigate the emergence of AMR. Since discovering new antibiotics is not an economically viable option, researchers are focussing on options that prolong the life span of conventional antibiotics by using antibiotic adjuvants. Antibiotic adjuvants are compounds used along with an antibiotic that sensitizes drug-resistant bacterial pathogens to the respective antibiotic. Polyphenols, abundant in plant food matrices, have emerged as potential candidates for antibiotic adjuvants.

Despite the abundance of polyphenols in date seeds, limited research has explored the antibiotic adjuvant potential of individual date seed polyphenols. Since the emergence of AMR is a particular threat in *Salmonella,* we used the multi-drug resistant strain *Salmonella* Typhimurium DT104 as our model organism. Since DT104 was resistant to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (the ACSSuT phenotype), we tested the adjuvant properties of 24 date seed polyphenols against the said antibiotics. None of the tested 24 tested polyphenols exhibited any antimicrobial properties against DT104. However, eight of the 24 polyphenols, *viz.* coumaric acid, itaconic acid, 4 hydroxycinnamic acid, cinnamic acid, syringic acid, vanillic acid, β- carotene, and gallic acid made DT104 sensitive against the tested antibiotics. Of these, 4 hydroxycinnamic acid, cinnamic acid, and itaconic acid made DT104 susceptible against four tested antibiotics and were therefore named 'broad spectrum' antibiotic adjuvants.

Subsequent checkerboard analysis revealed that the antibiotic adjuvant activity was polyphenol concentration dependent. All the broad-spectrum polyphenols exhibited effective antibiotic adjuvant activity until 0.25x MIC levels, beyond which the polyphenols were too diluted for adjuvant activity. Time-kill kinetic studies of 4 hydroxycinnamic acid against chloramphenicol revealed an overall 4-fold drop in DT104 cell density. Overall, we show that the adjuvant activity of the date-seed polyphenols can be explored as a potential strategy to mitigate AMR emergence.

P-136

IMPACT OF TEMPERATURE AND NACL CONCENTRATION ON THE GROWTH OF *E. FAECIUM* STRAINS AND THE TRANSCRIPTION GENES RELATED TO ENTEROCIN PRODUCTION, ANTIBIOTIC RESISTANCE AND VIRULENCE FACTORS

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Enterococci are integral to raw or thermized milk cheeses' microbial flora; however, their use in foods is controversial due to the occurrence of genes linked to antibiotic resistance and virulence factors.

The aim of the study was to investigate the influence of temperature and NaCl concentrations on growth of ten strains of *E. faecium* previously isolated from raw sheep milk and artisanal Feta and Kefalograviera cheeses and the transcription of genes associated with enterocin production, antibiotic resistance and virulence factors.

The strains of *E. faecium* with confirmed antimicrobial activity against the pathogenic microorganism *L. monocytogenes* were inoculated into commercial U.H.T. milk. Subsequently, they were incubated at three temperatures (20, 30, 37 °C) and exposed to two NaCl concentrations (1.5%, 3.5%) for 24 hours. Population counts were conducted by standard plate counting on MRS agar, and their ability to acidify milk was assessed by measuring the pH of the liquid nutrient substrate. Their antimicrobial activity against a mix of three *L. monocytogenes* strains and resistance to 8 antibiotics were evaluated using well and disk diffusion method, respectively. The high-quality draft genome data of the strains revealed that the strains harbored 25 genes associated with bacteriocin synthesis, immunity, transport and regulation of expression, 10 genes associated with antibiotic resistance and 2 genes associated with virulence factors. RT-qPCR analysis was applied to assess the examined parameters in the transcription of genes related to bacteriocin production in four representative strains.

The population, milk acidification capacity, and antibiotic resistance increased with the rise in temperature, while NaCl concentration did not significantly affect them. Antimicrobial activity tended to increase at 20 °C in the presence of salt, whereas in the absence of salt, most strains inhibited the growth of the pathogenic microorganism after incubation at 30 °C. Although the examined factors primarily did not influence the transcriptional response of genes, overall, it appeared that the temperature increase had the most significant impact.

More research is required to fully evaluate the biotechnological and safety aspects of *Enterococcus* strains for potential use as co-starters or bioprotective cultures in the food industry.

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EFFECTIVE SUPPRESSION OF *STAPHYLOCOCCUS AUREUS* ATCC 25923 GROWTH THROUGH PERIODIC DOSING OF PASTEURIZED MILK-DERIVED EXTRACELLULAR VESICLE

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Background

There is rising interest in extracellular vesicles (EVs) as natural antimicrobials against food spoilage and ensuring food safety. These membrane-bound particles exhibit bioactive properties, making them intriguing for scientific exploration. Our investigation outlines the antimicrobial efficacy of pasteurized cattle milk-derived EVs against *Staphylococcus aureus*, offering novel insights into natural antimicrobial agents for addressing microbial food quality and safety.

Material/methods

EVs isolated from commercially available pasteurised milk by centrifugation and tangential flow filtration. *Staphylococcus aureus* ATCC 25923, in the planktonic phase (1 × 107 CFU/mL), was co-cultured with these EVs (1 × 109 EVs/mL) in Muller Hinton broth. Subsequent two doses of EVs were administered at 3-hour and 6-hour time points and incubation continued for 48 hours at 35 °C. Bacterial growth was assessed by measuring absorbance at 620 nm. A negative control was maintained using a phosphate buffer solution. The percentage difference in bacterial growth, in comparison to the negative control, was determined and expressed as mean ± standard error of the mean. Reduction in CFU was observed at various time points (0, 3, 6, 12, 18, and 24 h) by subculturing bacteria-EVs co-cultures on blood agar. The study involved three biological replicates.

Results

Co-culturing *S. aureus* ATCC 25923 with EVs revealed their ability to inhibit bacterial growth. The CFU/mL decreased from 2.4 × 105 (single dose) to 2.3 × 105 (double doses) and further to 7.4 × 104 (triple doses) after 12 hours of incubation. Additionally, milk EVs extended the lag time, increasing it from 6 hours (single dose, $p = 0.0208$; double doses, $p = 0.0202$) to 9 hours (triple doses, $p = 0.0109$) compared to the control's 4 hours. Periodic milk EV dosing also prolonged the overall generation time, from 2.8 hours (single dose) to 3.5 hours (double doses) and 4.8 hours (triple doses), in contrast to the control's 3.4 hours.

Conclusion

While pasteurized milk EVs exhibit promise in impeding the growth of planktonic phase *S. aureus* by prolonging the lag phase, the elucidation of bioactive molecules and the precise mechanisms underlying this antimicrobial effect remains to be ascertained. Addressing these knowledge gaps could give innovative solutions to enhancing food safety and food preservation.

P-138

GENETIC AND PHENOTYPIC INSIGHTS INTO ANTIMICROBIAL RESISTANCE AND VIRULENCE OF *CAMPYLOBACTER* ISOLATES FROM POULTRY, SLOVENIA

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Campylobacter, one of the main causes of foodborne illness, leads to campylobacteriosis, a widespread zoonotic infection. Due to its adaptability, it thrives in a wide variety of environments, from poultry and livestock to pets and humans. This versatility often leads to complex cases of gastroenteritis, which are exacerbated by the bacterium's

increasing resistance to antibiotics. The global challenge of antibiotic resistance, particularly in pathogens such as *Campylobacter*, and its transmission via poultry, underlines the urgent need for effective food safety measures and has a significant economic impact on agriculture and healthcare. To investigate the dynamics of antimicrobial resistance and virulence factors in *Campylobacter*, we analysed 27 poultry meat and 28 poultry faecal samples, and isolated a total of 40 *Campylobacter* spp., namely 23 *C. jejuni* and 17 *C. coli*. These isolates were subjected to whole-genome sequencing (WGS) analysis to obtain a detailed genetic characterization. In addition, we performed phenotypic antimicrobial susceptibility testing (AST) using broth microdilution as well as CaCo-2 invasion testing to assess their virulence.

Our comparative analysis of genotypic data from WGS and phenotypic results from AST showed a strong concordance for antibiotics ciprofloxacin and tetracycline. Interestingly, the presence of genes associated with antimicrobial resistance did not always predict resistance to beta-lactams and aminoglycosides, indicating a complex relationship between genotype and antibiotic resistance phenotype. In addition, we identified important virulence determinants in the isolates from poultry meat and faeces, including cytolethal distending toxin, flagellar components and protein adhesins. However, the presence of these virulence factors did not directly correlate with the extent of invasion observed in CaCo-2 cells, suggesting that the relationship between specific virulence factors and pathogenicity may be more nuanced than previously thought.

Understanding the genetic basis and molecular mechanisms underlying antimicrobial resistance and virulence, and their interrelationships is crucial for the development and application of effective strategies to control and monitor antimicrobial resistance in virulent *Campylobacter* strains in the food chain, contributing to the improvement of overall food safety.

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EMERGENCE OF BIOCIDE RESISTANCE IN *SALMONELLA TYPHIMURIUM* VIA DIRECTED EVOLUTION EXPERIMENTS

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Salmonella spp. is a biotic agent responsible for foodborne outbreaks. It is the most reported toxi-infection, accounting for more than half of all hospitalizations related to foodborne. The use of antibiotic treatments for clinical cases of salmonellosis may lose effectiveness due to the emergence of antimicrobial resistance.

The phenomenon of antibiotic resistance is considered a global health problem and is being widely studied. However, it is uncertain whether enhanced resistance might occur against other antimicrobial compounds, such as biocides.

Peroxyacetic acid (PAA) is one of the main biocides used as surface disinfectants in the food industry. The mechanism of its biocidal activity consists of the oxidation of components of its cell membrane components, mainly membrane proteins, which leads to instability and subsequent cell lysis.

This study aims to determine whether PAA exposure could lead to the emergence of resistant variants in *Salmonella enterica* serovar Typhimurium populations.

Five evolution assay lines were conducted where *S.* Typhimurium wild-type strain (WT) was exposed to a subinhibitory concentration of PAA (800 µL/L) for 330 cell generations in tryptone soy broth. Minimum inhibitory concentrations (MIC) of PAA were determined against WT and evolved populations to characterize their direct resistance.

Results indicate that the resistance of the five evolved populations against PAA increased. MIC of WT against PAA was 1600 µL/L. while MIC of evolved populations were 2000 µL/L. Therefore, evolved populations were 33% higher resistant than the WT. However, further investigation is necessary to isolate clones from each evolved population to evaluate their individual resistance against PAA, and to identify the responsible mutations.

In conclusion, prolonged exposure to subinhibitory concentrations of PAA can lead to the emergence of direct

resistance on *S.* Typhimurium populations. Therefore, proper use of biocides in the food industry is suggested to mitigate bacterial resistance phenomena.

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ENVIRONMENTAL AND GENETIC FACTORS SHAPING THE PERSISTENT PHENOTYPE OF *L. MONOCYTOGENES* IN AUSTRIAN FOOD PROCESSING ENVIRONMENTS

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Listeria (*L.*) *monocytogenes* thrives in diverse natural and built habitats and poses significant challenges to the food industry due to its role as the causative agent of listeriosis. In food processing environments certain strains can survive cleaning and disinfection protocols for months up to years, causing a threat of contamination of food products. Despite extensive research by scientific and industrial partners into its genome, mobilome, and co-occurring microbial community, a comprehensive explanation of persistence remains elusive. Our study aims to unravel the complex interplay of genetic and environmental factors influencing the persistence of *L. monocytogenes*. Two Austrian food processing facilities (dairy and fish) were swab-sampled at food contact and non-food contact surfaces during both dirty and clean states over 1.5 years. Environmental parameters, including temperature, humidity, surface material, and size of swabbed area, were recorded during sampling. Swab samples underwent DNA extraction for microbiome sequencing, and *L. monocytogenes* isolation was performed using a two-step enrichment protocol, followed by whole-genome sequencing. After screening 16% of *L. monocytogenes* positive samples for persistent strains, we identified one strain (three repeated isolations), belonging to ST4 (CT4605, core genome MLST typing) as a persistent strain. We used whole genome SNP analysis to compare 39 genomes from previously isolated strains in this dairy facility, revealing a maximum of 10 SNP difference across all genomes. In the next step a in depth analysis of genetic factors, co-occurring microbiome and environmental parameters will follow.

We hypothesize that persistence of *L. monocytogenes* is linked to specific environmental parameters and genetic factors. Key environmental conditions create a niche for *L. monocytogenes* colonization and survival. Genetic elements, notably resistance genes, contribute to the bacterium's ability to endure challenges such as cleaning and disinfection. In summary, our research elucidates the environmental parameters defining the *L. monocytogenes* niche and genetic factors enabling its survival in food processing environments for long periods of time. By providing a holistic perspective, our study will enhance the understanding and management of the persistent phenotype of *L. monocytogenes,* ultimately contributing to more effective control strategies in the food industry.

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THE ROLE OF ΣB IN STRESS ACCLIMATION OF *LISTERIA MONOCYTOGENES* TO DYNAMIC HEAT TREATMENTS

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Although the general heat stress response of *Listeria monocytogenes* has been described, additional research is necessary to determine the specific mechanisms involved in the response of this species. A major knowledge gap is the ability of *L. monocytogenes* to initiate a physiological response when exposed to sub-lethal temperatures, resulting in increased resistance to subsequent treatments. This phenomenon (known as stress acclimation), is of great relevance for the design of thermal processes, as it has been

observed during dynamic treatments at low heating rates (< 10 °C/min).

This study advances the analysis of stress acclimation focusing on the heat resistance of exponential phase cells of three strains of *L. monocytogenes* EGD-e: a wild-type strain and two mutant strains (ΔsigB and ΔrsbL, R2, R3, R4). Heat treatments were applied under isothermal conditions (55 to 60 °C), as well as dynamic conditions with heating rates between 2 and 14 °C/min before holding at 58.0 °C. The data was analyzed using an innovative modelling approach that separates the effect of the instantaneous temperature (Bigelow model) from dynamic stress acclimation.

Heating rates lower than 10 °C/min induced dynamic stress acclimation, even in the two mutant strains. The amount of stress acclimation was quantified using the acclimation model, concluding that this phenomenon could induce up to a 2-fold increase in the *D*-value with respect to the one observed under isothermal conditions. This is of great importance for industry because models based on isothermal conditions could underpredict the microbial concentrations during treatments with complex profiles. Hence, the fitted models were translated into a two-dimensional diagram that enables the design of inactivation treatments considering stress acclimation. The diagram also facilitates the comparison between the three strains, emphasizing the role of σB on dynamic stress acclimation.

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INFLUENCE OF SPORE INNER MEMBRANE FLUIDITY ON RESISTANCE AND GERMINATION OF *B. SUBTILIS* SPORES

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Control of bacterial spores is one of the greatest challenges for the food industry because they are very resistant to physical and chemical hurdles used for food preservation and can germinate during food storage causing spoilage and foodborne illnesses. Thermal processing is the most effective method to inactivate spores, but it has a negative impact in nutritional and sensorial properties of foods. Therefore, a better understanding of the structural and molecular factors involved in spore resistance and germination may shed light on the development of novel control strategies of sporulated bacteria.

Spores are formed by multiple layers including the inner membrane (IM). This layer contains germinant receptors and other molecules involved in the start of germination and it is believed to provide protection to heat and chemicals. The aim of this work is to evaluate the effect of changes in the IM fluidity on heat inactivation and germination kinetics of spores. IM composition was synthetically modified using a *B. subtilis* mutant lacking the *des* gene (*des::kan*), encoding the phospholipid desaturase, and a strain (*desK::kan* P_{Km}*-desR,* DesK/DesR being the two-component system that regulates the expression of the *des* gene) with upregulated expression of Des when induced with kanamycin. Spores of the former strain were cultured in the presence of L-valine while the latter were sporulated with L-isoleucine to increase the proportion of iso- and anteiso-branched-chain fatty acids, respectively.

The absence of Des, which led to a more rigid IM, increased heat resistance, while the strain *desK::kan* P_{Km}*-desR* displayed a more fluid IM and decreased heat resistance. Although the *des::kan* mutant showed 1.7-fold higher D_T values than the strain *desK::kan* P_{Km}*-desR* at all the temperatures tested (97.5-105.0 °C), both showed a similar thermo-dependence (z value). On the other hand, germination kinetics in

L-alanine, L-valine, AGFK mixture (ariginine, D-glucose, D-fructose and potassium) and Ca2+-dipicolinic acid were similar in both strains. Our results demonstrate that changes in phospholipid composition of the spores' IM affects heat resistance but not germination.

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DIRECTED EVOLUTION EVIDENCES THAT HEAT RESPONSE MECHANISMS OBSERVED UNDER LABORATORY CONDITIONS MAY BE DIFFERENT FROM THOSE RELEVANT FOR INDUSTRIAL PASTEURIZATION

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Listeria monocytogenes is one of the most common foodborne bacteria. This microorganism poses a particular challenge due to its capacity to adapt and survive in various environments and adverse conditions, including refrigeration temperatures, higher temperatures (around 50 °C) and acidic conditions. Cells respond rapidly to external stresses by transmitting signals to provide appropriate responses to that stress. The transmission of such signals is a complex and articulated concept, requiring cell-sensing mechanisms and signaling pathways to direct appropriate responses to environmental changes. These mechanisms are finely regulated and allows rapid adaptation to changing external conditions, thus promoting bacterial survival through modifications at both the individual cell and the entire population level. This adaptative mechanism can lead *L. monocytogenes* to survive even at higher temperatures.

To study this adaptation mechanism, two heat resistant variants of *L. monocytogenes* CECT 4032 were isolated by applying two different treatments, a long-mild treatment (30 min at 58 °C) and a short-harsh treatment (5 min at 62 °C). The selection was performed by alternating rounds of exposure to the heat treatments and outgrowth of the surviving population at the end of each treatment. After 15 cycles, the variant obtained at 62 °C showed no difference in thermal resistance compared to the wild type, whereas the one obtained through directed evolution at 58 °C showed an increased thermal resistance (1 log-increase in the number of survivors). However, when this variant was exposed to 62 °C, it did not show an increase in thermal resistance with respect to the wild type. This could imply that the defense mechanisms that define bacterial survival to heat is dependent on the intensity of the treatment. This is relevant for the interpretation of pasteurization treatments because industrial ones are often at temperatures above 65 °C. To support this, a genome-wide association study (GWAS) was done comparing both adapted strains to wild-type strain, to determine the putative mechanisms involved on such adaptation.

Our results would imply that bacterial survival at these temperatures would not be ruled by the mechanisms observed under laboratory conditions at milder temperatures.

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DRY ADHESION CAPACITY OF *CRONOBACTER SAKAZAKII* ON PRE-CONDITIONED SURFACES

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Cronobacter is among the main pathogens of concern in low moisture foods (LMF), especially in powdered infant formula (PIF). These products can be contaminated by raw material or the processing environment.

The aimed of this study was to evaluate the impact of pre-conditioning on the dry adhesion of a cocktail of five *Cronobacter sakazakii* strains on stainless steel (SS) and polypropylene (PP) coupons. The surfaces were pre-conditioned with sterile soil, wheat flour, and PIF at 300 rpm for 24 h. Subsequently, the coupons were transferred to sterile vials containing soil inoculated with *Cronobacter sakazakii* and were incubated at 25 °C for up to 7 days.

Counts of planktonic cells (PC), cultivable sessile cells (CSC) and total viable sessile cells [CSV = CSC + viable but non-cultivable cells (VBNC)] were determined after 0, 2, 5 and 7 days.

The initial count of *Cronobacter* in the dry carrier (soil) varied from 5.9 to 7.2 log CFU/g. No signficant differences ($p > 0,05$) were verified among PCs for all analyzed treatments, except for PIF+SS at day 0. The counts ranged from 3.1 to 4.7 log CFU/cm^2. Overall, the contact time of the pre-conditioned coupons with the dry carrier (soil) did not influence ($p>0.05$) the bacterial adhesion on both surfaces. The initial CSC was between 2.1 and 3.8 log CFU/cm^2, whereas CSV ranged from 4.2 to 6.4 log CFU/cm^2.

After 7 days, CSCs were between 1.8 and 3.6 log CFU/cm^2 and CSVs between 3.5 and 6.1 log CFU/cm2. The highest dry adhesion of *Cronobacter sakazakii* was observed on PP. However, for CSC, there was a significant difference ($p< 0.05$) only between the surfaces conditioned with wheat flour for all contact times. While for CSV, significant differences were observed for PIF and soil at day 0 and wheat flour at day 5 ($p< 0.05$).

In addition, there were no significant changes ($p>0.05$) in the a_w values of all pre-conditioned coupons throughtout the experiment. Therefore, surface pre-conditioning can favor *Cronobacter* dry adhesion, particularly on PP surfaces commonly used in LMF industries.

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HEAT AND UV-C LIGHT RESISTANCE OF *SALMONELLA ENTERICA* SUBSP. *ENTERICA*, STRAIN 14028S (SEROVAR *TYPHIMURIUM*) ISOGENIC MUTANTS FROM A SINGLE-GENE DELETION MUTANT LIBRARY

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Salmonella is responsible of the second most frequent zoonotic disease in Europe and the United States. Furthermore, some serovars are considered as emergent pathogens. This Gram negative is a well-known foodborne pathogen. Thus, one of the *Salmonella* most characteristic feature is the great geno- and pheno-typic diversity. However, and even though its stress resistance has been widely studied, many aspects regarding are still largely unknown.

A large library of isogenic mutants has been generated by Porwollick et al. in 2014 by the targeted deletion of genes of interest, including sRNAs and non-essential ORFs. This is of the highest interest since it can be used to evaluate the effect of the individual gene deletion in different aspects of salmonella physiology, including stress resistance. In order to do so, we have developed a methodology to efficiently screen the resistance of the 3331 mutants from the SGD-K collection to heat and UV-C light treatments.

This protocol has been optimized to work with the 96-wells plates of the library and involves the application of treatments of different intensity in order to identify those genes whose absence modifies the resistance of *Salmonella* Tyhimurium ATCC 14028s strain.

By the moment, we have identified sensitive and resistant mutants for both processing technologies and we are characterising individually them in order to further understand the role of each gene/ORF in *Salmonella* heat and UV-C resistance.

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COMPARATIVE ANALYSIS OF COMMERCIAL CLEANING AND DISINFECTION FORMULATIONS AND PROTOCOLS FOR EFFECTIVE ERADICATION OF *PSEUDOMONAS FLUORESCENS* BIOFILMS

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Pseudomonas spp. is the main spoilage genus of meat stored under chilling and aerobic conditions. Its ability to adapt to different environmental conditions and to form biofilms would explain the establishment of this genus as part of the residential bacteria in the food industry. Biofilms are complex and structured communities encased in a self-produced exopolysaccharide matrix which show higher resistance against antibiotics, disinfectants, and dynamic or hostile environments, so they can create a persistent

source of product contamination. Hence, there is a need for the design and implementation of control strategies that are effective against biofilms in food industry. The aims of this study were to characterize the biofilm formation of a *Pseudomonas fluorescens* strain isolated from a poultry industry, and to assess the efficacy of different cleaning and disinfectant (C&D) procedures in its eradication.

Biofilm formation in 316L stainless steel 24-well plates at 25 °C under static conditions was quantified over time (24-96 h) by plate counting and optical density (595 nm) measurement after a sonication process to disaggregate the biofilm cells. Then, different C&D procedures were tested against mature *P. fluorescens* biofilms. C&D agents included detergents (alkali or enzymatic), detergent-disinfectants (based on tertiary alkylamines or on sodium hypochlorite), and disinfectants (based on peracetic acid), all of them kindly provided by OX-CTA S.L.; and application methods included immersion and fogging. The bactericidal activity of these C&D procedures was measured by comparing the counts of sessile cells before and after the procedure.

P. fluorescens demonstrated a strong biofilm-formation ability, and the point of maximum formation was established after 96 h of incubation. Regarding C&D procedures, the sole application of detergents, detergent-disinfectants or disinfectants proved ineffective against *P. fluorescens* biofilms. However, the combination of detergents and disinfectants in two separate steps led to the complete eradication of *P. fluorescens* biofilms. Furthermore, there were no statistical differences between applying the disinfection step via immersion or fogging, being both application techniques effective in biofilm eradication.

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CHARACTERIZATION OF BACTERIAL BIOFILMS FORMED BY THE MICROBIOTA PRESENT IN A POULTRY SLAUGHTERHOUSE

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During poultry slaughter and evisceration, there are numerous potential sources of contamination, including cross-contamination of chicken carcasses with microorganisms present at different slaughterhouse's surfaces, tools, or equipment. In recent decades, several control measures have been implemented to reduce contamination with pathogenic microorganisms. However, some of these microorganisms can persist in processing environments due to its ability to form biofilms, which are structures that keep bacterial cells adhered to surfaces.

The aim of this study was to characterize the biofilm-forming microbiota present on 22 surfaces of a poultry slaughterhouse, in northwestern Spain, after carrying out the cleaning and disinfection protocols. The microbiota present on each surface was grown at 12 °C in biofilms for up to 72 hours. Then the biofilm structures were examined using confocal laser scanning microscopy (CLSM) in conjunction with fluorescent dyes for all types of bacteria (DAPI) and for Gram-positive bacteria (Red-TEXAS), and its microbiological composition was described using metagenomic techniques (Oxford Nanopre sequencing).

All microbiomes developed robust biofilms at 72 hours, with biovolume values that ranged between 22.106,8 ± 5.544,3 μm^3 and 376.760,1 ± 43.513,4 μm^3 in the observation field of 16,078.2 μm^2. Nine predominant genera were found (some of them exceeding 45% of the total composition of the biofilm): *Pseudomonas* (present in all 22 samples), *Serratia* (17), *Citrobacter* (14), *Enterobacter* (13), *Klebsiella* (12), *Stenotrophomonas* (12), *Escherichia* (11), *Salmonella* (10) and *Shewanella* (9). It is also worth to highlight the presence of some pathogenic genera, such as *Clostridium*, *Enterococcus*, *Streptococcus* or *Yersinia*, although none of

them exceeded 3.0% of the total composition of each biofilm. These findings allow us to expand knowledge about the composition and main characteristics of the microbiota present on different surfaces of the slaughterhouse under study, which is of practical use, since it can serve as a basis for improving the cleaning and disinfection procedures.

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MONITORING OF ANTIMICROBIAL RESISTANCE AND VIRULENCE GENE DISTRIBUTION IN *SALMONELLA* SEROTYPES ISOLATED FROM HUMANS IN CENTRAL ITALY, 2005-2022

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Human salmonellosis is reported as the second most prevalent zoonotic disease in the EU. The increased incidence of this infection, often caused by resistant strains, requisites for the assessment of virulence factors contributing to *Salmonella* pathogenic potential.

This study was designed to monitor the prevalence and trends of both antimicrobial resistance (AMR) and virulence genes in human *Salmonella* strains (years 2005-2022) in Marche region, Central Italy. A total of 114 strains of five epidemiologically important serotypes (*S. Derby, S. Infantis, MVST, S. Enteritidis, S. Typhimurium*) were tested against 16 antibiotics (seven classes) through the disk diffusion method according to CLSI.

Results indicated that the highest resistance was found against sulfisoxazole (61.7%), followed by tetracycline (56.5%) and ampicillin (54.8%). Among these strains, 62% were multidrug-resistant (MDR), 12% showed resistance to less than three classes and 26% were completely susceptible. Among MDR strains, the highest percentage (37%) was observed against five classes and the lowest (6%) among seven classes.

Moreover, a rising trend for AMR was observed in all antimicrobial classes over the period investigated. After identity confirmation through genus-specific (ttrC) real-time PCR, the optimization of amplification protocols for the detection of twelve virulence genes was performed. SPI-1 (invA, sopB, sopD, sipA, sipB, sipD), SPI-2 (ssaR, sifA), a gene related to survival in the host (spvB), outer-membrane protein (tolC) and flagellar proteins flgK, flgL) were detected.

All isolates were found positive for sopB, sipA, and tolC genes. The frequency of virulence genes ranged from 32-100%. Detection of the 12 virulence genes classified the 114 isolates into 36 patterns. Pattern (I) with 10 genes was the most dominant (16.6%), followed by pattern (II) with 11 genes (14%) and (XIV) with all the 12 genes (12%). *S. Enteritidis* was the serovar carrying the highest number of virulence genes among those investigated.

Virulence genes can vary over time in different serovars. They can show stable, rising, or declining trends predicting the evolutionary feature of *Salmonella* spp. Our results are aligned with EU and global data and are important to define the trend of the pathogenic potential and public health significance of *Salmonella* in Central Italy.

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SOURCE TRACKING OF ANTIBIOTIC RESISTANCE AND FECAL INDICATOR BACTERIA ALONG THE WHOLE PRODUCTION CHAIN OF MUSSELS IN NORTHERN GREECE

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Antibiotic resistant bacteria (ARB) have been increased dramatically in all ecosystems where animals and human live. ARB can be transferred from inland to the aquatic environment through various sources, reservoirs, and pathways, and further to fish (finfish and shellfish) and the consumer's table.

The aim of this study was to reveal the sources of transferring ARB and fecal indicator bacteria from inland to Thermaic Gulf in Northern Greece where approximately the 80-90% of the total domestic production is produced and exported. To reach this aim, samples (e.g. water, soil, sewage) from municipal wastewater treatment plants, agricultural fields, animal farms, and river mouths (Axios River); water samples and mussels from three different mussel farms (near processed wastewater runoffs, near river mouths, and open sea) were collected.

All samples were analyzed via microbiological analysis (membrane filtration method for the water samples, plate-based methods for the rest of the samples) to isolate fecal indicator bacteria and potential foodborne pathogens. Afterwards, the isolated bacteria were identified using the MALDI Microflex LT (Bruker Daltonic GmbH, Bremen, Germany) following the on-target-plate protein extraction protocol used as recommended by the manufacturer (Bruker Daltonic) to increase spectrum quality. Spectra were obtained using the linear positive ion mode analysis, with a laser frequency of 60 Hz.

The mass range was m/z 2000 to 20,000. Each isolate was tested for resistance in 20 different antibiotics in discs using the antibiotic disc diffusion method. *Escherichia coli*, *Klebsiela oxytoca*, *Raoultella ornithinolytica*, *Roultella planticola*, *Serratia marcescens*, *Serratia fonticola*, *Aeromonas bestiarum*, *Enterobacter cloacae*, *Citrobacter braakii* were isolated from almost all samples including mussels, while *Enterococcus faecium*, *E. casseliflavus*, *E. mundtii* and *E. gallinarum* were found in environmental samples but not in mussels. Of them, *Enterococcus* isolates were found to be resistant in cephalosporins, while *E. coli* in Clindamycin, Vancomycin and Penicillin G. The rest of the isolated bacteria exhibited a multi-resistance profile to various antibiotics.

Finally, it is crucial to point out that ARB/potential pathogens and fecal indicator bacteria, were found not only in environmental samples of inland that end up in aquatic environment but also in the mussels farming water/aquatic environment and mussels.

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HEAVY METAL RESISTANCE IN *SALMONELLA* 4,[5],12:I:- AND ITS ASSOCIATION WITH ANTIBIOTIC RESISTANCE

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Salmonella 4,[5],12:i:- is one of the main food-borne pathogens transmitted through pork. Due to the increasing antibiotic resistance found in pathogens, the use of antibiotics as growth promoters in food-producing animals was prohibited in Europe in 2003 and were replaced by heavy metals in feed. Despite this, the prevalence of *Salmonella* 4,[5],12:i:- has increased in pigs in Europe, which may be associated with tolerance to the heavy metals.

The aim of this study was to analyse the evolution of mobile genetic elements conferring resistance to heavy metals and to analyse whether the use of metals may be causing a selective pressure favouring the increase of multi-resistant *Salmonella* 4,[5],12:i:-.

For that, a total of 57 *Salmonella* 4,[5],12:i:- isolates collected from 1999 to 2021 from human, food and pig origins were selected. Genomes of the strains were screened for antibiotic and heavy metal resistance genes presence using AMRFinderPlus. The core SNP alignment created by Snippy and the isolation date of each strain were used to construct a time-scaled phylogenetic tree based on the Bayesian method using BEAST.

The oldest strains, isolated between 1999 and 2012, were ST19 and in 86.2% of these strains the multi-resistance plasmid pUO-STmRV1 (harbouring resistance genes to arsenic silver and mercury) was detected with a significant positive association to 11 antibiotic resistance genes. In contrast, initial acquisition of the SGI-4 island (harbouring resistance genes to arsenic, silver and copper) was observed in 2008 and persisted in 96.3% of the ST34 isolates until 2021. Acquisition of MREL (harbouring resistance genes to mercury) simultaneously with SGI-4 was also observed in eight strains. Strains carrying SGI-4 showed a significant positive association with four antibiotic resistance genes. Interestingly, resistance genes to bleomycin, chloramphenicol and trimethoprim were lost in the most recently isolated strains.

Overall, the high prevalence of mobile genetic elements conferring tolerance to metals could be one of the reasons for the adaptation and evolutionary success of *Salmonella* 4,[5],12:i:- in swine. Following the prohibition on the use of antibiotics as growth promoters in food-producing animals, *Salmonella* 4,[5],12:i:- strains have evolved by losing several clinically relevant antibiotic resistance genes.

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AI-ASSISTED RISK NEGOTIATION. INTEGRATED RISK ANALYSIS FOR ONE HEALTH

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We are faced with a multitude of pressing global health risks with complex interdependencies that can only be effectively addressed in an integrated, participatory effort. Although risk analysis is a crucial tool to support decision-making, the compartmentalized nature of its current frameworks does not account for the complexity of multi-faceted systems.

We propose the participatory and interdisciplinary concept of Risk Negotiation to transform the way we tackle global health challenges, such as pandemics, physical and mental health inequities, environmental problems, and food security. To allow integrated, participatory risk analysis, we need to valorize risks and trade-offs and negotiate them with stakeholder groups that represent different disciplines and sectors.

This game theoretic approach becomes feasible through recent technological breakthroughs, such as artificial intelligence (AI)-assisted multi-agent negotiations or large language models (LLMs) in general. These are accessible, hold great promise in negotiating agreements and can be used to accommodate the complexity of real-world decision-making.

Risk Negotiation-based risk analysis could disrupt compartmentalized, silo oriented risk analysis processes, and thereby increase global health security, equity, and sustainability.

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SALMONELLA TYPHIMURIUM WITH ACQUIRED CIPROFLOXACIN-RESISTANCE SHOWS VIRULENT POTENTIAL

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The alarming appearance of *Salmonella* spp. resistant to ciprofloxacin (CIP) in food producing animals and human isolates indicates that the agri-food chain might serve as driver of antimicrobial resistant (AMR) bacteria towards the consumers. However, the acquisition of AMR in bacteria displays either positive or negative correlations with their virulence. Studying the virulence of CIP-resistant *Salmonella* spp. is of interest, as CIP remains the first-line drug for the treatment of multi-drug resistant *S. enterica*.

This study aims to determine and compare the virulence of *Salmonella* Typhimurium LT2 (SeWT) and a derived resistant variant to CIP ($SeRV_{CIP}$).

The CIP-resistant variant ($SeRV_{CIP}$) was previously obtained via submitting SeWT to evolution assays in presence of increasing concentrations of CIP. Virulence was studied performing dye-leakage and longevity assays in *Caenorhabditis elegans* as a test organism. Briefly, up to 12 synchronised L4 nematodes were fed *ad libitum* with *Escherichia coli* OP50 (control), SeWT and $SeRV_{CIP}$ for 21 days. At day 7, erioglaucine was used to denote morpho-functional alterations of the intestinal barrier under the microscope.

Observations of SeWT-fed nematodes after dye-leakage assays revealed abnormal and enlarged intestinal tubes, with a substantial loss of integrity. $SeRV_{CIP}$-fed ones exhibited more regular physiology although more compromised than compared to the controls (OP50-fed nematodes). Despite these slight divergences, longevity assays revealed no significant differences ($p > 0.05$) between SeWT and $SeRV_{CIP}$ in overall lifespan (0.1% and 1.2% at day 19, respectively). Furthermore, the median lifespan was 6.94 ± 0.79 days for SeWT and 8.05 ± 1.44 for $SeRV_{CIP}$, entailing no significant differences between the two *Salmonella* bacteria ($p > 0.05$). To conclude, mutations occurred in $SeRV_{CIP}$ during evolution towards CIP-resistance did not significantly affect its potential for virulence. Thus, CIP-resistant *Salmonella* spp. appearance in the agri-food chain may result in a hindered antibiotic efficacy whether reaching the end consumer via contaminated food products.

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FITNESS COST OF ANTIBIOTIC RESISTANCE IN *SALMONELLA TYPHIMURIUM* LT2: INSIGHTS FROM GROWTH PARAMETER ANALYSIS

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Antimicrobial resistance (AMR) currently represents a significant public health challenge. *Salmonella* spp., the main pathogen implicated in foodborne disease outbreaks, has shown AMR to antibiotics used in both human and veterinary medicine, including colistin (COL), amoxicillin (AMX) and erythromycin (ERY), among others. The impact of these genetic determinants of resistance on metabolism and, consequently, on their capacity to grow in the conditions frequently present in the industry remains uncertain. Therefore, the aim of this study was to evaluate how the acquisition of resistance to COL, AMX and ERY may affect the growth parameters of *Salmonella* Typhimurium LT2 (WT).

To achieve this objective, resistant variants (RVs) to COL, AMX and ERY were obtained via evolution assays in presence of those antibiotics. Hence, 9 COL, 2 AMX and 7 ERY RVs were obtained. Growth curves of these RVs were performed to compare their growth parameters with those of the WT strain. RVs were grown in tryptone soya broth and yeast extract medium at an initial concentration of 105 CFU/mL and incubated at 37 °C for 18 hours. Absorbance measurements were taken at 595 nm (OD_{595}) every 15 minutes. The curves were then fitted to a linear model using a modified Gompertz formula to obtain the lag time, maximum specific growth rate, and maximum absorbance value of the RVs.

None of the RVs to ERY showed significant modifications in growth parameters compared to the WT strain. However, RVs to COL and AMX showed reductions in growth rate of up to

75%. In addition, an increased lag time was observed in all the RVs to AMX; while in the RVs to COL, such prolongations were detected in 6 RVs, increasing by up to 5 additional hours as compared to WT. The maximum absorbance value was generally reduced in the RVs to COL and AMX in comparison to that of WT.
Further research on how reduced cellular metabolism affects growth in different conditions is crucial for understanding its impact on food safety. Exploring its potential effects on cell resistance to other treatments is also important, as it could enable cells to survive treatments and maintain infectivity.

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EARLY DIAGNOSIS OF SOME DISEASES AND PESTS IN CUCUMBER CULTIVATION IN GREENHOUSE CONDITIONS

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In this work, healthy and diseased plant data will be collected for early discovery systems aiming to detect plant diseases and pests, whose most troubling problem is the shortage of correctly labeled data, by creating two different growth environments (healthy plant production, HPP and artificially diseased plant production, ADPP) and collecting data via a variety of sensors.
Data from both environments will be handled as a single-class classification problem instead of two or multiple-class as done in previous research in the field. HPP and ADPP data will provide required measurement range for healthy production and disease early symptoms respectively. Following the completion of of a deep learning application that processes all sensor data, a prototype will be designed for actual in-field experimentation.
In this study, cucumber of cucurbitaceae family is chosen as the test produce as it can be produced all year round within greenhouses. Proposal covers the early deep learning and artificial intelligence diagnose of Cucumber mosaic virus (CMV), *Pseudoperonospora cubensis* (cucurbitaceae mildew) and *Meloidogyne incognita* (root-knot nematode) diseases before observations of symptoms possible with naked eye, which cause considerable loss of yield and quality on cucumber produced in greenhouse.
With the development of sensor technology and accessibility, artificially intelligent solutions that are designed to analyze multidimensional data from those sensors are necessary to ease fast and accurate detection of such adverse effects, from plant cultivation to harvest.
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NEW METHODS IN FOOD MICROBIOLOGY

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EVALUATION OF THE CULTURE METHOD AND THE PCR METHOD FOR DETECTION OF *CLOSTRIDIUM PERFRINGENS* IN FOOD

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The purpose of this study was to sugget a method to replace the culture media for Clostridium perfringens, which was time consuming and laborious. The real time PCR and the conventional PCR method were compared with the traditional culture method for the detection of *Clostridium perfringens. Strain of C.perfringens* (NCCP 10347) was spiked in food(milk formulas, cheeses, powedered red pepper, processed marine product and cucumber pickle).
A total of 72 samples were enriched to cook meat media and TSC media with egg yolk for 24 h 36 °C in anaerobic condition. To identify *C.perfringens,* DNA was extracted from the enrichment culture, followed by conventional PCR and real time PCR analysis using cpa gene. The detection method was checked for specificity and sensitivity. The limit of detection was tested in TSC media, conventional PCR and real time PCR.
The results from the culture method, and PCR method for five sample types showed equivalent detection capabilities. Consequently, the tools of culture method, conventional PCR and real time PCR assay are reliable solution for the detection of *C.perfingens.* The results showed that the PCR assay can replace the traditional culture method.

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A NOVEL APPROACH FOR THE CULTURAL DETECTION OF *YERSINIA* IN FOOD

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The isolation of *Yersinia enterocolitica* from food is challenging, because the bacteria are readily overgrown by the concomitant microflora. In addition, thus far, no strictly selective medium for this species is available. The gold standard for cultural detection of *Y. enterocolitica* in food is described in the EN ISO 10273:2017. It includes a direct plating step that only enables the detection of large numbers of yersiniae. In addition, plating of the bacteria after selective enrichment for two days and a treatment with potassium hydroxide is mandatory.
This approach may be critical, if the sample is highly contaminated with bacteria. Due to these limitations we improved the ISO procedure by adding a filtration and centrifugation step together with shortening the enrichment to only 6 h. This approach allowed a (i) more sensitive cultural detection than direct plating, (ii) limitation of background bacteria and (iii) saving of two days.
The performance of the procedure was evaluated on minced meat samples, purchased at five supermarkets in Germany that were spiked with different numbers down to 4–6 cfu of a bioserotype B4/O:3 strain. Regardless of the background bacteria, the added strain could be isolated from every spiked sample. Moreover, biotype 1A isolates and other *Yersinia* species were recovered from some samples.
The new protocol is currently tested with tonsils of wild boars, which frequently contain *Yersinia*.

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TRICH-TRACKER FOR TRACING INBRED PARASITE OUTBREAKS

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When a *Trichinella* infection is found in pigs, it is particularly important to identify the source of the infection for the animals and stop further transmission of the parasite to next pigs and into surrounding areas. For this, an epidemiological investigation is used, which is a complex procedure including few stages: epidemiological interview, serological investigtions, species identification of discovered *Trichinella* larvae, and differentiation of isolates of the same species. The last step is practically not applicable due to the lack of available methods to distinguish isolates of *T. spiralis* larvae. And thus, in most cases, it was not possible to obtain an answer as to what could have caused the infection of pigs in a given outbreak of trichinellosis.

Here, we propose a method based on ddRADseq and bioinformatics analysis called - Trich-tracker. The methodology applies the ddRADseq technique during which DNA libraries are created using restriction enzymes. Such DNA libraries contain the sequences of multiple random loci distributed throughout the genome under analysis. The obtained in NGS raw sequence data is used for finding single nucleotide polymorphisms (SNPs) and then for phylogenetic analysis or genetic structure analysis. Both of the analyses are useful to distinguish isolates one from another, what is the most important during epidemiological investigation in *Trichinella* outbreaks on pigs' farms.

The discriminating power of this tool is tunable and scalable, allowing application in a variety of epidemiological contexts. The simplicity of the entire procedure, and the timeliness and cost effectiveness of Trich-tracker ensure the usefulness of its practical application in ongoing *Trichinella* outbreaks. Furthermore, Trich - tracker may be also used to track origin of cured meat containing *T. spiralis* which was a source of human infection. Additionally, this tool may be adapted to distinguishing other species of *Trichinella* and of any other parasites.

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COMPARISON OF TWO METHODS FOR THE DETECTION OF BUTYRIC ACID BACTERIA

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The main risk factors for presence of butyric acid bacteria in raw milk are low quality of the silage and poor hygienic practices on the dairy farm. Spores of butyric acid bacteria can survive pasteurization of milk which is used in cheese production. These spores can form gas in cheese during ripening ('late blowing'). *Clostridium tyrobutyricum* is considered the main causative species for 'late blowing', but also *Clostridium beijerincki* and *Clostridium sporogenes* are associated with this phenomenon.

In this study, two methods for the detection of butyric acid bacteria, the 'van Beynum and Pette'-method (NEN 6877) and the AMP-6000 method, are compared with each other. NEN 6877 is applied as a standard for the detection of butyric acid bacteria in raw milk in the Netherlands. AMP-6000 (SY-LAB Geräte GmbH) is an alternative method which has a shorter time for analysis (48 hours versus 96 hours). Both methods are qualitative, and obtain a positive or negative test result. In total 355 samples of 1 mL raw milk, which are randomly selected, are tested with both methods. The number of positive and negative samples are compared (sensitivity). The positive samples are further investigated with PCR for establishing the species of butyric acid bacteria detected with both methods (specificity).

NEN 6877 obtained 104 out of 355 samples, which is similar to 91 out of 355 positive samples that were obtained with AMP-6000. NEN 6877 and AMP-6000 both detect *Clostridium tyrobutyricum* in the same range (57 versus 51 positive samples respectively). However, NEN 6877 is more sensitive for *Clostridium beijerinckii* than AMP-6000 (15 versus 0 samples positive) whereas AMP-6000 is more sensitive for *Clostridium sporogenes* than NEN-6877 (19 versus 1 samples positive).

In conclusion, both methods have a similar sensitivity for the detection of butyric acid bacteria in raw milk, but the sensitivity for specific species is different. Due to the fact that *Clostridium tyrobutyricum*, *Clostridium beijerincki* and

Clostridium sporogenes are all associated with 'late blowing' during the production of cheese, complimentary application of the two methods may be beneficial throughout the cheese production chain.

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DETECTION OF *SALMONELLA* SPP. THROUGH AN IMPEDIMETRIC BIOSENSOR BASED ON *HECHTIA ARGENTEA*

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Salmonella spp. is one of the most frequent pathogens found in foods such as poultry, eggs, dairy products, fruits, and vegetables with important incidence in health safety. The detection and identification of *Salmonella* spp. are generally done by techniques such as traditional culture, biochemical and serological testing, immunomagnetic separation, ELISA testing or PCR. Although most of these methods are optimal for detection and identification at low concentrations, they are time-consuming and laborious. The use of biosensors can be a current alternative offering selectivity and short-time low-cost analyses. A biosensor uses a biological recognition element and a transducer. Impedimetric biosensors are the most-used alternative for microbial electrochemical analysis, due to their lower limit of detection and feasibility of miniaturisation.
In this work, a sensitive electrochemical detection method for *Salmonella* spp. was developed based on a *Hechtia argentea* lectin immobilised on a screen-printed gold electrode. The lectin was extracted from *Hechtia argentea*, belonging to the *Bromeliaceae* family, by stirring 10 g of dried leaves in 50 mL of a 150 mM NaCl solution. The lectin with molecular weight near 27.4 kDa showed selectivity towards D-mannose sugar, present on the lipopolysaccharide cell wall of *Salmonella* spp. The carbohydrate selectivity of the lectin was measured as a change in impedance according to concentration. Impedance increases were measured by electrochemical impedance spectroscopy and analysed using Nyquist plots. In this sense, the binding of the bacteria to the biosensor surface increased impedance with increasing concentrations of *Salmonella* spp., achieving a linear detection range of 15 – 2.57 × 107 CFU mL–1, with a detection limit of 5 CFU mL–1.
The biosensor was applied in analysis of hen egg samples, and the results were consistent with those obtained using the official analysis methodology, although more samples should be tested to corroborate its feasibility.

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RAPID DETECTION OF SPOILAGE MICROORGANISMS IN JUICES. FACTORIAL VALIDATION OF THE FLOW CYTOMETRY METHOD D-COUNT® 50 (BIOMÉRIEUX) ACCORDING TO ISO 16140-4:2020

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Introduction

Fast detection of spoilage microorganisms, including moulds, yeasts, lactic-acid and acetic-acid bacteria in low pH fruit juices is crucial to optimize microbiological testing and to guarantee food quality.

Objective

Validate the rapid alternative method D-COUNT® (bioMérieux) based on flow cytometry technology for detecting acid-tolerant spoilage microorganisms in juice.

Material and methods

The D-COUNT® protocol includes a pre-enrichment step of a 1:10 dilution in Malt Extract broth (3 d/30 °C), sample preparation by the automated module followed by flow cytometer analysis.
The reference method (International Fruit Union Ref.:IFU2C) includes the same pre-enrichment step, followed by isolation on Orange Serum Agar (3 d/30 °C).
In total, 144 tests were performed from twelve different food items, which were spiked with four acid-tolerant

microorganisms and tested considering four factors (technician, incubation time, culture media and sample size). Performance characteristics were estimated and compared with the acceptability limits established in the ISO 16140-4:2020 for paired factorial studies.

Results

All samples provided equivalent results for both methods, except for one false negative sample showing positive results but below the sensitivity limit.

Sensitivity (ability to detect the analyte) for reference and alternative method was 100% and 97,14% respectively; Relative trueness (correspondence between the two methods) was 98,16%, with no false positives. Negative deviations (ND = 1) and positive deviations (PD = 0) were below the acceptability limits.

The Relative Limit of Detection combined was 1,05, below the acceptability limits. In addition, differences between the four factors evaluated did not have a significant impact.

Inclusivity was positive for 34 out of 40 target microorganisms. Four negative results for moulds, became positive after applying a suitable homogenization protocol. Two negative results from vegetative *Alicyclobacillus* spp. cells, became positive when testing spores.

Exclusivity for 16 non-target microorganisms provided negative results when applying acidification of pre-enrichment broth.

Conclusions

The bioMérieux D-COUNT® alternative method for detecting spoilage acid-tolerant microorganisms in juice based on flow cytometry technology provided equivalent results to the reference cultural IFU2C method, allowing a significant reduction of time to results (from 6 days to 3 days). The different factors evaluated in a real industrial laboratory, did not impacted the performance characteristics of the method.

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A NOVEL REAL TIME-PCR AS RAPID SCREENING METHOD FOR *LISTERIA MONOCYTOGENES* CLONAL COMPLEX IDENTIFICATION DURING TWO EXTENDED OUTBREAKS OCCURRED IN ITALY BETWEEN 2022 AND 2023

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Listeria monocytogenes (*Lm*) still represents a serious health concern in Europe. According to the European Union One Health 2022 Zoonoses Report, the number of foodborne outbreaks of listeriosis has increased in some countries, including Italy, over the last years. Italian Public Health and Veterinary Authorities managed two large listeriosis outbreaks caused by *Lm* Clonal Complex (CC) 155 and CC1 respectively in 2022 and 2023. Overall, 109 cases (6 deaths), caused by CC155 outbreak strain and 34 cases (3 deaths) caused by CC1, were notified in several regions all over the country.

Epidemiological investigation, supported by Whole Genome Sequencing (WGS), revealed a link with frankfurters made with poultry meat and black olives consumption for CC155 and CC1 respectively. Although WGS offers the highest level of bacterial strain discrimination for food-borne outbreak, the availability of a screening method for rapid typing is significant in investigative contexts in order to select

strains likely related to the outbreak. A few years before the outbreaks' occurrence, the EU Reference Laboratory for *Lm* (EURL *Lm*), developed a novel real time multiplex PCR able to identify the major *Lm* CCs circulating in Europe.
The real-time PCR workflow includes a first step for the identification of the five *Lm* molecular serogroup followed by a second step for the CC identification. The method generates results in less than 1 day starting from a bacterial culture. The Italian National Reference Laboratory for *Lm* (IT-NRL *Lm*) performed the verification of the method (ISO 16140-3:2021) and has been participating in the Inter Laboratory Validation Trial organized by the EURL *Lm*.
The IT-NRL *Lm* used this real-time PCR as screening method during the above mentioned listeriosis outbreaks. Overall, 250 and 45 *Lm* isolates were screened for the CC155 and CC1 respectively and those belonging to the targeted CCs were selected as a priority for sequencing in order to compare their genomes to the outbreaks' reference ones. Neither false negatives nor false positives were obtained achieving a 100% agreement.
The assay proved to be a key tool for guiding laboratories and competent authorities during epidemiological investigations reducing the outbreak response and preventing new cases.

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DEVELOPMENT OF A CHEMICALLY DEFINED MEDIUM TO SUPPORT THE GROWTH OF DIVERSE *BACILLUS* SPECIES

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The *Bacillus* genus contains important microorganisms with significance for food products, including the *Bacillus subtilis* group, which has wide applications in food fermentation, and the *Bacillus cereus* group, which contains pathogens. Typically rich media like Brain Heart Infusion Broth (BHI) or Tryptic Soy Broth (TSB) are used to cultivate Bacillus species. However, these complex media are composed of undefined components that can vary in composition and are unsuitable to provide a controlled environment for experimental studies. Hence, developing a chemically defined medium for *Bacillus* species is crucial to improve the reproducibility and consistency of research on the role of *Bacillus* in foods.
The aim of this study was to develop a protein-free, chemically defined medium that supports the growth of multiple *Bacillus* species relevant for food safety and fermentation. Previous studies have mainly focused on the development of defined media for single species. In contrast, this work focuses on a medium suitable for accommodating a wider variety of species within the *Bacillus* genus. A chemically defined medium was developed based on an existing medium published by Wang, Greenwood and Klein (2021), through various adjustments. The new medium "PROF" was tested on two pathogens from the *Bacillus cereus* group and seven different *Bacillus subtilis* group members encompassing 11 different strains. The growth behaviour of these strains on different versions of the chemically defined media was analysed.
All 13 strains could grow on PROF, of which 12 displayed a similar or higher maximum OD600 on PROF medium compared to rich medium (BHI). Compared with the previous medium published by Wang, Greenwood and Klein (2021), PROF medium resulted in a higher OD600 for two *Bacillus licheniformis* strains, two *Bacillus subtilis* strains and a *Bacillus weihenstephanensisis* strain. Detailed analysis revealed a biotin requirement for *Bacillus subtilis* PRO64, and the necessity of including essential amino acids for the growth of *Bacillus weihenstephanensisis* and *Bacillus paranthracis* strains. The newly developed defined medium, tested on seven *Bacillus* species, offers a controlled and reproducible experimental environment for various studies with food safety and food fermentation relevance.

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MICROWAVE STERILIZATION OF CULTURE MEDIA: NEW INSIGHTS, SECURITY AND DEVELOPMENT OF NEW METHODS

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There are different sterilization methods currently available, although the most commonly used and recommended in laboratories is steam sterilization, i.e. in an autoclave. Culture media are sold in different formats but it is less expensive to purchase dehydrated culture media although they must be sterilized after resuspension in milliQ water.

Although autoclaving is a safe method, certain precautions must be taken such as closing the equipment completely or leaving the bottles open a minimum so that they do not explode inside, as well as taking care when removing all the media from the equipment. In addition, there is a drawback in the use of the autoclave which is the time required at home sterilization process and the existence of culture media with special sterilization requirements.

Therefore, a special stopper has been designed for bottles that allows the pressure generated inside the bottles to pass through while maintaining sterility. This novel stopper would allow sterilization of culture media in other equipment such as microwave ovens.

The advantage of microwave ovens over autoclaves would be their speed, as well as their ease of cleaning. In addition, a microwave oven is very inexpensive, which could help laboratories with limited resources to operate without the investment required to purchase large equipment.

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INTERACTION BETWEEN MICROORGANISMS IN ITEMS PREPARED FOR APTITUDE TESTS IN FOOD MICROBIOLOGY

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Proficiency tests are exercises carried out by a group of laboratories on an item that has identical characteristics for all of them.

Thanks to these tests, laboratories verify the quality of their analysis what is essential to ensure food quality.

The suitability test items for microbiological analysis of foods are presented in the form of vials with freeze-dried microorganisms.

A study has been carried out to see how different microorganisms interact when they are freeze-dried together.

For the study, a microorganism that is analyzed daily in food laboratories has been chosen: *Escherihia coli*, and it has been combined with *Staphylococcus aureus, Salmonella enterica* and *Listeria monocytogenes.*

The first thing that was done was to freeze-dry the microorganisms separately, so that, knowing the concentration inoculated in the vials, we knew what amount was recovered after freeze-drying.

Next, all possible combinations of two microorganisms were made between *Escherichia coli* and the rest of the strains chosen for the study: (*Escherichia coli - Salmonella enterica; Esherichia coli - Staphylococcus aureus; Escherichia coli - Listeria monocytogenes*). Since the concentration added to the vials was known, it was possible to know what amount was recovered after freeze-drying.

Afterwards, combinations of three microorganisms were made per vial, with *Escherichia coli* as the common microorganism in all of them: (*Escherichia coli -Staphylococus aureus - Salmonella enterica; Eschreichia coli - Stpahylococcus aureus - Listeria monocytogenes; Escherichia coli -Salmonella enterica* and *Listeria monocytogenes*).

Finally, a vial containing all the study microorganisms was prepared and its recovery after freeze-drying was analyzed.

The vials have been studied by being reconstituted with peptone water since the microorganism to be studied

is *Escherichia coli*, it is considered the most appropriate diluent. In addition, analysis have been carried out in different matrixes (meat and dairy) to verify the importance of the matrix in the recovery of the freeze-dried strains.

It has been seen that there are differences in the recovery of *Escherichia coli* when it is lyophilized with the different microorganisms. However, no differences in recovery have been observed in the different matrixes.

ONE HEALTH

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ESTIMATION OF THE ENZYMATIC PROSPECTS OF FUNGAL CONSORTIUM FOR THE DECOMPOSITION OF PADDY STRAW: AN *IN-VITRO* STUDY

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The burning of the paddy straw has observed a concerned problem in Haryana. This act is responsible for air pollution in Haryana and NCR regions. The fungal communities play a dominant role in paddy straw decomposition due to their lignocellulolytic potential and can adapt to adverse conditions of the soil ecosystem. Soil is known to be the excellent habitat for fungi. The lignocellulolytic enzyme helps in the formation of humus and thus increases the health of soil and its fertility. The humus formation of paddy straw is supported by the lignocellulolytic enzymes secreted by fungi that increase the fertility of the soil and its health simultaneously.

The paddy straw samples were collected from the Mullana village; Ambala, Haryana, India and a total of five fungal forms were isolated from the taken paddy straw that are not repetitive and identified by microscope later they sent to the CSIR-IMTECH for further the confirmation by MALDI-Biotyper. These five fungi belong to Deutromycotina (*Aspergillus fumigatus, Aspergillus terreus*), Ascomycota (*Fusarium*), Zygomycotina (*Mucor, Rhizopus*).In-vitro testing of the fungi to produce extracellular enzymes *viz.* cellulose, pectinase and laccase was done.

The observations revealed that *A. terreus* shows higher lignolytic activity, but the rate of decomposition is less in comparison to the other fungus. *A. fumigatus* and *Rhizopus* showed more pectolytic activity as compared to the other. The *Fusarium, A. terreus, Mucor plumbeus, Aspergillus terreus, Rhizopus* showed all three activities *(CMCase, pectolytic and lignolytic).* The*Mucor* did not show lignolytic activity, but paddy straw decomposition is more. Since *A. fumigatus* and *Fusarium* were found potential decomposers of paddy straw *in-vitro.*

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ONE HEALTH APPROACH TO CONTROL ANTIMICROBIAL RESISTANCE IN FOOD PRODUCING ANIMALS

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Introduction

The emergence of antimicrobial resistance (AMR) is a global health problem without geographic boundaries. This increases the risk of complications and, thus, makes it harder to treat infections, which can result in higher healthcare costs and a greater number of deaths. Antimicrobials are often used to treat infections from pathogens in food-producing animals, making them a potential source of AMR. Overuse and misuse of these drugs in animal agriculture can lead to the development of AMR bacteria, which can then be transmitted to humans through contaminated food or direct contact.

Purpose

It is therefore essential to take multifaceted, comprehensive, and integrated measures, following the One Health approach.

Methods

To address this issue, many countries have implemented regulations to limit antimicrobial use. To our knowledge, there are previous studies based on AMR in food-producing animals; however, this paper adds novelty related to the AMR pathogens in livestock, as we include the recent publications of this field worldwide.

Results

In this work, we aim to describe the most critical and high-risk AMR pathogens among food-producing animals, as a worldwide health problem. We also focus on the dissemination of AMR genes in livestock, as well as its consequences in animals and humans, and future strategies to tackle this threat.

Significance

This paper offers a fresh perspective, updated and comprehensive overview of the most critical AMR pathogens among food-producing animals and the widespread dissemination of AMR genes in livestock, as well as to discuss the potential consequences for both animal and human health and propose future strategies to combat this threat.

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GENOMIC DIVERSITY OF FINNISH *YERSINIA PSEUDOTUBERCULOSIS* STRAINS

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Yersinia pseudotuberculosis is a psychrotrophic, facultative anaerobic, foodborne pathogen, whose reservoirs and epidemiology are partially unknown. The bacterium has caused several yersiniosis outbreaks in Finland, especially via carrots, lettuce, and raw milk.

The aim of our study was to analyse the population genetics of Finnish *Y. pseudotuberculosis* strains using a WGS approach. We studied 93 strains isolated between 1974 and 2022 and originating from wild animals (n = 27), food production animals (n = 20), companion animals (n = 18), and humans (n = 12) as well as food chain niches (n = 16). Four and 15 of the strains of human and food chain origin had been isolated during outbreak investigations, respectively. All strains were serogrouped in silico with blast. Sequence types (STs) and core genome sequence types (CTs) were determined by using schemes of seven housekeeping genes (Achtman scheme) and 1,553 core genes (EnteroBase), respectively.

We found four serogroups, six STs and 72 CTs. The most frequent ST-serogroup combinations were ST42 - O:1a (n = 36), ST43 - O:1b (n = 20), and ST19 - O:3 (n = 11); the first two groups also included strains associated with outbreaks. The virulence gene profiles were related with the serotypes and STs. *Y. pseudotuberculosis* strains isolated from humans had same STs with some strains of wild, companion, and production animal origin. According to the CTs, six human strains had differences of six (0.37%), eight (0.52%), ten (0.64%), 11 (0.71%), 12 (0.77%), and 12 (0.77%) alleles with sporadic strains isolated from a guinea pig, hare, wild bird, guinea pig, cat, and hare, respectively.

Production, companion, and wild animals shared highly related CTs. Most pig strains (69%) were ST19 - O:3 and belonged to a pig-specific group with only one cat strain but no human or wildlife strains. However, some ST42 and ST43 strains were isolated both from pigs and humans. In conclusion, wild animals, companion animals, and production animals can all be a source of yersiniosis.

More studies are needed to understand the population genetics of strains isolated from humans and the emergence of *Y. pseudotuberculosis* outbreaks.

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METAGENOMIC ANALYSIS AS A TOOL FOR ASSESSING MICROBIAL RESISTANCE IN POULTRY SLAUGHTERHOUSES EFFLUENTS. INITIAL INSIGHTS

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Poultry farming plays a significant role in global animal protein production. However, wastewater from the slaughtering process poses a major challenge in the food industry. These effluents may contribute to the spread of resistance genes, becoming a growing concern in environmental and One health contexts. Metagenomic analyses in samples of this kind are underexplored, but this approach stands out for

providing a comprehensive view of antimicrobial resistance profiles.

In this scenario, the research sought to describe and compare the resistance to the main classes of antimicrobials in the effluents from a conventional and an antibiotic-free slaughterhouse in the state of São Paulo, Brazil. Six samples, three from each slaughterhouse, were analyzed between January to December 2023. Genetic material was extracted with AllPrep® PowerViral® DNA/RNA Kit (QIAGEN) and libraries prepared using Illumina DNA prep kit (Illumina). Samples were processed on a 150-cycle flowcell for NextSeq equipment (Illumina). Bioinformatics analyses were conducted using the ARGS_OAP software with the recommended pipeline.

Results were reported as percentage of genes for each antimicrobial class normalized by cell number estimations. We identified 24 antimicrobial classes, with 7 classes being more represented (aminoglycoside, bacitracin, beta-lactam, macrolide-lincosamide-streptogramin, polymyxin, tetracycline, and multi-resistance gene groups). In these classes, a median percentage ranging from 13.39% to 61.12% was found for the conventional slaughterhouse, and 0.54% to 7.38% for the antibiotic-free. The two classes with the highest percentages for the conventional slaughterhouse were macrolide-lincosamide-streptogramin (MLS) (61,12%) and tetracycline (47,58%). For the antibiotic-free, they were MLS (7.38%) and bacitracin (3.16%).

When comparing the two distinct industries, no statistically significant difference ($p < 0.05$) was observed in the percentages found for any of the classes. This lack of statistical difference can be attributed to the still limited sample size (n) evaluated so far. During the research, we observed that the majority of compounds tend to be more present in the conventional system compared to the antibiotic-free system.

In conclusion, these initial indications underscore the need for more comprehensive research to fully understand the nuances of potential differences and their implications in antimicrobial resistance in industrial effluents.

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COMPARATIVE ANTIBIOTIC RESISTANCE PROFILING THROUGH NGS: ARE *E. COLI* ISOLATES FROM CONVENTIONAL AND ANTIBIOTIC-FREE POULTRY DISTINCT?

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Antimicrobial resistance (AMR) is a global health threat, notably impacting low to middle-income countries. Overusing antimicrobials in livestock leads to drug-resistant infections in animals and humans. We examined 284 frozen poultry samples in Botucatu, São Paulo—141 from the antibiotic-free chain and 143 from the conventional chain—to assess *E. coli* prevalence in both production chains.

Following the identification of positive samples, disc-diffusion testing was conducted, and Next-Generation Sequencing (NGS) was conducted on 39 *E. coli* isolates to examine the presence of antimicrobial resistance genes in both production chains. Notably, instances where isolates from the same sample exhibited divergent phenotypic resistance profiles were included in the NGS analysis.

The study aimed to ascertain concurrence in resistance gene profiles between the two production chains and explore if antibiotic-free poultry had a lower prevalence of antibiotic resistance. This comparative analysis provides valuable insights into the potential impacts of antibiotic usage on antimicrobial resistance in poultry production.

Of the positive samples, 18 (comprising 19 isolates) were derived from the conventional chain, while 21 (comprising 26 isolates) originated from the antibiotic-free production chain. The analysis spanned eleven antibiotic classes, including Aminoglycoside, Beta-Lactam, Bleomycin, Fosfomycin, Lincosamide, Phenicol, Quinolone, Streptothricin, Sulfonamide, Tetracycline, and Trimethoprim.

The conventional chain had higher resistance gene prevalence in Aminoglycoside (42.11% vs. 30.77%), Beta-Lactam (94.74% vs. 76.92%), Lincosamide (5.26% vs.

3.85%), Quinolone (15.79% vs. 11.54%), and Sulfonamide (42.11% vs. 26.92%) classes. Conversely, the antibiotic-free chain not only reported resistance genes for these but also displayed higher prevalence in Bleomycin (0.00% vs. 3.85%), Fosfomycin (0.00% vs. 3.85%), Phenicol (10.53% vs. 15.38%), Streptothricin (0.00% vs. 3.85%), Tetracycline (26.32% vs. 30.77%), and Trimethoprim (10.53% vs. 26.92%). This underscores complex antibiotic resistance profiles in distinct poultry practices, highlighting the need for further exploration into influencing factors.

While conventional wisdom assumes that antibiotic-free production reduces antimicrobial resistance, previous studies have identified numerous resistance genes in the antibiotic-free poultry chain, challenging this notion. Therefore, further research remains essential to comprehensively grasp the dynamics of resistance in these agricultural practices.

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METAGENOMIC ASSESSMENT OF THE RESISTOME AT DIFFERENT STAGES OF CHICKEN PRODUCTION

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In order to deepen the understanding of the presence of antimicrobial resistance genes in the chicken farming environment, the next-generation sequencing (NGS) technique can be utilized as a non-invasive approach to assess the dissemination of these antibiotic resistance genes and potential sources of contamination.

Thus, this study aimed to characterize the resistome in conventional chicken farms in the state of São Paulo, Brazil, through metagenomic analysis. Two conventional broiler chicken farms in the state of São Paulo, were evaluated from July to November 2023. Pooled samples from the farm (end of rearing – internal and external environmental swabs) and the slaughterhouse (swabs from clean and dirty areas, cecum, and carcasses) were obtained. Totaling 10 samples for resistome. The DNA from each sample was extracted and purified using specific extraction kits, following the manufacturer's conditions.

The samples underwent next-generation sequencing (NGS) following the manufacturer's protocol (Illumina). The ARGS_OAP software was used to evaluate the resistomes. It was possible to detect resistance to 23 antimicrobial classes, with a notable presence of aminoglycosides, chloramphenicol, macrolides, lincosamides, streptogramin, tetracycline, and genes conferring resistance to more than one class of antimicrobials (> 40%).

When comparing the presence of resistance genes between the two evaluated periods (farm vs. slaughterhouse), a decrease in the distribution of these genes in the slaughterhouse samples was observed, with statistical significance ($p < 0.05$) for the aminoglycoside, bacitracin, bleomycin, chloramphenicol, florfenicol, fosfomycin, fusidic acid, macrolide, lincosamide, streptogramin, novobiocin, pleuromutilin, polymyxin, quinolone, rifampicin, trimethoprim, and multi-resistance gene groups. The detection of bacitracin, even at low levels (median: 14% at farm and 0.3% at the slaughterhouse), is a cause for concern, as its use in animal production in Brazil has been officially restricted since 2018. The significant reduction of resistance genes demonstrates that the implementation of good manufacturing practices and appropriate self-control programs in the slaughterhouse environment play a crucial role in reducing the spread of resistance genes and can contribute to effective approaches in antimicrobial management in animal production. This also emphasizes the need for rigorous surveillance and the implementation of practices to ensure compliance with current regulations. FAPES – Process n.º 2022/03062-6,2023/01195-1.

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ISOLATION, CHARACTERIZATION AND *IN VIVO* EFFICACY OF LYTIC BACTERIOPHAGES FROM SEWAGE-TREATMENT PLANTS AGAINST CIPROFLOXACIN-RESISTANT *SALMONELLA TYPHI* IN MBARARA DISTRICT, UGANDA

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The emergency of ciprofloxacin-resistant *Salmonella typhi* infection among majority of the developing countries has elevated morbidity and mortality rate. The present study aimed at determining the *in vivo* efficacy against ciprofloxacin-resistant *Salmonella typhi* of lytic bacteriophages from sewage treatment plants in Mbarara District, Southwestern Uganda.

Twelve samples obtained from two sewage treatment plants within Mbarara District, Southwestern Uganda; Kakoba and Taso were used for isolation of bacteriophages using the double agar overlay plaque assay. The isolated phages were assayed for both biological and physico-chemical characteristics. A mice infection model that included; bacteria + phage, bacteria + SM buffer, bacteria + ciprofloxacin antibiotic groups and phage only was used to assess the *in vivo* efficacy of bacteriophage cocktail against ciprofloxacin resistant *Salmonella typhi* after intraperitoneal administration. The bacterial loads and phage titres from the lungs, intestines, liver, and kidney were determined using surface spreading and double agar overlay plaque assay. Data was analysed using SPSS-26 to compute descriptive statistics and statistical significance was considered at $P \leq 0.05$.

The four isolated phages exhibited lytic activity against the ciprofloxacin-resistant *S. typhi* with an average host range limited to *S. typhi* species with exception of phage TA001 with activity against *Shigella* species. The adsorption time of phages ranged from 5-15 minutes, with latent period of 10 – 20 minutes and burst sizes ranged from 286 to 163 PFU/mL. The phages exhibited high stability at temperatures up to 60 °C and pH 4 -12. The phage had an average host range of 8.3% to 50.0%. The phage cocktail rapidly decreased the *S.* Typhi counts in blood, liver, spleen, intestines and kidney to 0 CFU/mL within 48 h as compared to ciprofloxacin treated mice. The phages were completely cleared at a rapid rate in the spleen, kidney, and blood within 60 h, 96 h and 120 h respectively. Treatment with phage cocktail caused a significant reduction in the weights of liver and intestines as compared to ciprofloxacin treated mice.

Sewage harbored virulent lytic phages against ciprofloxacin resistant *S. Typhi* and the cocktail exhibited potent *in vivo* therapeutic efficacy in mice model.

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CORE-GENOME MULTILOCUS SEQUENCE TYPING (CGMLST) AS A TOOL TO CARRY OUT RISK MANAGEMENT OF *LISTERIA MONOCYTOGENES* PRESENTS IN READY TO EAT FOODS: A CASE REPORT

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Introduction

Listeriosis is the fith most commonly reported zoonosis in humans in the EU and one of the most serious foodborne disease under EU surveillance. Refrigerated ready-to-eat food continue to be of significance from a food safety perspective. Listeria monocytogenes is a ubiquitous organism and persistence in food processing environments is considered to be the major source of RTE contamination.

The use of next-generation sequencing (WGS) evidence for a high variability regarding the virulence potential and pathogenicity of Listeria monocytognes isolates. In cgMLST hundreds of loci are used for gene-by-gene comparisons of assembled genomes for studying the genetic diversity allowing uncover of hypo- and hypervirulent strains and new virulence genes.

Methods

Through the Commission's Administrative Assitance and Cooperation Network (AAC) two Member States were involved performing several communications. During 6 months an official investigation of *Listeria monocytogenes*

present in smoked salmon was carried out on the market and in the processing plant. 23 official samples were analising including final product, raw matherials and food processing environment. A cgMLST was conducted of the isolated strains according to the guidelines provided by Pasteur Institute. The bioinformatic and biostatistic analysis data was also performed according Pasteur Institute. Phylogenetic tree was built from cgMLST allelic differences detected.

Results and discussion

2 *Listeria monocytogenes* strains were isolated from food on the market and 6 from the processing plant: 3 from raw matherials and 3 from food processing environment. Two clusters of *Listeria monocytogenes* strains were obtained; one of them from the surface samples, which turned out to have genetic proximity to the persistent detected in the food on the market. This strain contains resistance genes but not virulence and belongs to a hypovirulent clone. However, the strain isolated from the raw matherial was not persistent but, without being clearly hypervirulent, it contains some virulence genes.

Persistent and hypovirulent strains of *Listeria monocytogenes* don't have the same risk to public health as hypervirulent strains so guidelines will be necessary to interpret the results in a harmonized manner and establish proportional official actions.

P-173

THE INFLUENCE OF ESSENTIAL OIL COMPOUNDS IN ANIMAL FEED ON THE PRESENCE OF MULTIDRUG-RESISTANT *ESCHERICHIA COLI* AFTER CONTINUED EXPOSURE TO SUBTHERAPEUTIC ANTIMICROBIALS WITHIN FOOD PRODUCTION SYSTEMS

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Antimicrobial agents, such monensin sodium, are commonly used as a cost-effective feed additive in dairy production systems to ensure growth and health of the animal. However, a shift towards reducing the use of antimicrobials as animal growth promotors due to concerns of antibiotic resistant pathogens led the investigation into natural alternatives namely garlic, capsaicin, carvacrol, and cinnamaldehyde. These compounds may prove to be beneficial without an increased risk of antimicrobial resistance in dairy systems.

The purpose of the study was to determine whether a blend of essential oil compounds in the diet of dairy calves could influence the prevalence of multidrug-resistant (MDR) faecal *Escherichia coli* while yielding similar animal growth and health benefits when compared to a conventional antimicrobial, monensin sodium. The holistic use of these essential oil compounds was compared to that of monensin at a subtherapeutic dosage, and the cumulative effect on MDR *E. coli* investigated during two separate animal trials. These trials represented two distinct food production systems commonly in use by dairies within South Africa.

Antibiotic resistant *E. coli* was present across the diet groups. Nonetheless, diets containing monensin sodium increased the abundance of MDR *E. coli* (71%) compared to that of essential oil compounds (46%; $P = 0.02$). Furthermore, this trend remained consistent over two different food production systems, indicating that the use of essential oil compounds may prove to be a low-risk alternative to monensin sodium in dairy calves.

This study adds to the concern of the increased risk of multidrug resistance associated with the use of subtherapeutic antimicrobials in food production systems. Furthermore, the combination of essential oil compounds could be an adequate replacement in the diet of dairy calves where previous long-term antimicrobials were used at subtherapeutic levels in these food producing systems.

P-174

WASTEWATER BASED EPIDEMIOLOGIC STUDY OF ENTERIC VIRUSES

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Wastewater-based epidemiology (WBE) is considered as a useful tool that allows to track the community spread of different enteric and respiratory microorganisms. This tool

has historically been used to track viruses such as poliovirus, and more recently, it is widely employed to monitor SARS-CoV-2. Similarly, it is available to assess the presence of different enteric viruses who are excreted through our feces, saliva, and other fluids and then collected by our sewage systems and delivered to wastewater treatment plants.

To this end, we quantified the concentration of genomic material of the following enteric viruses: Noroviruses genogroups I and II, Human Astrovirus, and Rotavirus from samples collected in the Burgos's sewage system throughout two years. An aluminum-based precipitation method was used to concentrate the viruses from each wastewater sample, previously seeded with a known concentration of Mengovirus as a process control. This was followed by an RNA virus extraction with a commercial kit (Qiamp® Viral RNA Mini kit), and finally measured by a specific RT-qPCR for each target.

As a result, it was possible to detect the seasonal behavior of the four different viruses under study, in accordance with general clinical results. Human noroviruses and astrovirus were more prevalent during autumn and winter. However, it was spring and summer when rotavirus had a higher presence. Additionally, it was observed that the concentration of norovirus genogroup II is generally lower than genogroup I during the two-year period.

To summary, the epidemiology of enteric viruses based on wastewater samples could be a cheaper and complementary tool to the clinical information, because there is a good correlation between them. Moreover, this type of tracking allows us to understand the real health status of the population, unaffected by the symptomatic or asymptomatic bias and the willingness of individuals or the doctors to conduct clinical tests.

P-175

MICROBIOLOGICAL ANALYSIS OF URBAN WASTEWATER IN BURGOS: RESISTANCE OF PATHOGENIC BACTERIA TO METHICILLIN

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Urban wastewater often contains a range of pathogenic microorganisms which reflects the health population status of its inhabitants. Furthermore, the emergence and spread of antimicrobial resistant bacteria (AMR) is a major problem on a global scale due to its health and economic impact. In fact, the OMS has declared in 2019 that the AMR is one of the 10 public health threats facing humanity. Moreover, in 2022, the HERA listed it in the top 3 priority health threats. Bacteria may develop resistance to antimicrobials, "naturally" through mutations or by "acquiring resistance" due to external gene acquisition by horizontal transfer. Nowadays, drug resistance is driven by the overuse and misuse of antibiotics in humans as well as in animals.

A major example of drug resistant bacteria is the methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA are associated with several human diseases either by direct tissue invasion or by the action of exotoxins released by this bacterium. In this study, wastewater samples are collected from different points in Burgos city over three years and analyzed. Samples are first grown in RPF selective medium and then isolated in TSA medium. Subsequently, DNA is extracted from strains, their identity as *Staphylococcus aureus* is confirmed by qPCR and finally, it is determined whether they have any of the methicillin-resistance genes (mecA, mecB, mecC, and mecD) by PCR.

Our results allowed us to identify several strains of methicillin-resistant *Staphylococcus aureus*. Such results confirm the fact that wastewater is regarded as reservoir for a wide variety of antimicrobial resistant strains. Urgent measures should be taken in order to increase awareness, to control its prevalence and to protect public global health.

P-176

THE EFFICACY OF METAL-FREE PHOTOCATALYSTS FOR THE SOLAR-DRIVEN ELIMINATION OF *ESCHERICHIA COLI* AND *LISTERIA MONOCYTOGENES* FROM WATER

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Water is an essential aspect of life through agriculture, personal consumption and industrial use. However, one in four people do not have access to safe, clean water. Novel water disinfection techniques are needed to address this issue, particularly in developing countries lacking adequate water treatment infrastructure.

Metal-free photocatalysts offer the potential to address the water shortage that billions of people are facing. Upon excitation with solar-light these materials produce electrons, leading to the formation of antibacterial reactive oxygen species (ROS), such as superoxide, and/or hydrogen peroxide. These compounds lead to membrane destabilization, enzyme disruption as well as protein denaturation, oxidatively deactivating microorganisms.

Solar-driven disinfection experiments to eliminate *L. monocytogenes* and *E. coli* from water samples, in the presence of these photocatalysts, showed a reduction in the bacterial load to below the limit of detection (10 CFU/mL). This solar disinfection treatment shows promise in terms of agricultural water treatment, as well as implementation at food processing facilities for wastewater treatment.

P-177

A JOINED-UP APPROACH TO THE IDENTIFICATION, ASSESSMENT AND MANAGEMENT OF EMERGING FOOD SAFETY HAZARDS AND ASSOCIATED RISKS (FOODSAFER)

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In Europe, foodborne hazards, which include biological and chemical hazards such as bacteria, parasites, toxins, and allergens, cause already approximately 23 million cases of illnesses and 5,000 deaths per year. Food safety is further under pressure by a range of drivers like climate change, resource scarcity, emerging raw materials, an increase in susceptible population groups, and dietary changes. This shows the need for an adaption of food safety management systems to make them more robust in the face of changes that affect our global food systems.

To achieve such an adaptation, the EU-funded project FoodSafeR was established, which brings together academic working groups, industry, SMEs, and policymakers. In total, the project unites a consortium of 19 organisations from 14 European countries. FoodSafeR has the goal to design, develop and test the building blocks of an innovative, pro-active, and holistic food safety management system with a focus on emerging risks at its core. The project embodies integrated approaches to food safety risk identification assessment and management in a comprehensive suite of future-oriented frameworks, tools, methods, strategies, models, guidance, and training materials.

An open and accessible digital hub will be set up as a 'One-Stop-Shop' vehicle targeted at risk managers and assessors, food safety authorities and the relevant actors and stakeholders operating in the European food system. FoodSafeR will contribute to preventing food safety incidences which are caused by biological and chemical hazards in the

European food system. Drivers of emerging microbial food safety risks have already been successfully identified.
Further goals to improve the safety of food include among others the identification and monitoring of microbial risks in alternative food networks and novel or low regulated food production systems like novel protein foods from insects or microalgae. In addition, animal viruses in food products of animal origin will be analyzed to assess the presence of emerging foodborne animal viruses.

P-178

THE BIOTECHNOLOGICAL POTENTIAL OF SPORE-FORMING BACTERIA OF SPANISH OLIVE GROVES FOR USE IN AGRICULTURE AND FOOD PRODUCTION

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Microbial communities play a central role in maintaining healthy soils conducive for plant growth, ensuring a continuous food production and delivering high-quality food. Spore-forming organisms (i.e. the sporobiota) are one of the predominant members of the soil microbiome and given their extraordinary properties and versatility they have received increasing interest as candidates for use in novel biotechnological applications.
Thus, the aim of this work was to determine and characterise the culturable sporobiota of Spanish olive orchards. To do so, a collection of 417 heat-resistant bacteria was isolated from five olive orchards, termed the "olive sporobiota", and its resistance properties towards common environmental/agricultural challenges were analysed, including heavy metal resistance and fertilizer tolerance. Furthermore, the antimicrobial activity profile of the olive sporobiota was determined against food-borne and plant pathogens. Notably, several candidates were identified with antimicrobial activity against highly detrimental plant pests, such as *Xylella fastidiosa* and *Verticillium dahliae*. The most promising candidates were analysed in further detail regarding their genetic make-up using an omics approach as well as their spore ultrastructure.

The characterisation of the olive sporobiota increases our understanding of the role of food microbial communities in commercial agriculture and can provide the basis for the development of sustainable biocontrol measures, ultimately supporting the transition to a more sustainable agriculture.

P-179

TRANSFER OF FOODBORNE BACTERIAL PATHOGENS FROM CONTAMINATED SEEDS TO MICROGREENS

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Introduction

The consumption of novel salad greens such as microgreens has increased due to the presence of bioactive compounds that confer several health benefits on their consumers. Microgreens are often grown in controlled environmental agriculture (CEA) conditions and can be established as conducive systems to understand the survival of foodborne pathogens that may enter the CEA continuum. Contaminated seeds have been implicated in several microgreen related recalls.

Purpose

Seeds of microgreens have various concentrations of bioactive compounds. During germination, these compounds may influence persistence and proliferation of foodborne pathogens. This study aims to establish the transfer trend of these pathogens when microgreens are cultivated using contaminated seeds.

Methods

Seeds of daikon, mustard, broccoli, and red cabbage microgreens were inoculated with cocktails of two strains of *Salmonella enterica*, and *E. coli* O157:H7 or three strains of *Listeria monocytogenes* at low and high inoculum levels and

allowed to germinate on soil beds. The baseline prevalence level was established in the seeds on Day 0. On Days 7 and 14, microgreen and soil samples (n=288) were harvested, pummeled in TSB (5X volume), and plated on XLT4, CT-SMAC and MOX agars. Results from three independent replicates were analyzed for significant differences due to microgreens and contamination level using ANOVA model with α=0.05 LSD pairwise means comparisons.

Results

Red cabbage, daikon and broccoli showed a decrease of 1 log over 14 days when inoculated with 3 log CFU/g of *Salmonella* compared to 2 log decrease in mustard microgreens. A significant decrease in daikon and mustard was observed when seeds were inoculated at 5 log CFU/g *Salmonella*. Similarly, *E. coli* was significantly decreased in microgreens over 14 days when inoculated at 3 log CFU/g. However, microgreens inoculated with *Listeria* either increased or remained constant when seeds were contaminated with both inoculum levels.

Significance

Controlled-environment agriculture is helping to manage climate change related uncertainties, wherein resources and environmental conditions can be monitored.

P-180

EVALUATION OF THE MICROBIAL DIVERSITY IN A CONVENTIONAL PRODUCTION SYSTEM OF BROILER CHICKENS

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This study aimed to evaluate the microbial diversity present in a conventional production system of broiler chickens. Samples from two conventional broiler chicken farms in the state of São Paulo, Brazil, were collected at two time points: during the end of the rearing period and during the acquisition of the product in the slaughterhouse. A total of 10 samples, represented by swabs from both internal and external areas of the barn at the end of the rearing period, and swabs from different areas in the slaughterhouse, including cecal content, surfaces of both dirty and clean areas, and carcass rinse, underwent metagenomic analysis by NGS.

The DNA sequencing protocol followed the manufacturer's recommendations (Next seq - Illumina). Raw data were assessed with FASTQC and trimmed with Trimmomatic. Kraken2 was used for the taxonomic evaluation and classification of reads. The data were expressed in relative frequency (RF), and alpha and beta diversities were calculated through Qiime2, considering RF above 1%.

The analyses revealed that, at the end of the rearing period, seven bacterial families were predominant, including *Corynebacteriaceae*, *Dermabacteraceae*, *Lactobacillaceae*, *Staphylococcaceae*, *Aeroccaceae*, *Brevibacteriaceae*, and *Enterobacteriaceae*, with nine distinct genera. In the slaughterhouse environment, eight families stood out, namely *Intestiviridae*, *Barnesiellaceae*, *Suoliviridae*, *Rikenellaceae*, *Bacteroidaceae*, *Tannerellaceae*, *Oscillospiraceae*, *Helicobacteraceae*, and *Enterobacteriaceae*, with 10 different genera. *Corynebacterium stationis* (RF = 16,8%) and *Diorhovirus intestinalis* (RF = 12,4%) were the predominant species at the end of the rearing period and in the slaughterhouse, respectively.

The samples showed high taxonomic diversity. Alpha diversity analysis indicated higher diversity in the slaughterhouse than at the end of the rearing period (observed-features metric p = 0.01). Beta diversity analysis showed statistical differences between the slaughterhouse and the end of the rearing period (Jaccard metric p = 0,007; Bray-curtis metric p = 0,008).

The observed high taxonomic diversity suggests complexity and variability in the microbiological environment during these production stages. However, identifying predominant species in these processes can guide strategies to promote a microbiome favorable to the quality and safety of poultry products.

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STRAWBERRY PROTECTION AGAINST *BOTRYTIS CINEREA* BY ENDOPHYTIC BIOLOGICAL CONTROL AGENT *BACILLUS VELEZENSIS* SB10

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Food security presents a significant challenge that 21st-century agriculture must address by improving alternative solutions to chemical pesticides. By 2050, the global population is projected to reach 9.7 billion people, and contemporary consumers are increasingly focused on the consumption of fresh fruits. Among these fruits, strawberries hold a prominent place due to their beneficial properties, boasting a rich array of bioactive compounds such as vitamin C and E, phenolic compounds, anthocyanins, and β-carotene (Saeed *et al.*, 2021), all of which contribute to their positive effects on human health. Furthermore, it's noteworthy that Spain ranks the sixth globally in strawberry production (Aoki and Akai, 2023).

However, strawberries are highly susceptible to attack by phytopathogens, with the ascomycete Botrytis cinerea being considered the primary fungal pathogen, causing grey mold—the most significant disease affecting harvested strawberries (Kozhar and Peever, 2018). This pathogen poses a threat throughout the entire food chain, from the field to storage, transportation, and ultimately the market, resulting in a reduced shelf life for fresh strawberries (Chen et al., 2018). Traditionally, pesticide treatments have been the preferred management strategy for controlling fungal pathogens. Without such treatments, more than 80% of strawberry blooms and fruit may succumb to disease during storage, transportation, or marketing (Díaz-Urbano et al.,2023). However, the use of chemical methods can lead to serious environmental pollution and increase the resistance of pathogenic microorganisms, posing threats to human health and food safety (Wang *et al.*, 2021).

Microbial biological control agents (MBCAs) have emerged as a promising and effective technique against major fruit postharvest decays. Our study aims to assess the potential of *Bacillus velenzensis* SB10 as a biological agent against three distinct phytophatogens: *Botrytis cinerea*, *Alternaria alternata* and *Colletrotrichum acutatum*. SB10, isolated from strawberry fruit and identified as *Bacillus velenzensis*, has exhibited antagonistic activity against the three fungal phytopathogen in vitro assays.

Furthermore, these capabilities were confirmed *in vivo* assay against *B. cinerea*, where the bacterial strain SB10 significantly reduce the incidence of grey mold on strawberries fruit. These findings establish *Bacillus velenzensis* SB10 as a safe and effective alternative to chemical pesticides, enhancing strawberry production.

PREDICTIVE MICROBIOLOGY AND MICROBIAL RISK ASSESSMENT

P-181

LISTERIA MONOCYTOGENES GROWTH IN LOW-TEMPERATURE ENVIRONMENTS

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This study aimed to determine the growth rate of Listeria monocytogenes in a low-temperature environment and investigate the impact of various genetic and environmental factors. Five models, namely Baranyi, Easylinear, Gompertz, Spline, and Trilinear, were employed to simulate the growth of L. monocytogenes. The Spline model exhibited a significantly higher maximum growth rate compared to other models, while the growth rates fitted by the remaining four models were comparable. Consequently, the average growth rates from the four models, excluding the Spline model, were adopted as the final growth rate.

Comparative analysis revealed that at 4 °C, ST18 strains and ST836 strains exhibited higher growth rates, while strains ST1 and ST5 displayed lower growth rates. Notably, strains collected from seafood processing facilities demonstrated significantly higher growth rates compared to those from other environments. At 7 °C, strains from vegetable environments exhibited markedly lower growth rates than other strains. The increase in temperature from 4 to 7 °C resulted in a significant overall growth rate enhancement, with strains from clinical environments showing a faster growth rate increase than other strains. Regardless of the temperature (4 or 7 °C), strains of lineage 2 consistently exhibited significantly higher growth rates than those of lineage 1.

Furthermore, the study aimed to identify factors influencing the growth rate of Listeria monocytogenes in low-temperature environments through genetic analysis. Fifty-two genes related to Listeria monocytogenes growth in low-temperature environments were identified from published literature. It was found by gene alignment that these genes were present in all strains, albeit with sequence variations. TreeWAS was employed to statistically assess the association between phenotype and genotype at all loci, to identify significant associations.

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VALIDATION OF PREDICTIVE MODELS FOR HYBRID MEAT PRODUCTS

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One of the main messages in the green transition is that we all, especially in the western industrialized part of the world, need to change our diet and eat less meat. To accommodate the transition many food manufacturers have increased their interest in hybrid products, e.g. products that combine meat and plant-based ingredients. A challenge for the manufacturers is the limited knowledge on how the addition of vegetables affects the shelf life and food safety of these products.

DMRIpredict is a collection of predictive models that can be used, for example, to document that a meat product is microbiologically safe. We have conducted a range of studies in which the current predictive models are validated against hybrid meat products.

The focus is:

- Microbial and sensory shelf life of MA-packed minced pork combined with 50% vegetables.
- Growth of the pathogens Listeria monocytogenes and Clostridium botulinum (toxin-producing) in ready-to-eat hybrid products with varying preservation.

The results have shown that minced pork with 50% vegetables has a shorter shelf life than traditional minced pork without vegetables. The hypotheses for impaired shelf life are multiple: 1) Plant ingredients increase carbohydrate levels (sugars) and may boost nutrition for typical spoilers in

modified atmosphere (MA) and vacuum-packaged food. 2) Addition of plant proteins often results in products that have a higher pH which favors microbial growth 3) A higher pH also reduces the preservative effects of e.g., organic acids.
Growth of Listeria monocytogens in processed meat products containing vegetables and/or plant proteins is also a concern due to the higher pH and increased level of carbohydrate. Growth of L. monocytogens in sausages with or without vegetables, at varying levels of sugar (dextrose 0-4%) and pH (6,3-6,6) is being tested in new challenge tests. The results will be presented in the poster at Food Micro 2024.

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REDUCE FOOD WASTE BY PREDICTIVE GROWTH MODEL FOR *LEUCONOSTOC* SPP. IN DELI-MEAT

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Large quantities of deli meat products are discarded annually due to unwanted gas production caused by spoilage by microorganisms (usually lactic acid bacteria, i.e. *Leuconostoc* spp.). This spoilage results in blowing of the packaging and by such reduces the shelf life. Introduction of a predictive model for growth of gas producing bacteria will help to prevent spoilage and by such reduce food waste. Currently, predictive models for growth of *Leuconostoc* spp. are not available. Therefore, the aim of this study was isolation and identification of microorganisms responsible for blowing deli meat products as well as generation and validation of a predictive model that assists deli meat manufacturers in optimising their preservation strategy.
Isolation and identification of gas producing organisms were performed by collecting blown deli meat from retailers, from which isolation and identification were performed by whole genome sequencing (WGS).Eleven strains were chosen for the development of the predictive model. The deli meat was manufactured at in-house pilot plant facilities, sliced under sterile conditions, inoculated with the 11-strain *Leuconostoc* cocktail, MA-packaged (30% CO_2/70% N_2) and stored at temperatures between 3 and 8 °C. Subsequently, data for the model were collected by analysing growth on the inoculated deli meat slices produced with varying combinations of pH, NaCl, nitrite, Na-lactate, and Na-acetate.
Spoilage resulting in blown packages was mainly caused by Leuconostoc carnosum and Leuconostoc mesenteroides. The challenge test showed that high concentrations of preservatives are needed to inhibit growth of the Leuconostoc cocktail. For example, at pH 6, an 8-log growth within 60 days at 3 °C was observed, although the deli meat was preserved with 4% NaCl, 3% Na-lactate and 0.5% Na-acetate. Furthermore, the studies showed that gas production did not occur until the Leuconostoc count had been at a maximum level for 1-2 weeks.
The validation of the model (n=57) showed that it was acceptable with a bias factor (B_f) of 0.90 and an accuracy factor (A_f) of 1.43.

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COMPARATIVE QUANTITATIVE ASSESSMENTS OF THE MICROBIAL RESPONSES OF *P. PHOSPHOREUM* AND *P. ILIOPISCARIUM* AS A FUNCTION OF DISSOLVED CO_2 IN A SEAFOOD MODEL PRODUCT

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P. phosphoreum is considered a common specific spoilage organism (SSO) in marine fish originating from cold waters following chilled storage under various atmospheres. Recent studies have shown that *P. iliopiscarium* has a similar spoilage potential as *P. phosphoreum* but its contribution to seafood spoilage is probably underestimated. While *P. phosphoreum* is acknowledged as a CO_2-tolerant bacterium, information regarding the CO_2-tolerance of *P. iliopiscarium* is currently unavailable.
Therefore, this study aims to comparatively analyse the impact of different packaging atmospheres on the growth inhibition of *P. phosphoreum* and *P. iliopiscarium*. Sterile fish pate was inoculated with either *P. phosphoreum* or *P. iliopiscarium* and stored at 4 °C. Four packaging atmospheres were selected (O_2:CO_2:N_2): 0:0:100, 20:0:80, 0:70:30 and 20:70:10.

The headspace gas composition, the dissolved CO_2 and the Photobacterium counts were investigated. CO_2 quickly dissolved in the aqueous phase of the fish pate, reaching an equilibrium by the 3rd day of storage.

The observed disparities in dissolved CO_2 concentrations between *P. phosphoreum* (1500 ± 25 ppm) and *P. iliopiscarium* (1720 ± 32 ppm) inoculated samples could be associated with the concurrent weight variations, where the fish pate samples with *P. iliopiscarium* were, on average, 5 grams heavier than those with *P. phosphoreum*. Both strains reached maximum cell density (7.5-8.5 log cfu/g) after storage day 4 for the group 0:0:100 and storage day 6 for the group 20:0:80. In a CO_2 atmosphere (0:70:30), the time to reach maximum cell density was extended to 9 and 12 days of storage for *P. phosphoreum* and *P. iliopiscarium*, respectively. By introducing oxygen and CO_2 in the gas mixture (20:70:10), the time was additionally extended by three days for both strains.

The findings suggest that the presence of CO_2 in the packaging atmosphere significantly reduced the *Photobacterium* spp. evolution. However, the discrepancies in growth between Photobacterium species might be attributed to the observed differences in dissolved CO_2 rather than inherent differences in CO_2-tolerance among *Photobacterium* spp. Integration of the dynamic changing levels of the dissolved CO_2 in predictive microbial modelling approaches are applied to compare the Photobacterium species' growth variation.

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ACCELERATING INNOVATION IN MEAT AND PLANT-BASED MEAT ANALOGUES PRODUCTS THROUGH MODELING *L. MONOCYTOGENES* GROWTH; THE INDUSTRIAL PERSPECTIVE

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Listeria monocytogenes control in food is regulated worldwide. Both meat and plant-based meat analogues products are among the most risky food categories regarding potential growth of *L. monocytogenes*, due to their close-to-neutral pH, vacuum or MAP packaging, and storage at refrigerated temperature. Its growth and control depend on specific food characteristics, such as moisture content, pH, sodium chloride, sodium nitrite, water activity, organic acids, and storage conditions (dynamic and static temperature). Driven by consumers' demands and new trends, the meat industry is constantly seeking preservative solutions targeting product (re) formulation, such as increasing meat pH, lowering salt content, and replacing current preservatives with clean-label alternatives. These trends, however, represent a challenge for the industry, which needs to perform several challenge tests to (re) formulate their products without any indication of success potential.

By using modeling tools to predict *L. monocytogenes* growth in specific food matrices, food manufacturers can greatly reduce R&D cost and time, increase the success rate in challenge tests, and speed up innovation time to market. This study aims to present the industrial perspective on the benefits of using a Listeria control predictive model tool by: (i) comparing how different product parameters may affect the growth of *L. monocytogenes* in meat and plant-based meat analogues products, (ii) performing target application validation studies in specific matrices, (iii) keeping up developed predictive model with new trends, through constant updates and validations. As a result, modeling *L. monocytogenes* growth can strongly help the industry meet

L. monocytogenes safety requirements when designing new product formulations, accelerating innovation.

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PRESENTING BIORISK, AN R PACKAGE THAT FACILITATES THE IMPLEMENTATION OF (2D) MONTE CARLO MODELS FOR QUANTITATIVE MICROBIOLOGICAL RISK ASSESSMENT

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Quantitative Microbial Risk Assessment is nowadays a standard tool for research, food industries and regulatory agencies. Since risks are both variable and there is also uncertainty, these assessments are often stochastic for example making use of Monte Carlo simulation. However, the implementation and analysis of Monte Carlo simulations for QMRA models can be complex, especially when trying to separate variability from uncertainty using a two-dimensional Monte Carlo model. This poses an important barrier for the adoption of the methodology, as the implementation of these models requires advanced skills on scientific programming.

This presentation introduces the R package biorisk. It is an Open Access software (accessible from its GitHub page: https://github.com/albgarre/biorisk/) that facilitates the definition of QMRA models. It uses a modular approach based on Modular Process Risk Models (MPRM). The modules in the package contain pre-implemented common QMRA elements (growth, inactivation, dose-response, re- and cross-contamination, dynamic conditions, models with correlated variables…) and predefined distributions for variables (Normal, Poisson, Pert…), so the models are defined simply by linking these modules. This facilitates model definition, as equations do not need to be defined by the user. The package also simplifies model simulation, with a single line of code changing between point estimates (e.g., means of normal distributions), one-dimensional MC or two-dimensional MC.. It also includes functions for different types of visualizations (results, model structure, model convergence…), for comparing the results of different models or for generating summary tables of the simulations. The capabilities of biorisk will be shown using a case study of Salmonella in a vegetable-based beverage. The case study is analyzed using a scenario-analysis and sensitivity analyses to identify the most relevant variables for the risk and the number of cases. It emphasizes how biorisk can simplify the definition, simulation and interpretation of QMRA models. Therefore, biorisk can help overcome barriers to the implementation of (2D) Monte Carlo models, promoting the adoption of this methodology among food microbiologists.

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IMPACT OF WEAK ORGANIC ACID SALTS AND PH ON THE INFLUENCE OF GROWTH RATE OF *LISTERIA MONOCYTOGENES*

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Introduction

Listeria monocytogenes, a foodborne pathogen with significant public health implications, poses a persistent challenge in the food industry. Understanding the predictive microbiology of *L. monocytogenes* is crucial for devising effective control strategies. The effects of pH, propionate, lactate, and acetate salts on the growth dynamics of *L. monocytogenes* in broth are basic requirements for predictive model development.

Material and methods

A systematic approach was employed to assess the growth kinetics of *L. monocytogenes* under varying conditions. A polynomial model was developed for predicting the growth

of *L. monocytogenes* (NCCB 92025) in BHI broth at 10 °C at different levels of pH (5.8, 6.3 and 6.8), sodium propionate (0.1-0.9%w/w) sodium lactate (0.5–2.5%w/w) and sodium acetate (0–0.9%w/w). Collection of the growth data was done using absorbance measurements in broth cultures. Absorbance values were natural log transformed. Values of maximum specific growth rate were calculated by fitting the growth curves with the equation described by Baranyi (Baranyi and Roberts, 1994).

Results

The effects of pH, sodium propionate, sodium lactate and sodium acetate on the growth of *L. monocytogenes* were modeled. The results reveal pH as a critical factor influencing *L. monocytogenes* growth, with a notable sensitivity to acidic environments. Furthermore, combinations of pH values and acid concentrations demonstrated inhibition of the growth of *L. monocytogenes*. In the present study several interactions were significant ($p < 0.05$) to the growth rate of *L. monocytogenes*. This opens many opportunities for combinations that can help to reduce the risk of *L. monocytogenes* growth in food products. Using the developed model, various combinations may be tested virtually before doing expensive and time consuming inoculation studies on real product.

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LISTWARE-TOOL USING EXTENSION OF THE GAMMA CONCEPT FOR PREDICTING SHELF-LIFE OF SALADS

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There are several tools available for predictive modelling of Listeria growth in food. However, many of them are developed for academic use which require a lot of input data to give good predictions. Other tools are based on growth studies in broth which are less representative for real foods. The objective of the development of ListWare tool was to make a software that was adapted to the needs in the food trade sufficiently accurate predictions for targeted foods.
ListWare was developed for meat products and composed salads, both mayonnaise-based and fresh green salads. The user selects a product from the menu, and ingredients, pH, water activity, carbohydrate content and growth inhibitors such as lactate and acetate are suggested. The user can overwrite the default values. The user fills in packaging method and storage temperature, and a growth curve for Listeria is calculated. The number of days for Listeria growth to reach 100 cfu per gram is defined as the Listeria shelf life. The algorithm for prediction of Listeria growth is based on the maximum growth rate of Listeria in the product, from results of 100 growth trials, and a gamma module which estimates the impact of the main parameters in the product.
The results showed that pH decreased during storage if there were carbohydrates available in the mixed salads and, thus, inhibited the Listeria growth. This change in pH significantly affected the growth conditions, i.e. the gamma element, of the equation. The gamma models used in the ListWare tool were therefore expanded to include changing dynamic environmental factors. The pH in meat products is basically stable, but if the meat is mixed with vegetables, the background flora will grow in the vegetables and produce acids that reduce the pH and inhibit the listeria growth.
The user can print the shelf-life documentation for use at inspections and product development. The software can also be used for simulation, to find methods to increase shelf life. Longer shelf life is calculated with a few keystrokes, instead of performing challenge studies.

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A NEW MATHEMATICAL MODELLING APPROACH TO PREDICT THE QUALITY OF HAKE PACKED IN MODIFIED ATMOSPHERES ALONG THE FOOD CHAIN

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Food waste constitutes a significant environmental issue. This is particularly critical in the case of fish products due to their rapid degradation. It is estimated that inadequate storage conditions could lead to 35% of wasted fish. Mathematical models describing the evolution of fish quality along the food chain can be used to detect quality problems and react to minimize their impact. In this work, we propose a mathematical model that describes the evolution of the most relevant quality indicators -i.e. bacterial growth, total volatile basic nitrogen (TVB-N), nucleotide concentration, and the Quality Index Method (QIM)- for hake fillets packed in modified atmospheres. Two different approaches are considered: (i) mechanistic models are used to describe the biochemical indicators (bacteria, TVB-N, and nucleotide), whereas (ii) machine learning (ML) techniques are selected for the description of the QIM since it is a subjective indicator in which different complex phenomena are involved. In the ML approach, the input variables are the biochemical indicators and the output is the QIM.

Different modelling alternatives are considered and compared according to their capacity to describe the experimental data. In this regard, for the biochemical indicators, two secondary models (relationships between the process temperature and the growth/reaction rates) are used: Arrhenius and Ratkowsky. Primary models, which provide the evolution of concentrations with time, are selected according to the indicator being considered: (ii) For bacteria population, the Baranyi logistic and Gompertz expressions (vastly used in the literature) are selected; (ii) for TVB-N, we consider, on the one hand, an exponential model typically used in the literature and, on the other hand, we propose in this work a Power Law Model to account for the action of bacteria in the formation of TVB-N; (ii) Regarding nucleotide degradation, different first-order degradation routes with bacterial catalysis are considered. For the subjective indicator (QIM), two ML approaches are tested: Random forest and Neural Networks. Models are calibrated and validated using experimental data. The Akaike and the Bayesian Information Criterions are used to compare the different modelling options. Model performance is evaluated using the typical statistical criteria.

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DYNAMICS OF BIOACCUMULATION AND DEPURATION OF *ESCHERICHIA COLI* IN MUSSELS (*MYTILUS GALLOPROVINCIALIS*): EFFECTS OF TEMPERATURE, FOOD AVAILABILITY AND SALINITY

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The coasts of Galicia (NW Spain) are the main production area for bivalves in the European Union, and mussels (Mytilus galloprovincilis) account for about 95% of the production. The Galician coastline is highly populated and industrialized, which generates a large volume of wastewater, occasionally giving rise to faecal contamination and, eventually, to food safety problems due to the consumption of bivalves.

Official control of production areas is based on testing for *Escherichia coli* in bivalves, but this approach prevents detection of faecal contamination in real time. In contrast, high-frequency monitoring in the marine environment would allow early detection of such scenarios. For this purpose, linking the bacterial load in water to that of mussels would be necessary.

The uptake and clearance of *E. coli* in mussels depends on the concentration of *E. coli* in water and the time of exposure to contaminated water, but also on their physiological state, which in turn depends on factors such as temperature, salinity and food availability, among others. A pilot-scale

study was therefore performed to study the effects of temperature, salinity and food availability on the dynamics of *E. coli* accumulation and depuration in mussels, as well as on their physiological response. In addition, a numerical model was developed to link the concentration of *E. coli* in water and in mussels.

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IS VARIABILITY REALLY CONSTANT? THE RELATIONSHIP BETWEEN PH AND THE STRAIN VARIABILITY IN THE GROWTH RATE OF *LISTERIA* SPP.

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Variability is a keystone of modern Quantitative Microbiological Risk Assessment (QMRA), especially due to the low risk per serving of most products. However, there are still significant knowledge gaps on how to quantify variability and how to separate it from uncertainty. Currently, the most common approach, is to assume that the magnitude of variability is constant. This is done by defining an error term (with constant variance) that is added on top of the secondary growth model (for the square root of the growth rate). However, this may go against microbiological principles, which state that differences in growth fitness among bacterial strains may be more prominent in the vicinity of the growth limits than at optimal growth conditions. In other words, that strain-variability cannot be described by a constant variance.
This study evaluates whether variability is dependent on the growth conditions using 21 different strains of *Listeria* spp. The growth rate of these strains was estimated by optical density using the two-fold dilution method at pH 4.5, 5, 6, 7.2, 7.5, 9 and 10. The experimental design included two biological replicates and three technical replicates to account also for experimental uncertainty and within-strain variability. The data was analyzed using mixed-effects models to obtain estimates of the two sources of variability (within-strain and between-strain) and uncertainty (experimental) for each pH condition. The results show a clear relationship between variability and growth conditions. The lowest variability was observed at optimal growth conditions, with higher values at both sub-optimal and super-optimal pHs. The maximum variability estimates were obtained at both extremes.
This result would confirm microbiological hypotheses stating that differences between bacterial strains would be more relevant on harsh environments. This is of special relevance for QMRA, as it shows that an accurate assessment would require that risk models account for the relationship between strain variability and environmental conditions.

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PATHOGENS IN FOODS DATABASE: WEB RESOURCE FOR ASSESSING THE OCCURRENCE OF MICROBIOLOGICAL HAZARDS IN FOODS SURVEYED IN EUROPEAN COUNTRIES

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Studies on the occurrence of pathogens in foods surveyed in the farm-to-fork chain are essential for the development of risk assessment models, risk management tools and meta-analysis by food researchers and food safety authorities. However, the existing data are mostly dispersed, non-standardized or not easily accessible.
Pathogens-in-Foods (PIF) is a dynamic database of occurrence data (prevalence and enumeration) of pathogens in foods randomly sampled across Europe. Data are

periodically extracted from peer-reviewed articles retrieved through systematic literature searches using a publicly available protocol describing the search and screening process. Eligible primary studies are quality assessed and verified data are extracted into the database, according to a predefined categorization protocol that includes general study characteristics, food categorization (harmonized with EFSA's FoodEx2 system), pathogen information, microbiological methods, and outcomes (prevalence and/or enumeration results).

The PIF database contains results for fourteen hazards (*Bacillus cereus*, *Campylobacter* spp., *Clostridium perfringens*, *Listeria monocytogenes*, *Salmonella* spp., Shigatoxin-producing *Escherichia coli*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Cryptosporidium* spp., *Giardia* spp., *Toxoplasma gondii*, Hepatitis A virus, Hepatitis E virus and Norovirus) in different food categories. In 2025, PIF database will be populated with data on the occurrence of *Vibrio parahaemolyticus*, *Vibrio vulnificus* and non-O1, non-O139 *Vibrio cholerae* in seafood and of fish parasites of public health importance (including Anisakids) in fishery products.

The database is accessible through a web application (https://pif.esa.ipb.pt/) that facilitates data retrieval according to several relevant variables, and also generates dynamic graphs and summary statistics through interactive dashboards.

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TECHNOLOGIES FOR FOOD PRESERVATION AND SUSTAINABILITY

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THE IMPACT OF COMMERCIAL HIGH-PRESSURE PROCESSING PARAMETERS ON SPORES OF *BACILLUS AMYLOLIQUEFACIENS* IN COMPLEMENTARY FOODS

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The effect of the commercial HPP parameters (600 MPa at 9 °C for 180-240 s) combined with citric acid in the chilled infant foods (0.11 gL-1) on *Bacillus amyloliquefaciens* spores and, *B. subtilis* and *Clostridium sporogenes* was determined. Results showed that the HP-processed chilled infant foods were acceptable bacteriologically in terms of shelf life. However, in fruity chicken & brown rice as well as in sweet potato & cottage pie and apple & pear infant foods spore-forming *B. subtilis* was not inactivated. Acidification of chicken & tomato stew and veggies & lentils, as well as naturally low pH apple & pear in combination with the commercial HPP parameter, result in no spore recovery occurring after storage at 4 °C for 9 weeks.
The application of HPP in combination with citric acid could ensure the microbial quality of infant food products while maintaining the products' quality during cold storage by suppressing spore germination and outgrowth.

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INACTIVATION OF BACTERIA AND FUNGI USING BIOACTIVE NANOPARTICLES

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Pathogenic strains of microorganisms cause many diseases worldwide and have a significant social and economic impact. Biopreservation is focused on utilizing natural preservatives from bacteria, fungi, and plants that can ensure food safety due to their antimicrobial activity. They can be used as natural antimicrobials for the insurance of food safety. However, natural preservatives, such as bacteriocins and yeast killer toxins, readily lose their activity in foods due to interaction with food components, temperature, and inactivation by proteolytic enzymes. Nisin is the only bacteriocin licensed as a safe biopreservative. Fucoidan was chosen for the encapsulation due to its diverse bioactivity, biodegradability, biocompatibility, and increasingly growing applicability. This study aims to develop nisin-loaded fucoidan and nisin-yeast toxin-fucoidan nanoparticles and evaluate their stability and antimicrobial activity.
The antimicrobial efficiency of nisin-loaded fucoidan nanoparticles was evaluated against model Gram-negative (*Salmonella typhimurium* and *Escherichia coli*) and Gram-positive (*Bacillus subtilis* and *Listeria innocua*) bacteria using agar-diffusion method as well as viability assay. The highest antimicrobial activity was observed against *B. subtilis*. *S. typhimurium* was more resistant to the action of functionalized nanoparticles. The antimicrobial effect of nisin-fucoidan nanoparticles was the lowest against *E. coli* and *L. innocua* bacteria. The antifungal efficacy of toxin-loaded-fucoidan nanoparticles was tested against wine-related yeast contaminants from *Candida*, *Pichia*, *Torulaspora,* and *Saccharomyces* genera. The most effective inhibiting activity of the encapsulated toxin was detected against *Saccharomyces cerevisiae* yeast. The viability of other tested yeast strains, including potential human pathogen *C. albicans*, was also reduced under the action of fucoidan-toxin nanoparticles. The influence of pH and temperature on the stability of encapsulated biocides was demonstrated.
Our data suggest that encapsulated nisin and yeast toxin formulations could be used in the food industry for biopreservation.
The research has received funding from the Research Council of Lithuania (LMTLT), project No S-MIP-22-7.

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IMPACT OF PACKAGING TYPE ON OPEN SHELF LIFE OF UHT PLANT CREAMS

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Closed shelf life of ultra-high temperature UHT processed plant-based milk is mostly determined on quality and sensory aspects due to deterioration of food ingredients such as oxidation of fats. For UHT plant-based milk the consensus is that six to nine months of shelflife is considered the norm if kept unopened and stored at ambient temperatures. Open shelf life however is much more subject to debates. Food manufacturers generally recommend "after opening" to keep between 3 to 7 days under chilled conditions. However the type of packaging used to sell these products might have an impact on potential re-contamination from the consumer. In this study we investigated the different type of packaging opening tops and the impact on open shelf life. Three types of packaging were investigated including 200 mL pots with an aluminum seal and plastic lid (which needs to be manually ripped open), a brick type with a flat cap and a PET bottle with a screw cap. Spoilage and/or growth of potential pathogenic microbes were observed more rapidly in pots type compared to bricks or bottle type of packaging.

This was confirmed by our visual and microbiological testing up to 20 days after opening. Highest levels of microbial contamination were identified as *Enterobacteriaceae* spp., *Pseudomonas* spp. and spore-former *Bacillus cereus*. Pots require more consumer manipulation and are usually kept "open" in the fridge making them more vulnerable to the environment.

Food safety is crucial for our consumers who are relying on the pack guidelines to keep food safe after opening. Having the best packaging type linked with an appropriate guidelines for open shelf life can impact on the reduction of waste by allowing consumers to keep a safe product for longer if kept under the appropriate conditions.

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ALTERATION OF PROBIOTIC PROPERTIES, ANTAGONISTIC EFFECT ON PATHOGEN ANTAGONISM AND LAB-PROBIOTIC ORANGE *BACILLUS* SPP. INCORPORATED IN JELLY CANDIES

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The research developed a pigmented probiotic jelly candy prototype incorporating *Bacillus* sp. isolate 63-11, a strain producing an orange pigment. The *Bacillus* spores were incorporated into the candy providing an orange sparkling effect. Maltodextrin or sodium alginate were used in a simple coating process, involving wet and dried, and surface, and internal coating methods as well as NaCl supplementation. The impact on probiotic activities, antagonistic effects on food pathogens (*Escherichia coli* ATCC 25922, *Salmonella* enterica ATCC1331, and *Staphylococcus aureus* ATCC 25923) and cariogenic pathogen (*Streptococcus mutans* UA159*)* and LAB-probiotics (*Lactobacillus acidophilus* LA5 and *Bifidobacterium lactis* BB12) were assessed.

Results showed that coating methods did not reduce probiotic and antagonistic properties of the *Bacillus* strain. Coating process did not negatively impact the antimicrobial resistance (AMR) and/or antibiotic resistance genes (ARGs) properties of *Bacillus* strain. In addition, the inclusion of *Bacillus* sp. in probiotic jelly candies did not negatively impact the growth or viability of LAB probiotics, and vice versa, and thus, enhances the overall probiotic activity of the product. Maltodextrin-coated candies exhibited higher probiotic viability and gastrointestinal tolerance. NaCl did not provide a clear impact on this prototype. Over 6 months, all coating methods showed increased water activity without significant microbiological changes.

This study demonstrated the successful development of a jelly candy prototype that goes beyond the traditional candy product by incorporating additional functionalities.

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ANTIMICROBIAL ACTIVITY OF HONEY AND HONEY POWDER

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Honey is a natural sweetener made by bees that exhibits well-known antioxidant, antimicrobial and anti-inflammatory activities. Nevertheless, the uses of honey are limited because of its high viscosity, stickiness and glucose crystallization, which leads to handling problems. To solve these drawbacks, recent studies were focused on making honey powders. Honey powder is commonly obtained by different procedures, such as spray drying (SP), vacuum drying (VC) or freeze drying (FZ), using different carriers.

The aim of this study was to research the antimicrobial activity of raw honeys from different botanical origins (ling heather, lavender, thyme, vetch and multifloral), and honey powders made with them, by VC (60% honey) and FZ (75% honey), using maltodextrin (MD) as carrier. Antimicrobial activity was assayed by broth microdilution against *Staphylococcus aureus* CECT 435, *Escherichia coli* CECT 99 and *Listeria monocytogenes* CECT 934. Eight concentrations (70, 60, 50, 40, 30, 20, 10 and 5 g/100mL) were studied. The 96 bottomed plates were incubated at 37 °C, 24 h.

After incubation, 10 µl from each bottomed plate was incubated at 37 °C, 24 h, in plates with specific agar for each microorganism, thus determining the minimal bactericidal concentration (MBC), described as the minimal concentration at which no bacterium was visible on the plate. All honeys and honey powders showed antimicrobial activity. MD showed no antimicrobial activity. *L. monocytogenes* was the microorganism most resistant, while *St. aureus* was the most sensitive for most samples.

In general, the honey powders showed lower antimicrobial activities than those of their corresponding raw honeys, mainly due to dilution factor. With regard to the drying procedures, FZ samples showed higher antimicrobial activities than VC samples. This fact could be due, on the one hand, to the fact that during VC, samples were heated, so that some thermolabile components with antimicrobial activity could be destroyed and on the other hand, because FZ powders contain a higher honey concentration. Antimicrobial activities were not influenced by botanical origin.

The results show that instead of sugar, honey powder could be used as a preservative of different foods, such as bakery, meat or fish products, among others.

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CHANGES IN THE MICROBIOTA OF COOKED OCTOPUS TREATED WITH HIGH-HYDROSTATIC PRESSURE, HEAT AND BACTERIOCIN AS-48

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Cooked octopus is a convenient and popular food. After cooking, the octopus is cooled, sliced and vacuum-packed. The final product is sold under refrigeration, ready for consumption. Post-process contamination may compromise the product shelf-life and safety. In the present study, comercial cooked octopus was processed by high hydrostatic pressure (HHP) at 250, 300 or 350 MPa (at temperatures of 22 °C or 45 °C) singly or in combination with the bacteriocin enterocin AS-48. Controls without treatments and controls treated only with bacteriocin were included. The samples were refrigerated stored for 20 days. Total DNA was extracted periodically and bacterial diversity was estimated using amplicon sequencing.

The microbiota of the untreated octopus was composed mainly by *Bacillota* (*Carnobacterium*). *Carnobacterium* remained as the predominant group during storage, with relative abundances exceeding 80%. *Paenibacillus* was also relevant at time 0. *Pseudomonadota* were represented mainly by *Psychrobacter*, with low relative abundance both at time 0 and during storage. Samples treated by HHP had higher relative abundances of *Psychrobacter* (and *Paenibacillus* at some points), with exception of treatments carried out at 350 MPa and 45 °C (in which the member of *Bacillota, Jeotgalibaca* showed higher relative abundances). The single bacteriocin treatment reduced the relative abundance of *Carnobacterium* very considerably, while *Jeotgalibaca* and

Enterococcus increased. For the combined treatments with HHP-bacteriocin, *Enterococcus* became the predominant group in most of the samples, while *Carnobacterium*, *Paenibacillus*, *Jeotgalibaca* and *Psychrobacter* only were significant at some points. The results indicated that treatments combining enterocin AS-48 and HHP have greatest impact on the microbiota of cooked octopus.

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EFFECT OF MEAT-EXUDATE SEPARATION AFTER THAWING ON THE MICROBIOLOGICAL QUALITY OF COMMERCIAL CHICKEN BREASTS

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Chicken meat is a widely consumed food whose shelf life is largely limited by microbiological quality, especially in the absence of modified atmospheres. This process is usually accentuated by the exudate produced after thawing and which accumulates in the packaging, becoming in the perfect growth medium for these spoilage microorganisms.

Therefore, the objective of this research was to evaluate the quality of chicken breasts (*G. gallus*) using new-generation food containers specially designed to extend the shelf life of meat, based on a separation system between the meat and the exudate produced during thawing and comparing them against a control one.

For this purpose, two types of containers which were filled with 7 kg of fresh, filleted chicken breasts from retail were used: a conventional container and another with an intermediate grid to separate the exudate produced, both with an airtight lid. These containers were subjected to forced air freezing (25 hours to reach –18 °C in the container's centre). After 15 days, they were thawed in cold storage at 4 °C (48 h). Then, quality control was carried out at times 0 and 48 hours on samples from the bottom and top of both kinds of containers and also on the exudate: colony count at 30 °C, *Enterobacteriaceae* and *Pseudomonas* counts.

In addition, other analyses were performed: water retention capacity, exudate volume, moisture, a_w and sensory characterization. Microbiological counts did not show significant differences between the two types of containers, nor between the selected sampling zones, although an increase ($p < 0.05$) in bacterial counts was observed throughout refrigerated storage, as expected, with higher loads after 48 hours.

However, the counts obtained in the exudate of both types of containers were, on average, 2 logarithmic units higher. In conclusion, within the first 48 hours the use of perforated containers had no effect on the microbiological quality of chicken breasts. However, given the exudate counts, it may be worthwhile to test whether there is any effect of meat-exudate separation at higher storage periods (72 or 96 hours).

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BIOACTIVE COMPOUNDS FROM *ALARIA ESCULENTA* DERIVED BY GREEN EXTRACTION PROTOCOLS. ANTIMICROBIAL AND ANTIOXIDATIVE PROPERTIES

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The aim of the present study was to investigate the potentials of microwave- and pulsed electric field-assisted water-based extractions (MAE and PEFAE, respectively) as green and novel technologies for extracting bioactive compounds from the brown alga *Alaria esculenta*. Moreover, the study aimed to investigate the extract's bioactivity, including its antimicrobial and antioxidant capacity.

A range of different extraction parameters, including temperature (40-140 °C) and holding time (2-15 min), were tested for optimizing the MAE, whereas electrode voltage (0-24kV), pulse width (4-7µs), and number of pulses (10-400) were variables tested for PEFAE. To verify extraction yields, all extracts were analysed for the total polyphenol content (TPC), fucoxanthin, and pH. Moreover, the antioxidant activity was evaluated by *in vitro* colourimetric assays: 2,2-diphenyl-1-picrylhydrazyl radical scavenging (DPPH), 2,2'-azin-bis(3-ethylbenithiazoline-6-sulfonic acid) (ABTS), and ferric reducing antioxidant power (FRAP). The extract's antimicrobial properties against *Pseudomonas fluorescence*, *Listeria innocua*, and *Listeria monocytogenes* were examined using a 96-well plate inhibition assay.

The results highlighted the potential of MAE to extract bioactive compounds from *A. esculenta*, as higher yields of TPC and fucoxanthin were found compared to no microwave treatment. For the total polyphenol content, the optimized parameters were an extraction temperature of 120 °C with a holding time of 5 minutes. The optimized parameters for fucoxanthin are inconclusive, as the highest quantified fucoxanthin content was obtained by the highest tested extraction temperature and holding time. The pH in the extracts was affected by increasing extraction temperature. All extract exhibited *in vitro* antioxidant activity and was found to be strongly correlated with the fucoxanthin content (r=0.90-0.96) and less correlated with the total polyphenol content (r=0.71-0.82). The extracts were found to have antimicrobial effects towards both *L. innocua* and *L. monocytogenes* (log 1.5 and 2 reduction, respectively), but limited effect against *P. fluorescence*. Results from the PEFAE protocols are still in progress and will be presented at the conference.

MAE was found to be a promising technology for extracting bioactive compounds from *A. esculenta*. Moreover, these compounds have the potential to be utilized in applications within the food industry, such as, e.g., in active- and edible packaging concepts.

P-201

PHOTODYNAMIC INACTIVATION OF *SALMONELLA ENTERICA* ON CUCUMBERS AND TOMATOES USING CURCUMIN AS PHOTOSENSITIZER

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Salmonella enterica is one of the main causes of foodborne gastroenteritis in worldwide. Salmonellosis outbreaks associated with cucumbers and tomatoes have been reported over the past decades. The photodynamic (PDI) treatment uses a combination of a blue light-emitting diode (LED) and the photosensitizer curcumin. PDI has been proposed as a non-thermal technology for inactivating pathogens in food surfaces. The effectiveness of PDI to inactivate *S. enterica* in on cucumber and tomato surfaces is limited.

This study evaluated the inactivation of *Salmonella* by PDI-curcumin on these fruit surface using different exposure times. Cucumber or tomatoes cubes (2.5 x 2.5 x 0.2 cm; 3 ± 1 g) were inoculated with a *S. enterica* cocktail (*S.* Agona, *S.* Montevideo, *S.* Michigan, *S.* Newport and *S.* Typhimurium; final counts 7.5 log CFU/sample) and dried for 1 h in a biosafety cabinet. Dried samples were immersed in a curcumin solution (75 µM) for 2 min, dried for 30 min and exposed to PDI (LED at 430-470 nm wavelength and 33.6 mW/cm2 of light irradiance; 2.01 J/cm2 for min) for

8-, 10- and 12-min. *S. enterica* inoculated control samples treated with LED or curcumin alone were also evaluated. *S. enterica* cells were enumerated in samples before and after each exposure time onto Tryptic Soy Agar (TSA) incubated at 37 °C for 18 h. Decimal reduction were calculated as the difference in *Salmonella* counts before and after the PDI and expressed as log CFU/sample. The data was analyzed using ANOVA, followed by Tukey test considering $p < 0.05$.
Analyses were performed using Sigma Stat 3.5 software. PDI reduced 1 and 1.3 log CFU/sample of *Salmonella* after 8-min of exposure on cucumber and tomatoes samples, respectively. 10-min exposure to PDI increased decimal reductions slightly (1.2 and 1.5 log CFU/sample on cucumbers and tomatoes, respectively). 12-min exposure to PDI increased the decimal reduction to 2 and 3 log CFU/sample on cucumber and tomatoes, respectively. No pathogen reductions were observed in controls. The inactivation by PDI appears time and fruit-dependent, but only of limited effectiveness.
These findings can be considered when implement measures for reducing *S. enterica* in fruit surfaces.

P-202

OPTIMIZATION OF FERMENTATION CONDITIONS FOR BIOETHANOL PRODUCTION BY *PARAGEOBACILLUS THERMOGLUCOSIDASIUS*

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Aim

Revalorization of agriculture and food residues is attracting the interest of the industry and governments to boost sustainability and profitability while adopting circular economy models. Indeed, many countries have started to utilize these resources to obtain biofuels and food ingredients by microbial fermentation. However, common organisms used for precision fermentation, such as *Saccharomyces cerevisiae*, have some limitations to fully exploit all potential nutrients derived from lignocellulosic materials. The thermophilic bacteria *Parageobacillus thermoglucosidasius* is an attractive alternative due to its ability to consume a wide range of sugars and its rapid metabolism, besides the fact that high incubation temperatures reduce contamination risks and ease bioethanol recovery. Since there is little information about the optimal fermentation conditions for *P. thermoglucosidasius*, the aim of this work was to identify the most critical parameters for reaching maximal cell density and bioethanol production.

Method

Fermentation by P. thermoglucosidasius DSM 2542 was carried out in sealed bottles of 40 mL capacity, and the effect of changing temperature (50 °C, 60 °C), inoculum concentration (1%, 10%), shaking (0-230 rpm), volume (10 mL, 32 mL) and glucose concentration (0.5%-2%) on cell viability, biomass, glucose consumption and ethanol production was examined.

Results

Despite the high growth rate of this microorganism at the optimal temperature (60 °C), cell viability decreased when reaching the late exponential phase. This phenomenon was not related to the drop of pH or starvation, and still alive cells could consume most glucose and produce ca. 1 g/L of ethanol. Variations in incubation temperature or inoculum size did not affect biomass and ethanol production. However, reducing glucose concentration (from 1.0% to 0.5%) impaired ethanol and biomass production, while increasing it (from 1.0% to 2.0%) did not improve any of the two and favoured cell death because of excessive acidification. Reducing the volume of the medium (from 32 mL to 10 mL) delayed cell lysis and enabled to double ethanol production when adding the highest glucose concentration despite the drop in pH.

Conclusion

Our results suggest that both control of aeration and pH while increasing glucose concentration are critical to obtain maximal benefits in alcoholic fermentation by *P. thermoglucosidasius*.

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IDENTIFICATION OF OPTIMAL PROTECTIVE CULTURES IN A HOLISTIC VIEW OF COOKED HAM PRODUCTION INCLUDING SANITATION

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Due to the high level of food waste worldwide, the associated CO_2 emissions and the consumer desire for food that is as natural as possible, biological preservatives such as probiotic cultures are becoming increasingly important in food production.
However, probiotics should act very selectively and not permanently contaminate the environment, as otherwise resistance will develop in an ecological niche colonized by various microbes, including pathogens. It is important that probiotics are removed by cleaning surfaces and do not potentially recontaminate other products not intended for this purpose. A proven method to identify potential protective cultures by isolating them from natural foods to selectively enrich their beneficial effects in high concentrations in the product.
The aim of the study is to combine methods for analyzing the potential protective cultures and the holistic view of the production chain through to sanitation. Another goal is to use protective cultures both before and after heat treatment, for which there have been few successful studies to date. This concept could provide an additional barrier to food safety and shelf life.
In this study, the production of cooked ham by an Austrian manufacturer is examined microbiologically and molecularly along the entire production chain, including the product environment, the brine used and the products themselves. The microbiological analysis is performed according to ISO reference methods (lactobacilli, total plate count, Enterobacteriaceae), followed by the confirmation of an isolate set using target-specific PCR, 16s rRNA sequencing and MALDI biotyper system. The microorganisms from the screening that are potential probiotics are then tested for their resistance to antibiotics and detergents using MIC tests. In addition, the antimicrobial potential is evaluated using bacteriocin tests. The candidate isolates (lactic acid bacteria) will then be characterized by genotyping (PFGE) and WGS analysis to determine their prevalence at farm level and record their genetic markers, which are essential for testing their suitability as a protective culture.
In the future, the potential organisms can be tested in heated meat product models and possibly integrated into the production process as a biopreservation barrier.

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KIWIFRUIT BY-PRODUCTS: INNOVATIVE VALORIZATION ROUTES

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Agricultural biomasses are still rich in value-added molecules, which can be recovered and valorized using bioprocesses and green extraction techniques, promoting the circularity of agribusiness systems. This is one of the challenges of spoke 8 of the National Center for the Development of New Technologies in Agriculture (Agritech), funded by the European Union. Bioprocesses like Solid State Fermentation can be used to upcycle by-products, converting the constitutive organic compounds into primary and secondary metabolites. The recovery of value-added molecules can be also approached uOalue-added compounds from kiwifruit discarded for their size. After the preliminary separation of kiwi parts (pulp, seeds, and peels) and the application of pre-treatments like milling and homogenization, the proximate composition and the molecular characterization of the three parts have been studied to understand how to exploit them better. Fermentation has been applied to kiwi pulp to recover antimicrobial compounds. This process has been carried out with Lactic Acid Bacteria and a Design of Experiment has been built up. Moreover, to valorize the interesting protein content found in kiwi seeds, an Enzyme-Assisted Extraction protocol using a protease from *Bacillus licheniformis* has been employed to obtain a protein hydrolysate from kiwi seeds.
Fermentation seems to affect the antimicrobial activity of the fermented biomasses against some of the most common

foodborne pathogens, while the protein hydrolysate obtained showed a good hydrolysis degree, confirmed by the characterization of the protein fractions. Considering the worldwide problem regarding agricultural biomasses, the results obtained open up new prospects for the valorization of kiwifruit waste from harvesting and processing for applications in food, feed, agriculture and even cosmetics.

P-205

OPTIMIZATION OF THE PRODUCTION OF BACTERIAL CELLULOSE BY *KOMAGATAEIBACTER* SPP. ON BREWER'S SPENT GRAINS

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Bacterial cellulose (BC) is synthesized by specific acetic acid bacteria (AAB) and is a valuable biopolymer with noteworthy mechanical and technological characteristics. The primary hindrance to industrial production lies in the production cost of this biomaterial. In this context, Agrifood by-products emerge as cost-effective and naturally abundant nutrient sources suitable for microbial fermentation, facilitating BC production. This study examined the growth kinetics and bacterial cellulose (BC) production of *Komagataeibacter hansenii* 6DB, *K. rhaeticus* LM2, LM4, and SM1, and Komagataeibacter xylinus DSM 2004 using brewer's spent grains, and yeast as growth media. In addition, an optimization of the production of BC was carried out by modulating parameters such as the addition of a buffer solution, pH, quantity of spent yeast in the formulation and ratio spent grain/water.
The results revealed that BC productivity was affected by the AAB strain and the growth conditions. In the absence of a buffer, BC production for all strains was limited to less than 0.5 g/L and this was accompanied by rapid acidification of the growth medium. The addition of phosphate buffer to the growth medium allowed to stabilize the pH and to increase the production of BC for all AAB strains. The higher BC producers were DSM2004 and LM2 (1.5 g/L). It was also observed that cellulose production gradually increased with the increase of incubation time, but the rate of BC formation remained almost constant after 7 days. The optimization of BC production by DSM2004 and LM2 allowed to increase the production rate up to 3g/L in 7 days of incubation.
This study allowed to identify AAB strains of interest for the production of BC from brewer's spent grains as well as to optimize the BC production. These aspects are crucial in order to favour the industrial exploitation of BC as innovative biopolymer.

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HOW CAN MODELS PREDICT THE PERFORMANCE OF BIOPRESERVATION SOLUTIONS? A CASE STUDY ON THE CONTROL OF MICROBIAL SPOILAGE IN STRAWBERRY PUREE

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New preservation methods are needed to replace current transformation steps in food industry. Strawberry undergo washing and decontamination with chlorinated water and thermal treatment for transformation into fruit preparations to limit fungal spoilage. Biopreservation technology could be a more sustainable and natural solution, but potential applications suggested by in silico experiments are difficult to transfer with the same efficacy to real foods.
The aim of this work was to evaluate the performance of a predictive model to predict growth of spoilage fungi in strawberry puree in presence of organic acids, either added as preservatives or by fermented ingredients, as a tool for the development of biopreservation solutions. Model strains from strawberry spoilage, *Saccharomyces uvarum*, *Saccharomyces paradoxus*, *Rhodotorula glutinis* and *Botrytis cinerea*, were selected to build a cardinal model in a strawberry synthetic medium, mimicking strawberry composition, in presence of five organic acids. The model was built through the determination of minimal inhibitory concentrations (MIC) of acids (≥ 30 growth curves for each strain and acid) and the fitting of the model to data was

quite satisfactory in both strawberry synthetic medium and strawberry puree.

Application to fresh strawberry puree with endogenous microbiota was tested. Organic acid concentrations that delayed or totally inhibited the growth of *S. uvarum*, the more resistant model strain, exerted the same or better inhibition level on the endogenous strains. The chosen model strain therefore seemed representative of endogenous microbiota. After validation of the model with direct addition of organic acids, the addition of a fermented juice containing acids in strawberry puree, as a biopreservation model, was tested. The juice allowed a significant delay of growth but lower than expected by the prediction and lower than the addition of equivalent amount of organic acids. This difference was attributed to the addition of other compounds when adding the juice.

Finally, the construction of the model in silico, in conditions close to real application, allowed good predictions in strawberry puree, but its application with biopreservation solutions was more limited due to the necessity to consider compounds other than organic acids in the model.

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OPTIMIZING BREAD PRESERVATION: USE OF SOURDOUGH IN COMBINATION WITH OTHER CLEAN LABEL APPROACHES FOR ENHANCED MOULD-FREE SHELF LIFE OF BREAD

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Bread and baked goods hold a significant role as a dietary staple across many countries. However, the challenge of fungal spoilage poses a notable threat to bread production, leading to diminished shelf life, compromised quality, and safety concerns. The water activity of bread ranges from 0.92 to 0.98, creating an environment suitable for fungal growth. Currently, preservatives such as propionate are commonly employed to prevent bread spoilage. But as consumer preferences continue to shift towards cleaner labels, efforts focus on enhancing the antifungal properties of bread-making processes, such as sourdough. Microbial fermentation is a traditional technique for food production with lactic acid bacteria.

In this study, we compared the mould-free shelf life of sourdough bread produced with *Fructilactobacillus sanfranciscensis* to chemically acidified bread made with acetic and lactic acid. Resilient strains of *Aspergillus* and *Penicillium* were deliberately chosen as target organisms for the challenge study. These robust fungal strains exhibited minimum inhibitory concentrations of propionic acid and acetic acid that surpassed accepted levels. In breads with final pH values spanning from 4.02 to 5.35, the growth of *Penicillium roqueforti* remained uninhibited up to concentrations of 200mM acetic acid and 100mM propionic acid.

The impact of water activity in the control breads revealed a prolonged mould-free duration at lower water activity levels (0.92) compared to higher water activity (0.96). Under the challenge of 0.92 water activity, bread formulations incorporating calcium propionate extended the mould-free shelf life of both sourdough and chemically acidified bread, with a more pronounced effect observed in sourdough.

These findings suggest that the incorporation of sourdough enhances the effectiveness of antifungal ingredients and preservatives.

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INACTIVATION OF *SALMONELLA*, *SHIGA* TOXIN-PRODUCING *E. COLI* AND *LISTERIA MONOCYTOGENES* IN RAW DIET PET FOODS USING HIGH PRESSURE PROCESSING

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Introduction

Raw diet petfood formulated with raw meat proteins can pose significant health risks to both pets and humans and outbreaks have occurred.

Purpose

Evaluate high pressure processing (HPP) to achieve a 5-log reduction of *Salmonella*, *E. coli* STEC and *L. monocytogenes* in various commercial raw diet petfoods and to maintain a 5-log reduction throughout post-HPP storage.

Methods

Eight raw diet petfoods, consisting of three beef formulations (A-, S- and R-beef), three chicken formulations (A-, S- and R-chicken) and two lamb formulations (A- and S-lamb), were inoculated with 7 log CFU/g cocktails of *Salmonella*, *E. coli* STEC and *L. monocytogenes*, vacuum sealed in high barrier bags and pre-cooled to 1.7-4.0 °C prior to HPP (586 MPa for 1-4 min at 4 °C) and stored post-HPP at either refrigeration (4 °C) or frozen (-10--16 °C) for 21 days with microbiological analyses conducted at various time intervals.

Results

All A- formulations inoculated with *Salmonella* and *E. coli* STEC and treated at 586 MPa for 2 min, achieved a 5-log reduction and was maintained during storage. *L. monocytogenes* inactivation in lamb-based formulations behaved differently than *Salmonella* and *E. coli* STEC inactivation and formulations containing beef or chicken. No *L. monocytogenes* inoculated samples stored refrigerated post-HPP (4.10 ± 0.19 log CFU/g reduction) and only 30% of frozen samples (6.45±0.52 log CFU/g reduction) achieved a 5-log reduction and was impacted by formulation. HPP treatment (3 min for beef and lamb; 4 min for chicken) inactivated 5-log of *Salmonella* and *E. coli* STEC and frozen storage post-HPP maintained microbial reduction than refrigerated storage.

Significance

HPP treatment of raw diet petfood can inactivate 5-log of *Salmonella* and *E. coli* STEC and frozen storage post-HPP maintained microbial reduction. *L. monocytogenes* was the more difficult pathogen to inactivate. The study also supports current consumer recommendation to thaw only needed amounts or prior to use of product.

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BIOLOGICAL VALIDATION OF IR PLUS UV PASTEURIZATION COMBINED PROCESS FOR CASHEW NUTS

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Salmonella enterica has the ability to survive on dry cashew nut for long periods. Drying process is used to reducing the water activity associated with microorganism's development. Infrared (IR) radiations is used for drying combined with others technologies as UV. Researches suggests that IR heating is a promising method to obtain high-quality dried food products, but, 5 decimal reductions must be reached for target microorganism, however, since the use of pathogenic organisms in processing facilities is not advised, a surrogate non-pathogenic organism must be used, with a similar kinetical behavior to the target pathogenic when exposed to process parameters. Considering nuts, *Enterococcus faecium* ATCC8459 is reported as a suitable surrogate for *Salmonella*.

This research aimed validate the pasteurization of Cashew Nut-SLW1 in Curomatic Equipment 6D-750 and MLCS5D-750, in a Brazilian facility, in order to check if 5 (99,999) logarithmic cycles of *Salmonella* sp., were obtained by 3 biological validation processes. For this, 1500 g (500

g/process) of cashew nuts (initial humidity = 5.2%) were inoculated with *E.faecium* ATCC8459 (1.41 × 1010 CFU/mL) dried to final humidity = 5.5%. The impregnated population was > 107 CFU/g. For tests, 1 kg of colored non inoculated cashew nuts was introduce into the IR module between the addition of the mono layer inoculated samples, to restrict the inoculated sample position and to evaluate the combined effect IR + UV. The samples passed through IR + UV modules and were collected at the UV module output. These samples were kept under refrigeration until survivors' population enumeration performed on mEnterococcus Agar-Neogen (35 °C/48 h). The Number of Decimal Reduction-NDR was establish considering the log of untreated and survival populations. On average of processes runs tested, the IR + UV effect caused 8.34 (min = 8.13 and max = 8.64) NDR in the *Salmonella* population.

Process 2 was the most efficient with 6.2 minimum NDR for *Salmonella*. All 3 processes runs, considering the IR + UV effect, achieved more than 5 NDR for *Salmonella*, as required by government agencies for cashew nuts. Therefore, under the processed conditions: V = 3 m/min; IR = 75 °C/4 min + UV (254 nm)/7.57 min, all processes were microbiologically validated as a cashew nut pasteurization, reaching more than 5 decimal reductions of *Salmonella*.

These results prove that tested IR + UV system was efficient in producing high-quality and microbiologically safe cashew nuts.

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USE OF VIRULENT BACTERIOPHAGES TO CONTROL *SALMONELLA* DURING DRY FERMENTED SAUSAGE PRODUCTION

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Salmonella is a major cause of foodborne illness, with about 60 000 laboratory-confirmed cases of salmonellosis reported in Europe in 2021. Pigs are regarded as a significant reservoir of this pathogen, and raw or undercooked pork and processed pork products are commonly implicated in human infections. Dry fermented sausage is a ready-to-eat food product mostly made of pork, which is among one of the main meat-based traditional fermented foods produced and consumed in Europe and worldwide. Pork raw materials used in the production of dry fermented sausages can be contaminated with *Salmonella* following carcass contamination linked to inappropriate slaughtering or handling practices. Among the different decontamination strategies, virulent bacteriophages constitute a promising option to control pathogenic bacteria such as *Salmonella* during food processing.

This study aimed to evaluate the sensitivity of a large panel of *Salmonella enterica* subsp. *enterica* strains (n = 50) isolated from the French pork industry to an anti-*Salmonella* commercial phage product, then to challenge its lytic activity on pork raw materials (lean meat and back fat) as well as ground meat (80% meat/20% fat), spiked with cocktails of *Salmonella* strains exhibiting different phage susceptibility profiles. All the *Salmonella* strains tested were sensitive to the phage product, but with different levels of sensitivity i.e. 68%, 14% and 18% exhibited a high, medium and low phage susceptibility, respectively.

Early results showed almost 1 $\log_{10}$ CFU/g or cm2 reduction of *Salmonella* populations within 1h on lean pork, back fat and ground meat samples initially spiked with 5 $\log_{10}$ CFU/g of a cocktail of three *Salmonella* strains highly sensitive to the phage and subsequently treated with 108 PFU/g or cm2 (0.5% pick up level). Extending the phage concentration to 109 PFU/g or cm2 (0.5% pick up level) reduced *Salmonella* levels by more than 2.5 $\log_{10}$ CFU/g or cm2.

These preliminary results highlighted that the use of virulent bacteriophages could be an additional hurdle used in the production of dry fermented sausages to reduce contamination with *Salmonella*. Further experiments will permit to assess the beneficial of such a bacteriophage treatment on the behavior of *Salmonella* during ripening of dry fermented sausages.

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EVALUATION OF TECHNOLOGICAL TRAITS AND ANTIBIOTIC RESISTANCE OF LACTIC ACID BACTERIA ISOLATED FROM FOOD MATRICES IN SICILY, ITALY

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Lactic acid bacteria (LAB) are naturally present in several foods and are valued for their technological applications, particularly fermented foods. LAB are used as starter and adjunct cultures in the dairy industry, and novel strains are being characterized as possessing versatile technological features.

We aimed to assess the technological traits of 38 LAB, previously obtained from different sources in Sicily, Italy (orange, donkey milk and cheese) and identified based on molecular methods: *Lactobacillus rhamnosus* (n = 5), *Lactiplantibacillus plantarum* (n = 24), *Limosilactobacillus fermentum* (n = 5), *Lactobacillus delbrueckii* (n = 1) and other LAB (n = 3). Isolates were grouped based on rep-PCR and subjected to spot-on-the-lawn assay to identify their inhibitory activity against *Salmonella* Enteritidis, *Listeria monocytogenes, Staphylococcus aureus, Escherichia coli* and *Shigella flexneri*. Isolates were characterized based on their technological features: diacetyl production, proteolytic capacity and acidifying activity. Antimicrobial resistance (AMR) was assessed for ampicillin (AMP, 10 µg), oxacillin (OXA, 1 µg), trimethoprim/sulfamethoxazole (SUT, 23,75 µg / 1,25 µg), imipenem (IPM, 10 µg), vancomycin (VAN, 5 µg), tetracycline (TET, 30 µg), clindamycin (CLI, 2 µg), chloramphenicol (CLO, 30 µg), rifampicin (RIF, 5 µg) and erythromycin (ERI, 15 µg).

Isolates presented a high similarity based on rep-PCR, being grouped in four distinct clusters with at least 70% of similarity. None of the isolates exhibited antimicrobial activity against the tested pathogens. Concerning technological potential, 50.0% (n = 19) of isolates produced diacetyl, an important aromatic compound for the dairy industry. Presumed proteolytic activity was observed in 86.84% (n = 33) of isolates, indicating potential application in cheese ripening.

All isolates presented some ability to acidify milk, with weak pH decrease after 24h. AMR was observed specially to OXA (n = 37, 97.4%), TET (n = 35, 92.1%), VAN (n = 34, 89.5%), RIF (n = 31, 81.6%) and CLI (n = 25, 65.8%). Less than 30% of the isolates presented AMR to the other tested antibiotics, and 34 (89.5%) presented simultaneous resistance to OXA, VAN, and TET. Despite the high frequencies of AMR, the isolates presented interesting technological features that can be exploited by the food industry.

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INVESTIGATING TOMATO PLANT MICROBIOTA AS SOURCE OF BIO-PROTECTIVE MICROORGANISMS FOR APPLICATION IN FRESH PRODUCE

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Introduction

Food bio-protection is considered a potential alternative to extend food shelf-life using natural microbiota or antimicrobial metabolites. Exploiting the microbiota of agricultural residues is desirable as these strains would be better adapted to the microecological conditions of the target food and would better compete against phytopathogens and spoilage microorganisms as they are part of the same microbial ecosystem.

Purpose

The aim of this study was to evaluate the antimicrobial activity of bacterial isolates from different anatomical parts of tomato plant (i.e., leaves, stems, rhizosphere, and fruit) against foodborne pathogenic bacteria and spoilage bacteria and fungi relevant in fresh produce.

Methods

Lactic acid bacteria (LAB), *Pseudomonas* spp. and *Bacillus* spp. bacteria were isolated using specific agar media based on standardized methods. The antimicrobial activity of the isolates was evaluated by the spot-on-lawn method against *Salmonella enterica*, *Escherichia coli* O157:H7, *Listeria monocytogenes* (pathogenic bacteria), *Pectobacterium carotovorum* (spoilage bacterium) and *Rhizopus stolonifer*, *Aspergillus flavus* and *Fusarium oxysporum* (spoilage fungi). The antifungal activity of the isolated bacteria against *F. oxysporum* was additionally evaluated on tomato-based agar as an intermediate product-oriented approach. Finally, bacterial isolates showing antifungal activity against *F. oxysporum* on tomato-based agar were identified by ITS sequencing.

Results

A total of 266 bacteria were isolated. The experimental results showed that 6 *Bacillus* strains and 3 *Pseudomonas* strains isolated from rhizosphere and leaves inhibited the growth of *L. monocytogenes*. In addition, 3 *Bacillus* and one *Pseudomonas* strains isolated from leaves, inhibited the growth of *P. carotovorum*. Regarding the antifungal activity, 90 and 37 bacteria, belonging to *Bacillus*, *Pseudomonas* and LAB and isolated from rhizosphere, stems, leaves and fruit, reduced the growth of *R. stolonifer* and *A. flavus*, respectively. Finally, 3 bacteria isolated from tomato fruit and identified as *Bacillus* spp, *B. amyloliquefaciens* and *B. velezensis* reduced the growth of *F. oxysporum* on tomato-based agar.

Significance

The results demonstrate that the microbiota of tomato plant (mainly leaves) harbour bacteria with bio-protective potential. This information can serve as a solid base to develop bio-based approaches aiming at sustainably preserving foods from farm to fork and mitigating food waste.

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SUSTAINABLE PRODUCTION OF ANTIMICROBIAL BETA-CHITOSANS AND PROBIOTICS FROM SQUID PEN RESIDUES UNDER A BIOREFINERY APPROACH

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The endoskeletons (pens) of jumbo squid (*Dosidicus gigas*) are one of the most important by-products generated in the industrial processing of such food resource and which are usually managed as urban solid waste. However, those pens could be a valuable source of chitin derivatives and proteins useful for multiple uses in food industry (preservation, packaging, fermentation media, etc.).

Based on a circular economy approach, we have assessed the integral valorization of pens for the optimal production of b-chitosan and b-chitooligosaccharides with antibacterial properties and the recovery of the effluents, used in the purification of these polysaccharides, as substrates rich in protein (peptone) for microbiological cultures. In the first case, chitosan compounds demonstrated high ability in inhibiting surrogate food pathogen bacteria (*Escherichia coli* and *Bacillus cereus*). In the second one, the bioconversion of the effluents was validated by the fermentation of two probiotic lactic acid bacteria (*Lactobacillus plantarum* and *Leuconostoc mesenteroides*).

In this context, low-cost media formulated with squid peptones showed similar capacity to support growth (biomass) and metabolite formation (lactic acid) than that observed in commercial medium. These combinations of chemical/enzymatic/fermentative processes suppose a sustainable biorefinery strategy for the integral upgrading of squid wastes and corresponding production of high-value biocompounds (biocides, food starters, probiotics, preservative and chemical building blocks).

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ROLE OF COMPETITION-EXCLUSION FOR TRACE ELEMENTS IN THE ANTIFUNGAL ACTIVITY OF *L. PLANTARUM* L244 AND *L. RHAMNOSUS* CIRM-BIA 1759

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Dairy products are susceptible to contamination by and growth of acid-tolerant spoilage fungi, leading to important food waste and economic losses. In order to prevent fungal spoilage, the use of antifungal lactic acid bacteria (LAB) bioprotective cultures is of growing interest as an alternative to chemical preservatives and/or as a complementary hurdle technology. Among action mechanisms of these bioprotective cultures, competition-exclusion for trace elements is a newly discovered mechanism which plays an important role in their antifungal activity.

In this context, the aim of this study was to investigate this mechanism in two known antifungal LAB strains, namely *Lactiplantibacillus plantarum* L244 and *Lacticaseibacillus rhamnosus* CIRM-BIA 1759, using a yogurt model. To do so, skimmed milk supplemented or not with a single trace element, including 6 metals (manganese, copper, iron, zinc, nickel and cobalt) and 12 vitamins (vitamins B1, B2, B3, B5, B6, B8, B9, B12, C, D, E and K1), was fermented with a commercial yogurt starter culture as well as with or without one of the tested antifungal strains. Following fermentation, yogurts were artificially contaminated with different spoilage fungi (*Penicillium biforme*, *Galactomyces geotrichum*, *Mucor racemosus* and *Yarrowia lipolytica*) and fungal growth was evaluated during storage at 10 °C.

In yogurts containing the antifungal strains, manganese supplementation suppressed the growth inhibition of the selected *P. biforme* and *Y. lipolytica* isolates, suggesting that manganese depletion was involved in the antifungal activity. The addition of the other tested trace elements did not affect their antifungal activity. In parallel, scavenging of trace metal elements was determined using ICP-MS in cell-free lactoserum obtained from yogurts prepared with or without the antifungal LAB and stored for 7 days at 10 °C.

The obtained results showed that manganese concentrations were significantly lower in yogurt supplemented with antifungal LAB (2 ppb) as compared to the control yogurts (5 ppb), confirming that manganese was indeed scavenged by the antifungal strains. The role of competition-exclusion for manganese in the inhibition of a large diversity of spoilage fungi is currently under study to see whether this mechanism is generic among dairy spoilage fungi.

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EXPLORING THE CRABTREE EFFECT OF *METSCHNIKOWIA PULCHERRIMA*

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The Crabtree effect, observed in certain yeast species, refers to the phenomenon where under aerobic conditions, glucose repression of respiratory enzymes leads to a preference for fermentation over oxidative phosphorylation. This metabolic switch results in decreased respiratory activity despite the presence of oxygen, leading to increased ethanol production. Understanding the Crabtree effect is crucial in biotechnological and food applications such as biomass production and metabolic engineering, where optimizing yeast metabolism for desired product yields is paramount.

Reducing biomass yield due to the Crabtree effect can lead to financial losses in industrial applications reliant on yeast fermentation, where maximizing biomass conversion efficiency is essential for economic viability. *Metschnikowia pulcherrima* is a yeast species recognised both for its biocontrol potential against fruit pathogens

and in winemaking for its contributions to fermentation dynamics and flavour profiles.
However, despite its growing importance in both domains, its growth optimization remains an area requiring further exploration. While it may replace chemical pesticides, its growth dynamics, especially regarding metabolic pathways like the Crabtree effect, lack thorough research.
This study focuses on optimizing *M. pulcherrima* strain APC 1.2 growth, revealing a potential Crabtree-positive phenotype. Understanding such metabolic intricacies in alternative yeast species is vital for biocontrol and biotechnological advancements.

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LACTIC ACID BACTERIA AS PROTECTIVE CULTURES FOR PLANT-BASED FOOD

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The increasing popularity of plant-based foods has prompted a re-evaluation of food safety strategies to address new microbial challenges. Recent studies have shown that plant-based ingredients can have a variety of contaminants either coming from the endogenous microbial load of the raw materials or/and of the contamination during the manufacturing process. Considering this data, one of the most common contaminants found in plant-based ingredients are the spore-forming *Bacillus* spp., where *Bacillus cereus* and *Bacillus liqueniformis* representatives can lead to food-borne disease.
Several lactic acid bacteria (LAB) produce a variety of antimicrobial compounds, including bacteriocins, which target specific microbial contaminants, thus mitigating the risk of spoilage while minimizing the need for chemical preservatives or harsh processing methods. LAB as protective cultures have been an emerging solution in the dairy and meat industry. In this study, we aimed to develop protective cultures of LAB against *Bacillus* spp. that can be applied in our model plant-based matrix made out of Lupin beans. First, we determined the endogenous contaminants found in the Lupin matrix where we found a variety of *Bacillus* spp. Then, we screened in Agroscope's strain collection for LAB candidates that produced a selection of antimicrobial compounds known to inhibit *Bacillus* spp., either targeting their vegetative form or hindering spore germination. Then, under *in vitro* conditions the activity of the LAB against the contaminants was tested. Lastly, the promising candidates will be tested in competition against *Bacillus* spp. model strains in the Lupin matrix to analyse their performance under *in vivo* conditions.
By exploiting the antagonistic properties of LAB as protective cultures, we could effectively outcompete and suppress undesirable microorganisms in plant-based foods and ingredients, increasing their safety and ensuring food security.

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BIOACTIVITY OF AQUEOUS EXTRACTS FROM AGRI-FOOD BY-PRODUCTS: EFFECT OF PARTICLE SIZE

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Many spoilage moulds are well adapted to the environment of cheese factories and entail significant economic losses. To solve this problem, chemical preservatives authorized by FDA and UE are widely used. However, the increasing of resistances against these antimicrobial agents leads to search for new natural sources. By-products generated in the agri-food industry are still rich in bioactive compounds that show antifungal activity. This research has focused on the search for new sources of bioactive compounds to fight against cheese spoilage fungi. For this purpose, aqueous extracts (AEs) from agri-food by-products have been obtained and the influence of the particle size of these by-products on their physicochemical characteristics, antifungal and antioxidant activities have been studied.
Almond pericarps, pea pods, discards of broccoli, grape stems, and grape skins by-products were collected from different local agri-food industries (Albacete, Spain). A sixth, prickly pear peels, was supplied for a Sicilian juice factory.

All by-products were lyophilized, grinded and classified in two different sizes (between 1-2 mm and 2-4 mm). The AEs were obtained by dynamic solid-liquid extraction, filtered, and tested by the agar well diffusion assay against strains from *Penicillium* and *Aspergillus* spp. that were previously isolated and identified from Spanish cheese factories.
The Folin-Ciocalteu method (total phenolic content) and the inhibition activity (%) of DPPH radicals (antioxidant activity) were determined too. The AEs were also analyzed by HPLC-DAD. The highest total phenolic content was found in grape stems AEs, independently of the particle size used during the extraction. Among the AEs that showed antifungal activity, the main phenolic compounds found were ellagic acid in broccoli and caffeic acid in almond pericarp and grape stem AEs, respectively. Grape skin and almond pericarps AEs showed the highest antioxidant activities.
Only the extracts from almond pericarps, grape stems and broccoli discards showed antifungal activity. Broccoli was the only extract that showed activity against both genera. Particle size was only significantly relevant in the case of broccoli AE, with a greater antifungal and antioxidant activity in the AE obtained from larger material.

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ANTIFUNGAL AND ANTIOXIDANT ACTIVITY OF AROMATIC PLANT HYDROSOLS AND ITS POTENTIAL APPLICATIONS TO CONTROL FILAMENTOUS FUNGI IN CHEESE

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Filamentous fungi play an important role in the spoilage of different types of cheese by causing several defects in rind and pastes which important economic losses. There are several categories of preservatives authorized by the FDA or the EU to control fungal contamination, such as the use of weak acids and their salts (sorbic, benzoic, or propionic acids) or antibiotics such as natamycin. Currently, consumers demand to replace the existing preservatives by more natural alternatives, and the use of agri-food by-products is postulated as a good way to obtain natural antimicrobial compounds. Hydrosols are aromatic water distillates remaining after the manufacture of essential oils that still contain potentially bioactive compounds representing a good option for obtaining effective natural alternatives against spoilage moulds. This work aims to test the antifungal activity of different hydrosols against moulds isolated from different Spanish cheese factories. In addition, the composition and antioxidant capacity of these by-products have been studied.
Hydrosols of cinnamon, clove, cistus, lavender, thyme, and oregano, supplied by El Jarpil S.L. (Almería, Spain), were tested by the agar well diffusion assay (100 µl) against 5 strains isolated from 4 different cheese factories (*Aspergillus puulaauensis* 1A05, *A. jensenii* 501, *Penicillium crustosum* 1B01, *P. commune* 301 and *P. commune/biforme* 601), and inhibition haloes were determined at 3 and 7 days. The total phenolic content (TPC) was determined by the Folin-Ciocalteu method, and the antioxidant activity by the DPPH radical scavenging assay.
Hydrosol composition was characterized by HPLC-DAD. Among the hydrosols, only cinnamon showed activity against 4 of the 5 tested strains, with inhibitions between 8.4% (*P. commune* 301) to 19.3% (*P. crustosum* 1B01), but that antifungal effect decreased with time. The TPC oscillated between 4.8 to 415 mg GAE/g for cistus and clove hydrosols, respectively; and the percentage of DPPH showed that the most active hydrosols were clove, cinnamon, and oregano. The main compounds for the most bioactive hydrosols were epicatechin gallate and ellagic acid for cinnamon hydrosol, and catechin gallate and pyrocatechol for clove hydrosol.

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ANTIBACTERIAL AND ANTIBIOFILM ACTIVITY OF POTATO (*SOLANUM TUBEROSUM* CV. LAURA) PEEL-DERIVED EXTRACELLULAR VESICLES AGAINST *BACILLUS CEREUS* ATCC 11778

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Background

Bacillus cereus is commonly found in many foods and known for causing foodborne illness. Due to their spore-forming and biofilm-forming nature and contamination capability these bacteria are presenting a large challenge to the food industry. The bacterium has become increasingly resistant to multiple drugs necessitating novel preventive strategies. One promising solution is the use of plant-derived extracellular vesicles (EVs), specifically from potato peels, which carry complex bioactive cargos with potential antibacterial properties. Our research explores the effectiveness of these plant-derived EVs against both planktonic phase cells and biofilms of *B. cereus* ATCC 11778.

Methodology

Potato peel derived EVs were prepared using a combination of vacuum infiltration, sequential centrifugation, and size exclusion chromatography. *B. cereus*, cultured in the planktonic phase at a concentration of 1 × 106 CFU/mL, was co-cultured with potato peel derived EVs at a concentration of 1 × 109 EVs/mL in Muller Hinton broth. Two doses of EVs were administered at the 6 and 12hour time points thereafter, and the incubation period was extended to 24 hours at 35 °C. Bacterial growth was assessed by measuring absorbance at 620 nm. Reduction in CFU was observed at various time points (0, 3, 6, 12, 18, and 24h) by subculturing bacteria-EVs co-cultures on blood agar. The inhibition of biofilm formation and eradication of pre-formed biofilms were evaluated using crystal violet assay. A negative control was maintained using a PBS whereas ampicillin (11 µg/mL) was used as the positive control. Data are presented as mean ± SEM (n = 3).

Results

Potato peel EVs revealed their ability to inhibit bacterial growth by extending the lag phase. With periodic dosing, a CFU reduction of 87.2% ($p = 0.0084$, single dose) to 94.5% ($p = 0.0002$, two doses) and further to 95.7% ($p=0.0013$, three doses) was observed in comparison to the negative control after 18 hours of incubation. Potato peel EVs further showed eradication (16.02 ± 0.008%) and inhibition (28.9 ± 0.007%) potentials over preformed and initial attachment of biofilms, respectively.

Conclusion

Potato peel EVs exhibit antimicrobial efficacy against B. cereus planktonic cells and may impede biofilm development, indicating promising applications. Mechanism and bioactive compounds warrant thorough investigation for sustainable, non-toxic approaches.

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ANTIFUNGAL ACTIVITY OF *ORIGANUM VULGARE* ESSENTIAL OIL-LOADED NANOEMULSIONS AGAINST POSTHARVEST FUNGAL PATHOGENS

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This study aimed to evaluate the physicochemical characterization and the antifungal potential of polymer coating incorporated with oregano essential oil (OEO) nanoemulsion. OEO (0.75% w/w), water, and polysorbate 80 (0.75% w/w) (Ultra-Turrax, followed by ultrasound treatment 150 W/3min) resulted in nanoemulsion (ONE - F1). ONE (0.25% w/w) with a polymer solution of sodium alginate (1% w/w), carboxymethyl cellulose (0.5% w/w), and glycerol (F2), finally a polymer solution without ONE was used as a control (F3).

The formulations were store at 25 ± 2 °C for 28 days, and analyzed at 0, 7, 14, 21 and 28 days, regarding the physicochemical characterization such us particle size distributions (PSD), polydispersity index (PDI), zeta potential and color. The antifungal potential was tested against *Colletotrichum* sp., *Fusarium* sp., and *Cladosporium* sp. The radial fungal colonies diameters after 7th day was obtain to calculate the inhibition percentage (I%). The PSD for F1 remained stable over the 28 days of storage (<150 nm) and PDI between 0.2 and 0.4, showing the homogeneity of the particle size distribution nanoemulsion, while F3 showed high PSD (317-587nm) and PDI (0.94-0.99) values throughout storage.

For this reason, the gradual increase in oil droplets in the F2 over the 28 days of storage (157-468nm) was associated with an increase in PDI (0.54-1.0), possibly due to other components present in the polymeric formulation since the F3 showed high PSD and PDI values. F1 and F2 exhibited zeta potential values below -30 mV and had a homogeneous, milky appearance and showed no phase separation until 28 days. The whiteness index for F1 (64.78-61.95) and F3 (33.42-32.43) were stable throughout storage, whereas for F2, the whiteness index decrease (51.34-34.80), in this sence a transparent coating is desired to keep the original food. F1 completely inhibited all fungal growth *in vitro*. F2 showed better inhibition of *Colletotrichum* sp. Growth (100%), followed by *Fusarium* sp. (78%), and *Cladosporium* sp. (55%), showing greater efficacy than F3 which had no antifungal activity.

Thus, in the present work, the stability and strong antifungal activity of the F2 demonstrates its prospects as a nano-active coating agent for post-harvest fruit preservation.

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EXTRACTION OF PHENOLIC COMPOUNDS FROM *SORBUS AUCUPARIA* TREE LEAVES BY THIN-LAYER CHROMATOGRAPHY AND EVALUATION OF ANTIMICROBIAL ACTIVITY

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Sorbus aucuparia is a tree species belonging to the genus Sorbus, within the Rosaceae family, commonly known as the rowan tree or mountain ash. In Portugal, this plant is used as an ornamental tree, while in various European countries, the fruits are utilized in human food.

Concerning rowan leaves, scientific literature indicates the presence of various chemical compounds, including phenolic compounds. These compounds confer therapeutic properties to the leaves, such as antioxidant, anti-inflammatory, antimicrobial, and hypoglycemic effects. However, evidence remains limited regarding its therapeutic potential.

The primary objective of the current study was to optimize the extraction of phenolic compounds from *Sorbus aucuparia* leaves using thin-layer chromatography (TLC). The aim was to investigate their potential for use as a natural additive in food products. Multiple extraction solutions and mobile phases for TLC analysis (silica gel plates) were tested. The optimal procedures were selected, with hydro-acetone (20:80 v/v) identified as the most effective solvent extractor: Chloroform - Ethyl acetate - Formic acid (15:45:5 v/v/v).
Groups of phenolic compounds separated by TLC were isolated, and antimicrobial assays are currently being conducted to determine which ones exhibit the most promising biological properties. These extracts are also being analyzed to quantify total phenols, flavonoids, and DPPH.

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PRODUCTION OF HIGH-VALUED FOOD INGREDIENTS FROM THE FERMENTATION OF RED CHICORY (*CICHORIUM INTYBUS* L.) BY-PRODUCTS

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Red chicory (*Cichorium intybus* L.) is a leafy vegetable largely consumed in Italy as a raw salad with a distinctive bitter taste. Recently, its popularity has increased mainly due to the health benefits linked to its high content of fibers and phytochemicals with antioxidant activity.
Since the commercial production of red chicory includes the removal of outer leaves from heads, the high demand for this product leads to the accumulation of a large amount of by-products.
Fermentation by lactic acid bacteria and yeasts has been lately explored as a valuable strategy to transform vegetable waste into innovative value-added food products with low environmental impact and beneficial effects on consumers. Therefore, the aim of the present study was to investigate the effects of fermentation with *Lactiplantibacillus plantarum* VUCC206 and *Saccharomyces cerevisiae* VUCC561, previously studied as candidate starters for fermentation of dough fortified with grape pomace powder, on the polyphenol content of red chicory by-products. Moreover, *Lentilactobacillus hilgardii* ATCC 8290Tand *Lactiplantibacillus plantarum* ATCC 14917T were included as controls.
Almost all the strains, as single inoculum or combined, were able to decrease the pH by more than 2 pH units (from 5.57 to 3.5), with the lowest value (3.19 ± 0,04) obtained by the co-inoculum of the *Lpb. plantarum* strains; on the contrary, *Llb. hilgardii* ATCC 8290T was found unsuitable as fermentation agent for this substrate.
Among the trials, the chicory products fermented with both *Lbp. plantarum* strains combined with *S. cerevisiae* VUCC561 were characterized by the highest level of polyphenols (around 14 mgGAE /g of chicory powder), as revealed by the Folin Ciocalteau method.
Data obtained showed that *Lbp. plantarum* VUCC206 and *S. cerevisiae* VUCC561 are promising bioresources, with *Lbp. plantarum* ATCC 14917T, to produce a stable high-valued ingredient that could be used for the development of fortified food prototypes, meeting the circular economy perspectives and the consumers' preferences.
Future work will aim at the thorough safety assessment of these strains, as a requisite for their deliberate use in the food chain, and the evaluation of the antioxidant capacity of the final fermented product.

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VALORIZATION OF *BRASSICA* BY-PRODUCTS TOWARDS ISOTHIOCYANATES

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The production of *Brassica* vegetables involves a huge amount of by-products that are not well valorized and end up as massive waste which is usually disposed of by polluting processes such as incineration. Interestingly, those by-products are excellent sources of bioactive isothiocyanates (ITCs), which are typically for *Brassica* vegetables.
ITCs are generated by cleavage of relatively stable, odourless, sulfur- containing precursors named glucosinolates by

the endogenous enzyme myrosinase. Various physiological functions of ITCs have been reported such as anti-inflammatory, anti-oxidative, and antimicrobial activities.
However, ITC production is hampered by several factors among which the presence of epithiospecifier protein (ESP) in the plant cell induces the production of non-ITCs hydrolysates like nitriles, having no bioactivity, and the inefficient release of ITCs caused by inadequate exposure and the low myrosinase activity in some part of the plant tissues. Increased ITC production can be achieved during vegetable fermentation. It has been reported that some strains show myrosinase-like activity.
This research will show some interesting results about the development of a fermentation process to valorize *Brassica* by-products towards bioactive ITCs.

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NISIN-LOADED CHITOSAN/SODIUM ALGINATE MICROSPHERES ENHANCES THE ANTIMICROBIAL EFFICACY OF NISIN AGAINST *STAPHYLOCOCCUS AUREUS*

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This study presents the development and evaluation of nisin-loaded chitosan/sodium alginate (CS/SA) microspheres as an antimicrobial delivery system targeting *Staphylococcus aureus* strains. The microspheres, prepared by using a modified water-in-oil (W/O) emulsion cross-linking method, exhibited homogeneous spherical morphology with a size range of 1-8 μm and a surface charge of -7.92±5.09 mV, assessed by scanning electron microscopy (SEM) and Zetasizer. Fourier-transform infrared spectroscopy (FTIR) analysis validated the composition of nisin-loaded CS/SA microsphere.
Encapsulation efficiency (EE) and loading capacity (LC) of nisin in microspheres were determined as 87.60 ± 0.43% and 1.99 ± 0.01%, respectively. *In vitro* release kinetics over 48 hours indicated a controlled release pattern of nisin from the microspheres under varying pH and temperature conditions, following Fickian diffusion described by the Korsmeyer–Peppas model, with higher release rates observed at 37 °C and alkaline pH. Antimicrobial assays revealed enhanced efficacy of nisin-loaded CS/SA microspheres compared to free nisin, with lower minimum inhibitory concentration (MIC) values and larger inhibition zones in agar well diffusion assays. Confocal laser scanning microscopy (CLSM) and scanning and transmission electron microscopy (SEM, TEM) provided visual evidence of enhanced bacterial membrane damage and cellular disruption induced by the nisin-loaded CS/SA microspheres.
Overall, this study highlights the potential of nisin-loaded CS/SA microspheres as an innovative antimicrobial delivery system with improved stability and antimicrobial efficacy against *S. aureus*, overcoming limitations associated with application of nisin alone. This approach presents a promising alternative to conventional methods of food preservation, and laying the groundwork for future exploration into the practical applications in relation to food biopreservation and active food packaging.

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