FoodMicro 2022

27th International ICFMH Conference

Organized by

International Committee on Food Microbiology and Hygiene

Under the auspices

Hellenic Scientific Society of Mikrobiokosmos

ΓΕΩΠΟΝΙΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ
AGRICULTURAL UNIVERSITY OF ATHENS

Next Generation Challenges in Food Microbiology

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ATHENS, GREECE

Hybrid

Abstract Book

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Next Generation Challenges in Food Microbiology

Hybrid

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Welcome address

Dear colleagues and friends,

It is our great pleasure to welcome you to the FoodMicro 2022, which is taking place in Athens-Greece, at the Megaron Athens International Conference Centre (MAICC), on 28th to 31st of August. The congress is held in a hybrid format, which means fully physical and fully virtual at the same time. The Organizing Committee would like to thank all attendees, whether physical or virtual, for joining the FoodMicro 2022 despite the COVID-19 restrictions, and the understandable fatigue from the extended period of remote scientific meetings. We are very honoured by the commitment of our colleagues from across the globe, as well as the engagement of affiliated disciplines to this endeavour.

Keeping up with all new trends and data in the Food Microbiology sector, the Scientific Committee has been working hard to prepare an attractive and high-standard Scientific Program.

Indeed, we made every possible effort to create an interesting scientific program, in the context of One-Health that covers areas of Food Safety, Microbial Ecology, Microbial Ecosystem and Microbiome, Microbial diversity and Physiology, scientific diversity serving food microbiology and Controlling and predicting microbial behaviour in food ecosystems.

We are deeply honoured by your participation and proudly welcome you to the FoodMicro 2022 in Athens-Greece!

Have a great conference time while you may enjoy the opportunities to explore the rich heritage of our historic city, in which the foundations of the most cherished principles of our western civilisation were born 2500 years ago, are truly limitless.

Best regards,

Prof. George John Nychas
On behalf of the Organizing Committee

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About "ICFMH"

The International Committee on Food Microbiology and Hygiene was founded in 1953. The major scope of ICFMH is to contribute to food safety and controlling food spoilage internationally, by means of organizing conferences (e.g. FOODMICRO), symposia and workshops, supporting of international bodies in food microbiology issues, publications (e.g. the International Journal of Food Microbiology), and initiation of education and training in food microbiology.
The ICFMH particularly focuses on the food safety situation in developing countries, with a special mission towards the African situation.
Learn more about ICFMH Association by clicking HERE

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ORAL PRESENTATIONS Food Microbial Ecology

O1.1 Fermentation of pea protein-based matrices by synthetic microbial consortia as a lever to modulate metabolome and overall sensorial perception: an innovative way for new foods design

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Moving to a more sustainable food system requires to increase the proportion of plant protein (e.g. pea) in our diet. However, the use of pea protein in foods is limited due to the persistence of off-flavors and/or anti-nutritional factors which make it difficult to use as an ingredient in foods. Fermentation of plant product, such as pea protein, could thus be used to develop innovative and tasty food products. We investigated the impact of fermentation by synthetic microbial consortia (SMC) on the perception of pea protein-based gels, giving possible keys to better understand the origin of sensory perception (e.g., beany, bitter). A total of 55 strains from different microbial species (isolated from cheese or plants) were compared for their ability to: (i) grow on matrices containing 100% pea proteins or on a 50%/50% pea:milk protein, (ii) increase aroma quality and reduce sensory off-flavors; and (iii) compete against endogenous micro organisms. Based on these data, a selection of strains were possibly assembled as consortia. Several examples/projects were developed i) for SMC adapted to the pea matrix, ii) to improve sensorial properties (e.g. aroma perception) of pea-based fermented matrices, iii) to reduce bitter taste of the fermented product. In pea matrices, sensory analyses revealed that bitterness increased after fermentation, which could be due to hydrophobic amino acids resulting from protein hydrolysis, but also decreased pea note intensity. In mixed matrices, pea perception was similar whatever the SMC, whereas cheesy perception increased. Olfactometry experiments revealed that some specific "green" aroma compounds, responsible for green off-note were suppressed/reduced by fermentation. The data presented investigated to which extent, the design of SMC together with gels composition (pea gels versus mixed gels), could modulate sensorial perception and drive consumer acceptability. Fermentation with SMC, could be applied to other sustainable sources of food protein to generate a new generation and more diversified fermented products with target functionalities (e.g., improved taste and digestibility).

O1.2 Metagenomics unravel the microbial heritage of Italian typical fermented foods

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In modern times, many traditional fermentation technologies have been industrialized, relying on the use of selected microbial starters to standardize the process and the fermented food properties. However, this leads to the global diffusion of few microbial strains and to a huge decrease of the microbial diversity in fermented foods. The project FOODMICROHERITAGE has the goal to characterize the microbiome of typical Italian fermented foods by using shotgun metagenome sequencing and to create a collection of microbial genomes of lactic acid bacteria (LAB) species and strains from artisanal fermented foods, to preserve and depict the microbial diversity of these traditional products. More than 200 Italian fermented foods were collected from different producers, including fresh, medium- and long-ripened cheeses and fermented meats. Metagenomics sequencing and Volatile Organic Compounds (VOCs) analysis were carried out.
We identified microbial genes involved in the production of the typical sensorial properties of each fermented food, highlighting the presence of different LAB strains in each product. Microbial profiles of each food type are unique and can be used to track fermented foods origin and production according to traditional practices.
Acknowledgements: This work was funded by a grant from the Italian Ministry of Foreign Affairs and International Cooperation to the project FOODMICROHERITAGE–Quality and authenticity protection of artisanal fermented foods through the characterization and conservation of their microbial and genetic heritage, CUP E79J21002000001.

Food Microbial Ecology

O1.3 Tetragenococcus halophilus, a contaminant of cheese production or a potential adjunct culture

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The use of starter cultures is well-established in cheese production, but even in large-scale cheese production non-starter lactic acid bacteria (NSLAB) are able to thrive in the maturing cheese and influence its quality in a desired or undesired way. Most prevailing NSLAB in hard cheeses are Lacticaseibacillus paracasei and Lactiplantibacillus plantarum. To take benefit from the NSLAB regarding flavor formation, but to avoid batch variability, selected NSLAB can be added as adjunct cultures.

The microbiota of 30 batches of Gouda cheese was investigated at different ripening times by high-throughput amplicon-based sequencing, targeting the full 16S rRNA gene, which allowed the detection of bacterial species that could have been overlooked previously due to the limited discerning power of partial 16S rRNA gene amplicon-based sequencing or the use of culture-dependent techniques solely. Additionally, free amino acids, other organic acids, biogenic amines, and volatile organic compounds were quantified. For the first time, Tetragenococcus halophilus was found as the most prevailing species in some long-ripened cheeses. Two main lineages of T. halophilus could be discerned, of which one was positively correlated with Loigolactobacillus rennini. Both LAB species were abundant in the brine bath and in the rind parts of the cheeses, confirming the brine bath as an inoculation source. Tetragenococcus halophilus was positively correlated with high concentrations of free amino acids and short-chain fatty acids, which could be beneficial for the cheese flavor, but also with high concentrations of biogenic amines, which are undesired. Especially the T. halophilus lineage that was correlated with Loil. rennini resulted in high concentrations of putrescine and cadaverine, which was associated with a bad smell and crack formation in the cheeses when the relative abundances of both species was higher than 50 %. However, the lineage of T. halophilus that was not correlated with Loil. rennini did not lead to these cracks or bad smell, suggesting that a well-chosen T. halophilus strain that lacks the ability to produce biogenic amines, could be a candidate adjunct culture.

Food Microbial Ecology

O1.4 Modeling of cleaning and sanitizing of stainless steel in dairy powder processing

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Introduction: Thermophilic sporeforming bacteria (Anoxybacillus flavithermus and Geobacillus stearothermophilus) are the main contaminants of dairy powder, reaching a concentration up to 5 log CFU/g. Spores in raw milk can resist pasteurisation, grow during the process due to favourable conditions and persist on stainless steel surfaces by forming biofilm, giving a resistance against Cleaning In Place (CIP) procedures (caustic soda and nitric acid). Purpose: The study first aimed at I) assessing A. flavithermus biofilm formation in skimmed milk and II) evaluating the resistance of these biofilms to caustic soda and nitric acid for various conditions needed to inactivate adhering vegetative cells and spores. Methods:
Firstly, 31 strains of A. flavithermus originated from French dairy powders, characterized by molecular method, were tested for their biofilm coverage on submerged stainless steel coupons after 6 hours of growth in agitated skimmed milk using fluorescent microscopy coupled with an acridine orange stain by random photography analysis. Secondly, to assess the effect of different concentrations of caustic soda and nitric acid at different temperatures and durations of treatment (22 conditions per strains), A. flavithermus biofilm formed on stainless steel microplates (6h at 55°C) were brought into contact with sodium hydroxide and nitric acid solutions. Residual adhered bacteria were enumerated with a spot-plating counting method quantifying resistant vegetative and spores cells. Results: of the 31 A. flavithermus isolates tested, 20 were biofilm producers (> 5% covering) with a maximum median coverage of 35% and 12 strains were 3D biofilm producer. High amounts of spores (up to 5.9 log spores/cm²) were obtained in 6h in biofilms. Nitric acid appears to be more effective than caustic soda against biofilms formed by vegetative cells and spores from these strains. A. flavithermus seems more resistant to high caustic soda and low nitric acid concentration as compared to G. stearothermophilus in similar conditions (data not shown). The effect of temperature and time of treatment have also been measured. Significance: Data acquisition on the impact of biocides on biofilms destruction in "real life conditions" is essential to optimize CIP treatment. This study show different behaviour among thermophilic sporeforming bacteria contaminating dairy powders.

Food Microbial Ecology

O1.5 Strain-level monitoring of functional starter cultures during cocoa fermentation processes

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Cocoa fermentation is a key step in the production of chocolate. It is traditionally performed in a spontaneous and uncontrolled way on-farm. A better control of the fermentation process can be achieved through the addition of functional starter cultures, to obtain a more homogeneous fermentation and a higher fermented dry bean quality. A series of functional starter cultures, composed of different yeast strains (Saccharomyces cerevisiae IMDO 050523, Hanseniaspora opuntiae IMDO 040108 and IMDO 020003, and Pichia kudriavzevii IMDO 020508 and IMDO 060005), together with a lactic acid bacteria (LAB) strain (Limosilactobacillus fermentum IMDO 0611222) and an acetic acid bacteria (AAB) strain (Acetobacter pasteurianus IMDO 0506386), were developed and tested during cocoa fermentation processes carried out in vessels in Costa Rica. The microbial community dynamics in the fermenting cocoa pulp-bean mass were assessed through high-throughput sequencing of the V4 region of the 16S rRNA gene (bacteria) and the internal transcribed spacer (ITS1) region (yeasts), unravelling a successive prevalence of Enterobacteria (Pantoea and Tatumella), yeasts (Hanseniaspora, Pichia, and Saccharomyces), lactic acid bacteria (Leuconostoc, Weissella, and/or Limosilactobacillus), and acetic acid bacteria (Acetobacter and/or Gluconobacter). Moreover, the resulting high-quality amplicon sequence variants (ASVs) obtained with the DADA2 R-package were analyzed in detail and aligned to the whole-genome sequences of the inoculated strains to monitor the starter culture strains throughout the fermentation processes. This analysis showed that the most abundant ASVs were identical to the corresponding genomic regions of the inoculated strains. Hence, the inoculated strains prevailed at high relative abundances during all starter culture-initiated cocoa fermentation processes performed, thereby outcompeting the background microbiota and steering these processes. Further, the inoculation of functional starter culture mixtures containing S. cerevisiae IMDO 050523 and/or P. kudriavzevii IMDO 020508 and IMDO 060005 positively influenced the metabolite and volatile organic compound compositions of both pulp and beans during the fermentation processes, providing an extra tool to monitor the effects of successful starter culture strains. In general, the consumption of carbohydrates and citric acid and the production of ethanol, acetic acid, higher alcohols, and esters were enhanced during the starter culture-initiated fermentation processes.

Food Microbial Ecology

O1.6 Fermentation of plant-based ingredients using lactic acid bacteria as an emerging alternative to dairy-based fermented products

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The market for plant-based alternatives for meat and dairy products has grown rapidly. However, the publicly available knowledge about microbial characteristics of such products is still quite limited. This also relates to the application of lactic acid bacteria (LAB), traditionally used in dairy fermentations, in the production of plant-derived fermented foods.

The aim of this study was to assess the performance of 44 LAB strains in 6 different plant-based substrates and to evaluate key properties of the strains as relevant to fermented dairy alternatives. This included growth, acidification capacity, utilisation of carbohydrate sources, production of aromatic compounds and removal or reduction of undesirable (off-flavour) compounds. In total, 22 Lactiplantibacillus plantarum and 22 Lactococcus lactis and Lactococcus cremoris strains of dairy and non-dairy origin were cultured in oat, yellow pea and fava bean protein concentrate emulsions and additionally in rice, amaranth and quinoa flour emulsions for 24-48 hours. In addition, the ability of the strains to grow on different carbon sources was investigated for 4 sugars (glucose, sucrose, raffinose and galactose) that were added to plant-based emulsions at the concentration of 1%. All analyses were conducted in a high throughput screening system.

Most of the representatives of Lactiplantibacillus plantarum, Lactococcus lactis and L. cremoris demonstrated good capacity to grow in selected plant-based substrates with higher acidification rates when sugars were added. The further aroma formation analysis (performed by GC-MS) allowed for the identification of strains with the ability to increase desirable aroma compounds such as diacetyl, acetoin and 2,3-pentanedione, and strains that are able to decrease off-flavours levels, for instance, hexanal, pentanal, and nonanal. The next stage of our work will involve matching these phenotypes with genotypes (based on the whole genome sequences of the strains).

Our findings on the many variables (e.g. strains, plant substrates, carbohydrates) in relation to measured key outcomes of the plant-based fermentations will be used to develop new algorithms and predictive tools that can support the rational selection of LAB strains and fermentation conditions to obtain fermented plant-based products with desired properties.

Food Microbial Ecology

O1.7 Sensitivity of 240 Listeria monocytogenes isolates to common industrial biocides is more dependent on the presence of soils or biofilm than on genetic determinants

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Listeria monocytogenes continues to be a problem in ready-to-eat foods. Resistance and adaptation to biocides have been proposed to contribute to the persistence of L. monocytogenes in the food processing industry.
This study aimed to determine if resistance to biocides vary among L. monocytogenes isolates. The impact of soils, biofilm, and concentrations on biocide susceptibility as well as ability to adapt to biocides were also evaluated for a representative subset of isolates.
Food related isolates of L. monocytogenes (n=240) were whole genome sequenced and assigned to sequence types (n=53) and clonal complexes (n=32). Isolates were screened for presence of known resistance genes, and experimentally tested for their minimum inhibitory concentration (MIC) to benzalkonium chloride (BC), peracetic acid (PAA), sodium hypochlorite (SH) and ethanol (ET). Biocide resistance under dirty (high organic load) and biofilm conditions and in a broth suspension test (BST) were further tested for 19 isolates, while the ability to adapt to sub-MICs of BC, PAA or SH (18 days, 15°C) was tested for 15 isolates.
MIC for PAA (63 ppm) showed no variation, while for SH (47-94 ppm) and ET (4.7-9.4 %) values differed 2-fold among isolates. For BC, an 8-fold difference (0.3-2.5 ppm) was observed. Eighty-seven of 88 BC resistant isolates (2.5 ppm) harbored known BC resistance genes. Dirty conditions increased MIC values 8 to 33-fold for BC and 8-fold for SH but not for PAA and ET. Biofilm increased minimum bactericidal concentrations 4 to 8-fold but exclusively for BC. Survival of isolates with BC resistance genes were not significantly ($p>0.05$) improved in BSTs with PAA, SH or BC. Lineage I isolates (n=7) showed significantly ($p<0.05$) better survival than lineage II isolates (n=12) when exposed to PAA (100 ppm). Adaptation to BC (4-fold MIC increase) occurred only for originally sensitive strains, while no adaptation to SH and PAA were observed.
In conclusion, L. monocytogenes isolates showed limited variation in biocide MIC values. However, lineage I isolates were less sensitive to PAA than lineage II isolates. The efficacy of SH and BC were affected by dirt and/or biofilm, demonstrating the need for proper cleaning prior to biocide use.

Food Microbial Ecology

O1.8 Survival of 400 Salmonella enterica strains isolated from Brazilian food industries to environmental perturbations

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The microbiological safety of milk powder has a central importance in public health, since it has diverse applications in the food industry, including the manufacture of infant formulas which is consumed by children, who are extremely susceptible to microbial infections. Salmonella enterica are frequently associated with foodborne diseases involving these products likely because of their ability to adapt and survive in dry foods. Here, 400 strains of Salmonella enterica, previously isolated from Brazilian food industries in different production areas along the food chain, were exposed to different environmental stress to evaluate their survival. The 400 strains were submitted to an increase milk concentration (30% and 50% dry matter), heat treatment (62°C form 0 to 1min), and desiccation (ambient air for 24h and 48h). The inactivation was evaluated using the plating method and then expressed as log10(N/N0). A first analyze showed a significant effect of strain, serovar, and environmental origins on the survival of Salmonella enterica. The obtained values were also described by a Multiple Factorial Analyze to observe the correlation between the survival to stress and intrinsic parameter of strains, as their serovar and zone of sampling. Interestingly, some serovar are more correlated to the heat and desiccation resistance and other to the high milk concentrations. Also, the strains isolated from final products are more correlated to the survival of thermal and desiccation stresses. All these data permits to increase our knowledge and highlight some parameters in link with Salmonella enterica resistance along the food plant.

O1.9 Impact of phages on the dynamics and metabolism of a model microbial community inspired from a fermented beverage

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Model microbial communities are often studied to better understand interactions and fluxes during fermentation processes. However, models taking into consideration the potential impact of bacteriophages (phages), recognized as drivers of microbial communities, are scarce. This study aimed at investigating the behavior of a model microbial community inspired from cider, an alcoholic fermented beverage, including phages, and being subjected to dysbiosis. The model microbial community was composed of three lactic acid bacteria strains belonging to the species Liquorilactobacillus mali, Leuconosctoc mesenteroides and Oenococcus oeni, and of a Saccharomyces uvarum yeast strain. Two phages were selected, targeting L. mali and Ln. mesenteroides strains. Experiments were performed in MRS broth at 25°C and 15°C during 168 and 220 hours, respectively. Dysbiosis was created by a heat shock at 50°C for 10 min applied when the community reached 105 CFU/mL before adding phages. Microbial community was monitored by enumerating phages and bacteria, and measuring pH. The consumption of sugars and the production of organic acids, ethanol and volatile compounds were also assessed by HPLC and GC-MS.

At 25°C, the community with phages (P) was closer to the control community (C) than to the community without phages (D). Bacterial levels were all around 108 UFC/mL in conditions C and P, which were characterized by high concentrations in 2-phenyl-ethanol, 3-methyl-butanol and octanoic acid. In condition D, L. mali and Ln. mesenteroides were dominant while S. uvarum and O. oeni did not exceed 105 UFC/mL. The concentration of ethyl-lactate was higher in condition D than in conditions C and P. After 220h at 15°C, condition P differed from conditions C and D, as Ln. mesenteroides was not detectable while the other strains all reached approximately the same levels (about 107-108 UFC/mL).

The current study is the first report dealing with the impact of phages on a microbial model inspired from a fermented beverage. It showed the importance of phages on microbial fluctuations and metabolism, as phages seemed to contribute to restore microbial balance after dysbiosis, depending on temperature. Getting knowledge about phage impacts in microbial models is crucial to guarantee the production of safe and sustainable foods.

Food Microbial Ecology

O1.10 Study on prophages in Lactococcus lactis strains from a complex dairy starter culture provides new insights on bacteriophage-host interaction

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Bacteriophages are commonly present in complex microbial ecosystems such as the gastrointestinal tract or fermentation mixed cultures, and are considered to play important roles in shaping the ecosystem along long-term evolution. Knowledge on bacteriophage-host interaction and co-evolution can be provided using such complex ecosystems as study models.

In the study on a microbial community originating from an artisanal cheese fermentation starter, we demonstrated that bacteriophages not only co-exist with bacteria but also are highly abundant. From the Lactococcus lactis strains representing different genetic lineages in the culture, prophages were present in all strains. Remarkably, most analyzed prophages show disruptions in different tail encoding genes, and the prophages carry up to 3 different phage defense systems per genome that are potentially functional in protecting the host from foreign phage infection. Defective, tailless phage particles were shown to be released spontaneously from these strains, and upon prophage induction up to 10-fold increases in phage release were observed. In both cases, no obvious cell lysis was observed.

Moreover, we also demonstrated that the prophage activity, and particularly the prophage-encoded holin-lysin system contributed to extracellular membrane vesicle (EV) formation in L. lactis. The tailless phage particles were released along or enclosed by the EVs without causing massive cell lysis. The mutant with prophage-encoded holin-lysin system deleted showed reduced fitness compared to the wildtype strain.

Combining all observations, we propose a novel model of bacteriophage-host interaction in L. lactis, where the defective prophages offer advantage to the bacterial host by continuously producing the prophage-encoded proteins including the phage defense systems. At the same time, the prophage-encoded holin-lysin system conceivably leads to local weakening of the cell envelop, allowing release of EVs and removal of defective phage particles without lysing the host. These findings highlight the diversity and ecological significance of bacteriophage-host interaction and co-evolution. Obtained insights can be applied to other prophage-carrying beneficial Gram-positive bacteria with important roles in fermentations and probiotic formulations, to enable desired release and delivery of cellular components with nutritional values or probiotic effects.

Food Microbial Ecology

O1.11 Klebsiella pneumoniae in the marine setting – a challenge for seafood safety?

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Klebsiella pneumoniae (Kp) can cause challenging hospital- and community acquired infections predominantly among immunocompromised individuals and is identified as one of the ESKAPE pathogens of special concern. The primary reservoirs of Kp are not well described, but the human gastrointestinal (GI) tract is recognised as a major source. Furthermore, Kp is widespread in nature and has been isolated from the terrestrial, limnic and marine environment world-wide. When found in in the sea, it can be expected that Kp could also be found on marine organisms harvested for human consumption. Kp has indeed been isolated from a variety of seafood organisms, including fish, bivalve mollusks and crustaceans. We examined 578 bivalve samples, including blue mussels, oysters and scallops, 53 fish samples, 24 sediment samples and 17 seawater samples for the presence of Kp. Kp was recovered from 16 % of bivalve samples and 41 % of the examined seawater samples, whereas no isolates were recovered from fish and sediments. Among 99 Kp isolated from bivalve mollusks and seawater, identified by MALDI-TOF-MS, 11 of the isolates were identified as K. quasipneumoniae subsp. similipenumoniae, K. quasipneumoniae subsp. quasipneumoniae, K. variicola subsp. variicola and K. quasivariicola after whole genome sequencing. We found high diversity of the Kp present in examined samples, with 50 sequence types identified. Acquired antibiotic resistance genes were identified in six Kp isolates and one K. quasipneumoniae subsp. quasipneumoniae, while ten Kp isolates carried the yersiniabactin siderophore, associated with human infections. Our work shows that Kp carrying both antibiotic resistance and virulence genes may be present in coastal waters and seafood organisms. Although not considered a classic foodborne pathogen, our findings show that Kp should be considered of relevance for seafood safety.

O1.12 Bacteriophages biodiversity and quality of natural whey starter cultures in Trentingrana cheese production

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Natural whey starters (NWSs) are traditionally used in the production of Italian long ripened hard cheeses like Trentingrana. Daily maintained in the dairy factory from whey collected at the end of the cheese-making process, NWS is mainly characterized by specific niches of thermophilic lactic acid bacteria (LABs) together with the presence of bacteriophages, or bacteria-infecting viruses. Deriving from different sources but mainly from raw milk and dairy implant facilities, bacteriophage infection of NWS's LABs represents the most common cause of slow and/or incomplete fermentation, with negatively effects on the quality or the yield of the final product. In this study we report the dynamics of bacteria and phage biodiversity focusing on NWS collected just before addition to the vat milk and cooked not acidified whey (cNAW) collected at the end of curdle cooking, obtained through a massive sampling system in six cheese factories following different NWS technologies over 1 year of production.
L. helveticus represented the main isolated species and biotype in NWS, together with L. brevis, L. paracasei, L. plantarum, and L. fermentum. Differences in bacterial richness between NWS and cNAW were mostly associated with the dairy implants, rather than the sampling period. The 120 NWS isolated phages were all able to infect L. helveticus and they all belonged to Siphoviridae and Myoviridae families, whereas no lysogenic phages were found. The majority of phages (58%) were isolated from two dairy plants, which showed the lower L. helveticus biodiversity. This findings confirmed that the lower the bacterial biodiversity, the higher could be the phage presence with the consequent loss of NWS activity and generation of a defective cheese.
In conclusion, in this study we found a possible correlation among bacterial starter biodiversity and the number of recovered phages, underlying the importance of phage control strategies in the dairy industry to protect the excellence in cheese production as for Trentingrana.

Food Microbial Ecology

O1.13 Microbial and associated contamination in South African craft ales

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Beer is the most consumed alcoholic beverage worldwide and the third most popular drink after water and tea. Craft beer can be prone to microbial contamination due to a lack of pasteurisation and microfiltration in smaller craft breweries, which can have a negative impact on the quality of the beer. Additionally, improper raw material (grain) storage can result in toxigenic fungal growth and the formation of mycotoxins, which are heat stable and can survive the brewing process. The aim of this research was to determine the level of microbial contamination in South African craft beer and detect any associated compounds that influence the flavour profile or could prove hazardous to consumer health. Beers from 30 different craft breweries with production volumes ranging from 200 to ≥2000 L were studied. Microbial analysis using conventional cultivation methods on enriched and selective media were used to differentiate between spoilage-, pathogenic bacteria and fungi. Volatile phenol compounds and mycotoxins were analysed using GC-MS and LC-MS, respectfully. Spoilage by lactic acid bacteria (LAB) was detected in 52% of the samples analysed with microbial loads ranging from 25 to 1.7×105 CFU/ml. However, main volatile off flavours compounds 4-vinylguaiacol (4-VG), 4-ethylguaiacol (4-EG) and octanoic acid did not exceed the sensory threshold limits. No coliforms, Escherichia coli or Staphylococcus aureus were detected. Beers from 5 breweries contained mould at levels of up to 135 CFU/ml, but the occurrence did not coincide with the presence of mycotoxins. Deoxynivalenol (DON) was detected in beers from 3 breweries at concentrations ranging from 5.8 and 41.86 ng/l and Fumonisin (FB1 and FB2) was detected in beers from 2 breweries at concentrations of 0,15 and 0,35 ng/l. No aflatoxin, ochratoxins, zearalenone and nivalenol were found. The presence of LAB in the production and packaging environment can be addressed by improving sanitation procedures. The presence of mycotoxins may not be cause for alarm as levels detected were well below the allowed limits for cereal-based food products in South Africa.

O1.14 Characterisation of Simple Sequence Repeat polymorphism in Metschnikowia pulcherrima genome for typing and genetic diversity analysis of wine isolates

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The yeast Metschnikowia pulcherrima is a major component of the grape-associated yeast community and holds significant technological promise in winemaking. While different molecular typing approaches have been previously established for various yeast species, none has been designed for M. pulcherrima. Here, we describe a straightforward and fast technique for molecular typing and genetic diversity evaluation of M. pulcherrima isolates based on multilocus SSR analysis. The whole genomic sequence of M. pulcherrima was carefully examined for the existence of putative informative SSRs. Sixteen microsatellite regions were initially selected and evaluated on a group of M. pulcherrima vineyard isolates. Eight of them that showed the highest discriminatory capacity were selected further for use in genetic diversity analysis of M. pulcherrima isolates associated with five distant viticultural zones in Greece. This is the first report on the characterization of SSRs in M. pulcherrima. These molecular markers can be used for the analysis of wine yeast populations.

Food Microbial Ecology

O1.15 Principal Component Analysis as a powerful tool to cluster L. monocytogenes isolated from food and food-industrial surfaces based on their physiological and 3D-biofilms features

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Listeria monocytogenes is well-known to have the capability to form biofilms and persist onto Food Processing Surfaces (FPS). They can be transferred into foodstuffs due to sporadic contacts with contaminated FPS, and then, into the population, increasing the risk of a foodborne outbreak.
In this work, 22 L. monocytogenes isolates obtained from foodstuffs and stainless steel (SS) food contact surfaces form different food processing plants, were clustered according to their monospecies biofilm physiological and three-dimensional characteristics in an attempt to classify them based on their level of risk in a given scenario.
To achieve this, a Principal Component Analysis (PCA) and subsequent clusterization was conducted to find out underlying correlations between Confocal Laser Scanning Microscope micrographs' (CLSM)-3D microstructural features (biomass, maximum thickness, average thickness, surface area, rugosity and surface to biovolume ratio), minimum inhibitory concentration (MIC) and minimum biofilm eradication concentration to benzalkonium chloride (BAC) and peracetic acid (PA), and their ability to be transferred from biofilms grown onto SS into smoked salmon.
PCA correlation circle showed the relationships between variables. Biofilm surface area and biomass were found to be positively correlated, whereas were inversely related to roughness coefficient and surface to volume ratio. Interestingly, the percentage of biofilms cells transferred to smoked salmon fillets did not correlate positively with neither the 3D-parameters obtained in the CLSM analysis nor with the resistance to disinfectants, measured by MIC, MBEC-BAC, MBEC-PAA. Moreover, roughness coefficient and surface to volume ratio seem to correlate with MBEC to BAC, which poses concerns on the general assumption that there is a direct interrelationship between structural complexity of biofilms and resistance.
Finally, PCA revealed 3 well-defined L. monocytogenes clusters: cluster 1, with strains that form coarse biofilms with high resistance to BAC; cluster 2, isolates whose biofilms cells have a high ability to transfer from stainless steel to smoked salmon; cluster 3, strains characterized by biofilms with high maximum and average thicknesses.
Overall, this study demonstrated PCA could be a valuable data analysis tool to group L. monocytogenes strains according to the potential risk associated to cross-contamination in a selected scenario.

Food Microbial Ecology

O1.16 Revealing sourdough starters microbial communities interactions by metagenome-scale metabolic modeling

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Popular ideas associate high species richness with microbial communities of traditional spontaneous sourdough starters. However, recent studies found a more modest composition, with a median of three bacterial species and one yeast species per starter. Still, current research on microbial community building and characteristics focuses on even more elementary communities of only one yeast and one bacteria species. To better understand interactions within such microbial communities, we implemented metagenome-scale modeling algorithms for the first time in this field. We constructed a cross-feeding model and analyzed the effect of the bacterial secreted metabolites on the growth rate of Saccharomyces cerevisiae. Analysis revealed that secreted acetaldehyde and acetic acid were correlated with an increase in S. cerevisiae growth rate. Indeed, the addition of external acetaldehyde to a defined growth medium confirmed the correlation. Moreover, we modeled 120 microbial sourdough starters communities, each composed of one yeast strain and three bacterial species out of the ten most common species in sourdough starters worldwide. Community modeling reveals a correlation between specific bacterial species presence and the increasing growth rate of S. cerevisiae. In addition, a similar correlation observed between the same bacterial species to the secretion of carbon dioxide by S. cerevisiae indicates a better proofing ability of the specific communities. Further analysis was done based on the bacterial consumed metabolites, which defined the growth niche within each community. The same beneficial bacterial species showed significantly different growth niches from the growth niche of S. cerevisiae, indicating a non-competitive ecological interaction. Network analysis reveals more complex food-chain interactions, as the beneficial bacteria species have a variety of secretion fluxes of amino acids, which are later consumed by S. cerevisiae. The inferred interactions patterns were validated by an extensive dough fermentation assay by building and characterizing defined sourdough microbial communities. This was uniquely done in a natural sourdough system composed of sterile flour and water over the same in silico studied communities.

Food Microbial Ecology

O1.17 Listeria monocytogenes post-outbreak management- when could a food production system be safe again?

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In cases of outbreaks, food business operators face inspections, recall actions and delisting by retailers. This could have happened to an Austrian meat processor whose products have been associated with a cluster of seven cases of listeriosis spread over the years 2015-2017. Sequencing of clinical and foodborne isolates by public health specialists raised the suspect of a single source outbreak since all strains were of MLST 155, cgMLST 1234.
Since the family-driven business was highly motivated to save their business, a crisis management scheme was applied that was agreed upon with national authorities. An end-product–based approach testing every single lot for L. monocytogenes was set into power and only negative lots were released for delivery. We combined the active food lot controls of food authorities with a Listeria environmental transmission mapping procedure. The environmental monitoring approach included 19 sampling activities during 3.5 years resulting in 1632 samples. This scheme allowed to trace and mitigate the Listeria contamination but did not jeopardize the processing of meat products. In total, 14 measures were set into power that reduced the overall Listeria occurrence after sanitation of 50-75% (sampling event I, II) to 0.0-3.8% (sampling events XIII to XIX). The outbreak-associated ST155/CT1234 clone was not detected in the third sampling event onwards but popped up during the sampling event VIII again. From then on, the outbreak clone ST155/CT1234 was no longer detected in the food business operator (FBO). We conclude that an intense combined investigation of food lots and environmental samples is needed to control the contamination dynamics. Initially public health authorities suspected contamination of the slicer, but the monitoring approach has localized the source of ST155/CT1234 outbreak source in a Schnitzel sorting machine.
Other factors leading to the contamination scenario were inadequate conveyor belt hygiene. An inadequate crate washing system and an inadequate hygiene lock led to Listeria spreading between compartments. All transmission routes could be effectively interrupted. A rout cause analysis and preventive maintenance program implemented in the FPE is mandatory for food processing facilities.

Food Microbial Ecology

O1.18 Evaluation of toxinogenic potential of Bacillus licheniformis - a prominent species in plant protein-based ingredients and dairy powders

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Bacillus licheniformis has robust growth properties and strains can produce endospores with high level heat resistances. Such spores can be predominant in milk powders, but also in plant-based proteins used to produce dairy and meat alternatives. While the organism is well known as a spoiler of heat-treated food products, it can also be the causative agent of foodborne intoxication due to the production of the surfactant lichenysin.
We evaluated lichenysin production by 11 B. licheniformis isolates and determined their growth properties in high salt (1M NaCl), 4% ethanol, at 37 or 55°C, and under aerobic and anaerobic conditions. All strains produced the stable compound lichenysin, with total levels varying greatly between strains. Production was growth phase dependent: lichenysin was not detected at cell densities <5 log CFU/ml in milk and LB, and the highest levels were found in the stationary phase of growth. Total production of lichenysin was 4–20 times higher in LB (max 36 µg/ml) than in milk, demonstrating medium effects. In addition, two-fold higher amounts were produced at 37°C than at 55°C. We determined that the concentration of lichenysin needed to reduce cell viability by 50% (IC50) was 16.6 µg/ml for Caco-2 human intestinal epithelial cells and 16.8 µg/ml for pig ileum organoids.
Depending on the strain present, the composition of the product, and the storage condition of the food, a risk of foodborne intoxication may arise if growth of B. licheniformis to high levels is supported and such product is ingested. Considering the high concentrations of these spores in new plant protein-based foods and the fact that heat treatments of such ingredients are limited (to avoid loss of protein functionality), potential safety risks related to this bacterium will be discussed.

Microbiome; factors affecting microbial diversity and physiology

O2.1 A genetic mechanism for Salmonella resistance to bacteriophage used in pathogen biocontrol in food

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There are over 2,500 serotypes of Salmonella enterica, many of which are bacterial causes of food poisoning and represent a global food safety problem. In the USA alone, there are an estimated 1.35 million infections from Salmonella each year with ~26,500 hospitalizations and 420 deaths. Numerous foods have been associated with multistate outbreaks of Salmonella including meat, poultry, eggs, fruit, salads, powdered goods and nutbutter spreads.

Many methods are being explored to combat this threat including non-thermal food processing strategies that have minimal impact on food quality. One strategy is using Salmonella specific bacteriophage that selectively infect and lyse the pathogen. FDA approved commercial cocktails are available for this purpose and use broad-host range bacteriophage as a clean label food processing aid for foods and food processing surfaces. One such phage is Felix-O1 which reportedly lyses 98.2% of Salmonella and less than 1.4% of other Enterobacteriaceae. Whilst effective, the widespread adoption of bacteriophage by the food industry is a stress that may lead to phage resistance similarly to the emergence of antibiotic resistance in medical and veterinary sectors. Mechanisms of resistance can include restriction systems, CRISPR-Cas and abortive infection systems.

To address this risk, our group tested Felix-O1 against several serotypes of Salmonella and identified strains that are lysed at high multiplicity of infection but do not have visible plaque forming units (PFUs). This suggests that some strains have mechanisms to reduce phage replication and confer levels of resistance to the bacterial population. The most resistant strains we identified were Salmonella Enteriditis strains possessing a plasmid encoding a putative type-3 phage abortive infection system. We sub-cloned the abortive infection locus and demonstrated that this conferred increased resistance to Felix-O1 through dramatically reduced phage replication. This may enhance survival of Salmonella in food processing environments that utilize Felix-O1 or other susceptible bacteriophage. Our data suggests that the food industry should exercise caution in using phage technology and that efforts should be made to overcome the emergence of phage resistance systems in Salmonella and other foodborne pathogens.

Microbiome; factors affecting microbial diversity and physiology

O2.2

Seasonality of E. Coli O157:H7 and microbiome on fresh-cut cold-stored lettuce of two cultivars with short and long shelf life

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Outbreaks of Shiga toxin-producing E. Coli infection have been linked to lettuce and show seasonal trends in major production areas of the USA. Two romaine lettuce cultivars with different shelf life were planted in fields in California. The microbiome of lettuce harvested in the spring and fall was characterized by metagenomics in soil and on plants before harvest, and on cut lettuce after processing and throughout cold storage in MAP. Additionally, E. Coli O157:H7 (EcO157) was inoculated into MAP lettuce and population sizes quantified during storage. The soil microbiome, as well as the lettuce microbiome before harvest, after processing and during storage differed by season ($P<0.001$), while the microbiome on the two lettuce cultivars only diverged during storage ($P=0.0001$). The shift in the microbiome of the variety with short shelf life was characterized by a decrease in the relative abundance of Pseudomonas spp, and an increase in that of the Erwiniaceae during storage. On the contrary, lettuce with long shelf life hosted increasing and stable proportion of Pseudomonas spp and of Erwiniaceae, respectively. EcO157 survived at higher densities over 14 days at 6°C in lettuce with short shelf life, indicating that compromised leaf tissue increases the risk to human health. Recursive partitioning analysis also revealed that the factors 'season' followed by '% decay' were the most significant contributors to EcO157 survival during cold storage. More specifically, the pathogen declined 5.6x more on spring- than fall-harvested processed lettuce. Trends in the microbiome of fresh-cut stored lettuce correlated with changes in EcO157 population sizes. This was supported by LEfSe identification of common biomarker species in microbiomes based on EcO157 survival and harvest season. In particular, higher representations of Erwinia persicina and Pantoea agglomerans were significant predictors of spring microbiomes and lower EcO157 survival, and of fall microbiomes and enhanced EcO157 survival, respectively. These results suggest that seasonal characteristics inherent to lettuce and to the microbiome it harbors, and interaction thereof, are critical aspects to further investigate regarding seasonal trends in EcO157 outbreaks associated with this commodity.

Microbiome; factors affecting microbial diversity and physiology

O2.3 A novel functional herbal tea containing probiotic Bacillus coagulans GanedenBC30: an in vitro study using the Simulator of the Human Intestinal Microbial Ecosystem (SHIME)

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This study aimed at investigating the potential of a novel functional herbal tea to act as the carrier for the probiotic Bacillus coagulans GanedenBC30. Spores markedly survived to infusion treatments and the simulation of the gastrointestinal transit only slightly affected the survival, which was 94.8 ± 2.8%. An in-depth investigation using the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) which incorporated mucin-covered microcosms was performed. Q-PCR with targeted primers demonstrated that the probiotic quickly colonized mucosal compartments and persisted after 4 days of wash out. As estimated by plate count combined with 16S rRNA sequencing, B. coagulans GanedenBC30 showed almost the same behavior in luminal compartments both during herbal tea intake and wash out. By summing the luminal and mucosal values and referring to whole volume of colon bioreactors, the content of viable cells of B. coagulans GanedenBC30 was largely above the probiotic threshold.

O2.4 Development of chitosan-coated agar-gelatin particles for probiotic delivery and targeted release in the gastrointestinal tract

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This study reports the development of a simple formulation for probiotic delivery using chitosan-coated agar-gelatin particles. The method involves the production of agar-gelatin particles by thermally treating a mixture of agar and gelatin solutions at high temperatures (121°C) and subsequently coating with chitosan. The particles were able to protect the probiotic strain Lactobacillus plantarum NCIMB 8826 during incubation for 2 hours in simulated gastric fluid (pH 2) as no statistically significant loss ($P > 0.05$) in cell concentration was observed, and also resist dissolution in simulated intestinal fluid (pH 7.2). This protection is related to the fact that the intense thermal treatment affected the physicochemical properties of agars, and resulted in the formation of a strong and tight polymer network, as indicated by the X-ray diffraction (XRD) analysis. Using an in vitro faecal batch fermentation model simulating the conditions of the distal part of the large intestine (pH 6.7-6.9), it was demonstrated by quantitative real time PCR that the majority of L. plantarum cells were released from the agar-gelatin particles within 30 to 48 hours. This novel methodology for the production of probiotic-containing particles is simpler compared to current encapsulation technologies, and has a lot of potential to be used for the controlled release of probiotics and potentially other solid bioactives in the large intestine.

Microbiome; factors affecting microbial diversity and physiology

O2.5 Investigating the stress adaptation and response of food-related pathogens to ultrasound, cold atmospheric plasma, and nisin in structured food model systems

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An increasing consumer demand exists for minimally processed foods which retain their nutritional content and sensory characteristics. Novel non-thermal processing methods (e.g. ultrasound, cold atmospheric plasma (CAP), natural antimicrobials) are of great interest as they are milder than traditional thermal methods and can maintain fresh-like food characteristics (e.g. taste, texture, nutritional content). Synergistically combining non-thermal inactivation methods also presents the potential to increase processing efficiency by acting as a hurdle for microbial growth. However, the efficacy and mechanisms of action of combined treatments remains unclear. These technologies may instead represent a mild, sub-lethal stress, resulting in stress adaptation, post-treatment survival, and potential development of antimicrobial resistance (AMR).

Most studies on the inactivation of food-related pathogens by natural antimicrobials and/or ultrasound/CAP are conducted in liquid broths, or in/on specific food products. However, many foods are solid(like) e.g. soft cheeses, meats, and studies in real foods are informative only for the specific product studied. Furthermore, cells grown as colonies on the surface of a solid(like) system experience a completely different environment to cells grown planktonically in liquid, with diffusional limitations of nutrients, oxygen and (acidic) metabolites existing at a colony level which lends to an overall stressed state. This could lead to environmental stress adaptation and subsequent cross-protection may also lead to different levels of AMR.

This work presents a fundamental study on the efficacy of ultrasound, CAP and/or nisin as individual or combined treatments on the inactivation of Listeria or E. Coli. Inactivation efficacy is observed to depend on many factors including the microbial species, ultrasound frequency, treatment time, treatment order, system structure, and growth morphology (planktonic/colony). Furthermore, enhanced inactivation was observed in systems also containing nisin, i.e. the combination acts as a hurdle for microbial growth. Mechanisms of ultrasound inactivation are suggested to be mostly physical, while CAP mechanisms and efficacy depend on the chemistry of the plasma gases formed.

This study sheds light on the combined efficacy of novel processing techniques for the inactivation of food-related pathogens in structured food models, and highlights the importance of accounting for structural effects when designing novel inactivation processes for the food industry.

Microbiome; factors affecting microbial diversity and physiology

O2.6 Early prediction of food safety & spoilage incidents using microbiome profile

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Early detection of spoilage microorganisms and foodborne pathogens is important step in controlling food safety, avoiding food recalls and foodborne outbreaks. Monitoring the microbiome profile of food products, will provide the required database and knowledge about proper and normal vs. invalid profiles of food products.
Since most foodborne pathogens have low infection dose, the regulation challenge is to identify up to one colony forming unit (CFU) in food products. To reach the required sensitivity, pathogen detection in food sample usually relies on culture-based techniques that are time-consuming. Thus, it is necessary to find ways to detect food spoilage microorganisms and pathogens at early stages.
Although many new technologies are already available for assessment of food products, they are not sensitive, fast and accurate enough to determine microbiological hazards in foods. Culture-independent techniques, based on the analysis of DNA or RNA extracted from food products, can help in overcoming these limitations.
Our research hypothesis is based on the idea that under inappropriate conditions, there will be a change in the microbial profile of the food product and thus it will be possible to detect the changes in the profile efficiently and at near real time. Genomic indicators may also detect the specific reason of the improper food production.
Hear we describe calibration of a reliable and reproducible microbiome profiling protocols of the food products that distinct between live and dead bacteria. Based on the microbiome profile we have developed epidemiologic tools to identify the sources of contaminating bacteria and identify bacteria that serve as indicators of food spoilage or foodborne pathogens. The developed q-PCR tests for these bacterial indictors will serve as rapid early detection system for possible events of foodborne pathogens and food spoilage bacteria.

O2.7 How Listeria monocytogenes CC121 strains successfully adapt to the food processing environment

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L. monocytogenes isolates have a high variability regarding prevalence, pathogenicity and stress response. They can be grouped into clonal complexes (CC) based on multilocus sequence typing. CC121 is one of the most abundant CC, predominately isolated from food and food processing environment. There is evidence that CC121 strains are able to survive for months and even years in food-producing environment.
We have performed whole genome sequencing and analysis of 70 CC121 strains from different origin and identified several genetic feature essential for the survival of CC121 strains in the food-processing environment.
First, the transposon Tn6188, which is integrated chromosomally and consists of three transposase genes and genes encoding a transcriptional regulator and QacH, a small multidrug resistance protein family transporter. We showed that the presence of QacH confers tolerance to quaternary ammonium compounds like other efflux pumps. Secondly, we characterized the stress survival islet 2 (SSI-2), consisting of a transcriptional regulator lin0464 and a PfPI protease lin0465. The PfpI protease is involved in alkaline and oxidative stress responses, but not in acidic, gastric, heat, cold, osmotic, and antibiotic stresses. In parallel, deletion of lin0464 decreased expression of the PfpI protease and subsequently survival under alkaline and oxidative stresses. L. monocytogenes faces alkaline and oxidative stress during cleaning and sanitation procedures in the food-processing environment. Third we discovered a 12.5 kbp insertion in CC121 genomes that harbors a 3056 amino acid protein with rearrangement hotspot (Rhs) repeats and have named it Rhs insertion. Rhs proteins have been described to be involved in intercellular competition by inhibiting growth of neighbouring cells similar to toxins. We showed that expression of the Rhs C-terminus in Escherichia coli inhibited growth. These data suggest that the Rhs insertion supports the survival and growth of CC121 strains by inhibiting growth of neighbouring cells.
In conclusion, we demonstrated that CC121 harbor specific genetic feature supporting the adaption to the food processing environment.

Microbiome; factors affecting microbial diversity and physiology

O2.8 A point mutation in ribosomal gene rpsU enables SigB activation independently of stressosome and SigB regulator RsbV in Listeria monocytogenes

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Microbial population heterogeneity leads to different stress response and growth behaviour between individual cells in a population. Previously, a point mutation in the rpsU gene (rpsU-G50C) has been identified in Listeria monocytogenes LO28 variant 15, which leads to higher stress resistance and lower specific growth rate. In L. monocytogenes, the general stress response is regulated by the alternative sigma factor SigB and the activation of SigB is controlled by a series of Rsb proteins. Within these Rsb proteins, RsbR is a member of stressosome and responsible for stress signal detection. When a stress signal is detected, RsbV can bind with anti-sigma factor RsbW and release the SigB. The released SigB is activated and able to trigger the expression of many stress response genes. In the current study, the rpsU-G50C mutation was introduced to L. monocytogenes EGDe wild type and ΔsigB, ΔrsbV and ΔrsbR mutants. We combined the phenotyping and proteomics approach to investigate the acid and heat stress resistance as well as the growth rate of these mutants. As expected, the introduction of the rpsU-G50C in the ΔsigB mutant did not trigger a SigB-mediated robustness increase. However, the introduction of rpsU-G50C in the ΔrsbV and ΔrsbR mutants resulted in activation of SigB and its regulon members and concomitant increased robustness, indicating that SigB is not activated through this signalling pathway. However, all the rpsU-G50C mutants always had lower fitness even with the deletion of SigB, RsbV and RsbR. Therefore, the increasing stress resistance of rpsU-G50C mutants was due to the activation of SigB by an unknown mechanism which was different from the classical SigB activation model. The fitness changing of rpsU-G50C mutants was independent of the SigB and may be due to the downregulation of ribosomal proteins.

Microbiome; factors affecting microbial diversity and physiology

O2.9 Patient similarity networks of the gut microbiota as a personalized strategy to study obesity

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Background: The World Health Organization recognized obesity as a global epidemic in 2000. Up to 39% of adults aged 18 years and over were overweight in 2016, and 13% were obese. There is growing evidence suggesting that gut microbiota is a potential factor involved in the pathophysiology of obesity and related metabolic disorders. The construction of patient similarity networks integrating gut microbiota composition and the patients' nutritional habits may help develop personalized strategies to prevent obesity.
Methods: We analyzed the gut microbiota of 70 patients aged 21-58 years participating in the citizen science project #Pictureyourmicrobes (https://bit.ly/3wKuRYt), which consisted of a photovoice activity (https://bit.ly/3LrUHEF) accompanied by self-reported nutritional questionnaires and stool sample collection for microbial profiling through sequencing the 16S rRNA gene. Relative abundance of each ASV, alpha, and beta diversity were calculated. A patient's network was constructed using Aitchinson distance of the microbiome with the NetComi package, and network properties were analyzed. Nutritional data were analyzed to obtain information about macro and micronutrients.
Results: The resulting similarity network has two components. We applied knn clustering and found two main clusters, including 95.6% nodes. "Cluster 1" was characterized by higher BMI, waist circumference, and lower microbial alpha diversity ($P < 0.01$). "Cluster 2" showed a differential abundance in genera previously associated with weight loss (Akkermansia spp., Blautia spp), SCFA producers (Eubacterium spp.), or hepatoprotective effect (Ruminococcaceae UCG-010). We did not observe statistical differences in macro or micronutrient consumption regarding nutritional information. However, the healthy eating index was lower in "Cluster 1" patients (P = ns). We found an overall low consumption of fermented foods; only 10% of patients of both groups consumed kefir, kimchi, sauerkraut, or miso in a frequency greater than once a month. Finally, we evaluated the learning after photovoice activity, and participants increased their microbiome-related knowledge by 0.72 points ($P < 0.001$).
Conclusions: Photovoice contributed to adopting new healthy habits, including the consumption of probiotics and prebiotic foods. The constructed similarity network allowed us to discriminate between subgroups of patients without previous selection based on their gut microbiome.
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Microbiome; factors affecting microbial diversity and physiology

O2.10 Investigating plasmid uptake in Salmonella enterica in the presence of gastrointestinal stressors

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Salmonella enterica subspecies enterica serovars have a vast distribution across humans, animals, and the environment. Even within the serovar, members of S. enterica subsp. enterica vary greatly in virulence, host range, and genetic composition and individual strains within a serovar may differ from each other by the presence or absence of hundreds of genes. Due to their wide host range, propensity for horizontal gene transfer and environmental ubiquity, bacteria belonging to S. enterica subsp. enterica are critical components to gene transmission between bacteria of both the same and different serovars. Additionally, transfer of genes between Salmonella and commensal gut microbiota can potentially facilitate the spread of virulence or antimicrobial resistance (AMR) genes within the gastrointestinal tract microbial community.

Our understanding of how different abiotic and biotic factors of the gastrointestinal tract affect conjugation frequency is incomplete. By understanding how S. enterica subsp. enterica reacts under gastrointestinal stress, we may gain insight into what triggers conjugation events in the human gut microbiome. The stressors of the human gastrointestinal tract were modeled via acid shock (t = 1 hour, pH 3) and bile salt (2% weight/volume) challenges, as well as assays including exposure to both stressors, to simulate the passage of recipient S. enterica throughout the digestive process. The plasmid uptake of S. enterica under stress conditions was determined. Upon completion of the in vitro conjugation assays, conjugations were performed in the presence of a microbiota grown within a Simulated Human Intestinal Microbial Ecosystem (SHIME). We determined that S. enterica's response to gastrointestinal stressors, and S. enterica's ability as a recipient, were strain specific. Furthermore, the plasmid uptake of S. enterica seemed to increase after acid shocking.

By understanding how S. enterica subsp. enterica reacts under environmental stress, we may gain insight into what triggers conjugation events in the human gut microbiome. Gaining a better understanding of plasmid transmission dynamics in a complex ecosystem that is a mixing pot of different microbes, as well as part of a transient system, can help future researchers in making informed decisions on the topic of AMR.

Microbiome; factors affecting microbial diversity and physiology

O2.11 Impact of oligosaccharides from olive tree by-products on human gut microbiota

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Spain is the world's largest producer of olive oil. This agricultural production generates a high amount of waste so it is essential to carry out an efficient management of this waste. An example of a sustainable economy is the integral valorisation of these natural resources over-generated during industrial activities, producing high value-added compounds to develop functional food ingredients with benefits for human health.

The aim of this study was to evaluate the prebiotic potential of oligosaccharides (OS) from olive tree pruning (OTP) through fermentation by human gut microbiota.

OS were obtained from autohydrolysis from OTP biomass. The purified OS and human faeces were mixed, and human microbiota fermentation took place at 37 °C for 48 h. From the mixture, samples were taken at 0, 12, 24 and 48 h of fermentation to understand the microorganisms growth. All experiments were carried out inside an anaerobic cabinet. For the evaluation of in vitro human gut microbiota profile groups (Bifidobacterium spp., Lactobacillus spp., Clostridium Leptum and Prevotella spp.), genomic DNA was extracted and purified from stool samples using NZY Tissue gDNA Isolation Kit. The DNA purity and quantification were assessed with a NanoDrop spectrophotometer. Real-time PCR was performed in sealed 96-well microplates using a LightCycler FastStart DNA Master SYBR Green Kit and a LightCycler Instrument. Fructooligosaccharides (FOS) (Orafti® P95) were used as positive control.

In this work, FOS promoted the growth in all species (Bifidobacterium spp., Lactobacillus spp., Clostridium Leptum and Prevotella spp.). The relative differences to control of the OS showed positive effect upon Prevotella spp., Clostridium Leptum and Bifidobacterium spp. species, meaning that the OS tested could act as prebiotic. On the other hand, OS presented a negative effect for Lactobacillus spp. The most evident initial growth was obtained for Bifidobacterium spp., which showed an effect of ca. 18% after 24 h, and a slight increase after 48 h of fermentation up to ca. 22%.

The results demonstrated that OS from OTP promotes a positive effect on the growth of various gut microbiota species which could exert different health-related benefits. Thus, OS could be incorporated in food matrices as functional ingredient.

Microbiome; factors affecting microbial diversity and physiology

O2.12 Microbiome research as connecting force to link microbial dynamics across the food chain to food safety

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In food production, the microbial landscape is shaped by the source, facility characteristics and both natural and human interventions. Microbial dynamics in food are of utmost importance, because they influence product properties including shelf life and susceptibility to spoilage. It is estimated that 1.3 billion tons of food are discarded worldwide each year, equivalent to 30% of food products in primary processing. This food loss is mainly caused by microbial spoilage in primary processing due to re-contamination events. Here, we used an integrative approach along a meat processing line to sample the hygiene status from slaughter animals, which were followed along the slaughter- and cutting process up to the final products. In addition, we sampled personnel, equipment, machines and the facility environment and applied high-throughput full-length 16S rRNA gene sequencing for a subsequent source tracker approach to link specific taxa to particular environmental sources. A facility-specific transmission map of bacterial flows was created, indicating microbial transmission at all major production steps. Furthermore, we found autochthonous fungi to act as central components in microbial community structure in meat-associated end products and we will now use these co-occurrence patterns for metabolic interaction experiments between defined functional groups.

We further aimed to increase the resolution at which we can identify specific populations that are responsible for food spoilage. Towards this, we have applied a reverse ecology approach leveraging genomes and metatranscriptomes to show that animal-associated bacteria sharing an identical full-length 16S rRNA gene have distinct adaptions involving biofilm formation and core metabolism. Our results suggest that sequencing based approaches have great potential to contribute to food monitoring applications and that future efforts should continue to increase the resolution at which we identity and target contaminating microorganisms.

Microbiome; factors affecting microbial diversity and physiology

O2.13 Optimization of the in vitro model of gut microbiota culture and application to toxicomicrobiomics studies

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Gut microbiota (GM) play a vital role in maintaining human health. One of the important factors which can largely contribute to GM dysbiosis is chronic exposure to food contaminants such as bisphenols and phthalates which can result in structural and functional alternations of GM and can be a missing link between xenobiotics and microbiome-related human diseases. Therefore, there is now a great need to develop and optimize methods of in vitro culturing human GM to utilize it in a comprehensive toxicity evaluation of environmental xenobiotics and food additives. The use of in vitro models can provide a time- and cost-effective insight into microbiome responses and be a preliminary study providing guidance for further in vivo research.
Therefore, the objective of this study was to assess the potential of four commonly used microbial culture media (GMM, FM, SB, CFBM) to preserve the biodiversity and metabolic activity in in vitro GM batch cultures (24h) for future application in toxicomicrobiomics research. We also investigated application of individual or pooled frozen fecal samples from healthy donors (n = 15) as inoculum for fermentation studies to reduce number of variables and ensure reproducibility of tests. Herein, we applied omics technics such as 16S rDNA metagenomic sequencing and LCHR-MS/MS untargeted metabolomics supplemented with qPCR viability assessment and GC-MS derived SCFA profiling. Medium characterized by the highest parameters such as □ diversity, SCFA production and viability was utilized in toxicomicrobiomic preliminary evaluation of bisphenol A (BPA) in wide range of concentrations (10-5M – 10-11M). The 16S rDNA sequencing showed significant difference (P □ 0.05) in □ diversity and reduction in SCFAs concentrations in GM post fermentation fluids. Untargeted metabolome profiling revealed global impact of BPA treatment on gut metabolome. After 24h of GM culturing with different BPA doses, quantity analysis showed significant reduction of BPA concentration. Which indicates multidirectional effect of BPA on human GM.
Our workflow was successfully applied in study regarding BPA- induced changes of GM and enables extensive insight into xenobiotic-microbiome interactions, ensures reproducible results and high throughput which is essential for toxicomicrobiomics research.

Microbiome; factors affecting microbial diversity and physiology

O2.14 Pseudomonas fragi biofilm improves Campylobacter jejuni stress response in aerobic conditions

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Pseudomonas biofilm plays an important role in the spoilage of protein-rich foods and as a source of contamination on food-processing surfaces. This genus is characterized by extremely good biofilm formation on different surfaces, even under stressful conditions with low temperatures to which they are exposed in food-related environments. The Pseudomonas biofilm can thus create a new niche for other microorganisms that do not form abundant biofilms under these conditions, but are present in this environment. These include pathogenic bacteria such as Campylobacter jejuni – the causative agent of the most commonly reported zoonosis in the EU. In our work, we focused on the interaction between Pseudomonas fragi and Campylobacter jejuni in biofilms under different conditions, including those mimicking food-related environments. P. fragi enhanced the survivability and cultivability of C. jejuni in a wide range of temperatures, atmospheric conditions, and surfaces. In evaluating this specific interaction of P. fragi with C. jejuni in biofilm, we further examined the functional changes in C. jejuni induced by this interaction. To this end, we used a proteomic approach to compare the protein profile of C. jejuni biofilm alone with the protein profile of C. jejuni in a mixed biofilm with P. fragi. The results showed that C. jejuni in the mixed biofilm under aerobic conditions had a lower presence of proteins in biological processes crucial for stress response, such as the formation of iron-sulfur cluster assembly (NifU), translational elongation (FusA, Tu), cellular detoxification of oxidants (AhpC), refolding of damaged proteins (GroEL) and stabilisation of DNA to prevent its denaturation under extreme environmental conditions (HU). P. fragi biofilm thus enables better stress response of C. jejuni under aerobic conditions, which in turn can influence their survival in the biofilm. Thus, in order to successfully control these pathogenic bacteria, we must also have a good understanding of their interaction with bacteria ubiquitous in the food-related environment, such as Pseudomonas spp. which play a critical role in the survival of C. jejuni under stress conditions.

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Studying and enlightening food microbial ecosystem and microbiome

O3.1 Towards new strategies for extending the ground beef shelf-life: the cases of electrolysed water, bioprotective cultures and metataxonomic-based surveillance

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Ground beef is a perishable product with a shelf-life that varies in relation to the initial contamination level and microbiota composition. Meat industries are therefore seeking for new productive approaches capable to limit product losses through a shelf-life maximisation.

We tested on site the applicability of electrolysed water (EW; 100 ppm of free-chlorine) prior to grinding and autochthonous bioprotective bacteria (ABB) in ground beef. The impact of these antimicrobial strategies has been monitored by culture-based approach, sensory tests, metataxonomic and volatilomic analyses. More than a hundred metataxonomic profiles were collected from carcases after slaughtering up to the end of shelf-life and beyond. Genomic characterisation of the ABB was also conducted to decipher its antimicrobial mechanism.

Pre-grinding immersion of meat trimmings in EW has been ineffective and the following spoilage evolution varied among production runs. Discrimination according to the origin has been further observed within the same production run by metataxonomic analysis in carcases and ground beef, while microbiological and physical-chemical analysis did not show this fine discriminatory capability. Metataxonomic signatures of a faster spoilage tendency were identified from the original carcases until the late stages of vacuum storage, while manipulation phases before packaging determined only transient modifications in the ground beef microbiota. These recurrent signatures were the high □-diversity and co-occurrence of Carnobacterium and Pseudomonas. Moreover, Lactococcus (Lc.) piscium development and the related acetoin production were identified as the main shelf-life endpoint indicators in ground beef, while other Lactococcus, dominant in the early stages of vacuum storage, showed the capability to inhibit consortia of spoilage bacteria in vitro. Accordingly, a strain of Lc. lactis subsp. hordniae has been tested as ABB in ground beef. However, despite possessing genes encoding for several antimicrobial peptides and inhibiting Listeria monocytogenes growth in liquid substrate, it did not prevent or slow the spoilage in this solid product.

To conclude, both EW treatment and ABB adjunction apparently did not prolong the ground beef shelf life. Notably, metataxonomic-based profiling in the early stages of production might represent an effective approach to sharply discriminate between batches with faster or slower spoilage tendency.

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O3.2 DNA-based tools for authentication of Greek PDO "avgotaracho Mesolonghiou"

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Food traceability and authentication had become mandatory for food industry and global food trade. Numerous DNA based methods could contribute against food frauds, because of their advantages such as simplicity, accuracy and robustness. The aim of this study was to explore whether unique biological markers of a high valuable and popular Greek PDO (Protected Designation of Origin), avgotaracho Mesolonghiou" product could be indicated. The traditional avgotaracho (striped grey mullet fish roe) produced in Mesolonghi lagoon, represent an important food product with a significant need for analytical approaches that determine its geographical origin and guarantee products' authentication. The eggs from fishes (commonly referred as roe) caught in the Mesolonghi lagoon are known with the trade name "avgotaracho Mesolonghiou". The raw material of the product is derived from flathead mullet caught in Mesolonghi. Production, process and preparation of the final product is completed at the same region. Therefore, PCR-RAPD, PCR-RFLP and emulsion-PCR-DGGE were performed for Greek PDO product and potential biological markers were explored, based on either genomic DNA or bacteria communities. Band profiles resulted in molecular techniques, could be used as a "barcode" to certify the origin and authenticity of PDO product. In summary, this was the first study to successfully applied DNA-based methods for Greek PDO (Avgotaracho Mesolonghiou) traceability and authentication. Considering the results of this study, the adoption of DNA-based tools seems to be very promising for determination of food geographical origin or authentication among different samples. In terms of global food trade, the implementation of these techniques in food industry or in quality authority's laboratories could provide a great impact regarding quality schemes, food labeling, food safety, and food fraud incidents. Further DNA analysis and application to a variety of foodstuff in a large scale, may be the proper solution to manage adulterations and to strengthen consumers' confidence, as well.

Studying and enlightening food microbial ecosystem and microbiome

O3.3 Whole genome sequencing (WGS) and PCR-RFLP characterisation of Listeria monocytogenes isolates from ready-to-eat (RTE) hummus, fresh produce and the food processing environment (FPE) in South Africa

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Listeria monocytogenes is ubiquitous in agricultural environments and persistent in food processing facilities. Consequently, a variety of foods are contaminated and become serious health risks to immunocompromised consumers. The listeriosis outbreak experienced in 2017-2018 in South Africa (SA) was evidence of this. Different L. monocytogenes sub-types are associated with varying degrees of virulence, which influences their ability to cause listeriosis. Collecting surveillance data for foods less studied supports a precautionary approach to food safety; lack of awareness could have severe consequences to public health. The main objective of this study was to investigate the genetic diversity of L. monocytogenes from RTE hummus, fresh produce and the FPE in SA (2018-2021). PCR-restriction fragment length polymorphism (RFLP) lineage typing was performed for 60 isolates. A sub-set of 20 isolates was selected for WGS, using both Illumina and Ion Torrent technologies. The web server MLST-2.0 was used to identify sequence types (STs), and serotypes were inferred thereafter. A phylogenetic tree was created using the NDTree tool. Isolates analysed were found to be of two lineage types (I, II), three serotypes (1/2a, 1/2b, 4b) and seven STs (ST1, ST2, ST3, ST5, ST101, ST121, ST204). Lineage I was prevalent overall, and significantly associated with fresh produce (p=0.04). Both serotypes 1/2a and 1/2b were found in all three categories, while 4b (the most virulent serotype)(ST1 and ST2) was only recovered from fresh produce. ST204 (38%) was most prevalent in the FPE, ST5 (50%) in fresh produce, and ST5 (33%), ST101 (33%) and ST121 (33%) in RTE hummus. This sort of genetic profiling is very limited for L. monocytogenes in SA, especially from the food groups of interest. To the author's knowledge, these results constitute the first findings of their kind for RTE hummus. Contrary to global trends, lineage I is overrepresented in the SA food industry. L. monocytogenes ST1 and ST2 are considered emerging strains of concern. The recovery of these sub-types, as well as others associated with clinical listeriosis, is worrying and worth monitoring. This study emphasizes the need to research more novel foods to reduce the burden of foodborne disease.

Studying and enlightening food microbial ecosystem and microbiome

O3.4

Metatranscriptomics and metabolic modeling to identify bacterial metabolic interactions during the manufacture of a model pressed cheese

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In cheese production, lactic acid bacteria (LAB) and propionic bacteria are key players in the production of metabolites that confer nutritional and organoleptic qualities. However, the contribution of each species to the final quality of cheeses is not fully elucidated. The objective was to determine which species contribute to acidify and produce flavour compounds, by which metabolic pathways, according to which temporality and also to investigate the bacterial metabolic interactions contributing to the functioning of the cheese ecosystem.

We sequenced and annotated: Lactococcus lactis subsp. lactis biovar diacetylactis CIRM-BIA1206 (LL), Lactiplantibacillus plantarum CIRM-BIA465 (LP), Propionibacterium freudenreichii CIRM-BIA122 (PF). We reconstructed the metabolic pathways and developed a community metabolic model. Four semi-hard cheeses were made with LL, LP and PF and were analyzed throughout manufacturing. Bacteria, sugars, organic acids and flavour compounds were quantified and RNA sequenced.

The analysis of differentially expressed genes showed at which moment of the manufacturing process the genes involved in the catabolism of lactose and citrate and in the synthesis pathways of different flavour compounds: lactic, acetic, propionic, isovaleric (old cheese flavour) acids, diacetyl (buttery flavour) were induced. Lactose catabolism was performed by LL via the tagatose pathway during acidification and then via the Leloir pathway and only via this last pathway by LP. Diacetyl production was carried out by LL from the beginning of the manufacturing process and then moderately by LP. The synthesis of propionic and isovaleric acids were attributed to PF and the corresponding metabolisms were induced during the ripening step. Since the mixed fermentation pathways were induced during ripening, acetic acid was likely produced by LL, LP and PF at this stage. Implementation of the metabolic community model in the Smetana tool revealed the molecular basis of the previously discussed commensalism between LAB and PF. LAB produce lactic acid and potentially glycerol, serine and phenylalanine for the benefit of PF. These interactions identified in silico remain to be validated in vitro.

All these results make metatranscriptomics associated with metabolic models, tools of choice to better understand and control the metabolisms and interactions governing the functioning of cheese ecosystems.

Studying and enlightening food microbial ecosystem and microbiome

O3.5 The proteolytic profiles of lactic acid bacteria drive positive interactions in co-culture in a food-mimicking medium

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Microbial interactions govern natural and artificial environments. So-called positive interactions result in the fitness enhancement of at least one community member without harming the others. Positive interactions between microorganisms in co-culture are accompanied with an increase in the production or degradation of targeted molecules, as observed in fermented food products such as sourdough, kefir, and yogurt. In these products, the available peptides and free amino acids (FAA) are involved in the interactions but the effect of their nature has not been studied yet. This study aims to investigate how different proteolytic (donor) lactic acid bacteria (LAB) strains can stimulate or not the growth of non-proteolytic (receiving) LAB strains in a food-like medium. An increase in organic acids and aroma compounds and a decrease in sugars inducing intestinal discomfort are expected with the co-cultures compared to the mono-cultures of donor strains.

A synthetic medium, mimicking a food product, was first developed in order to provide all the necessary nutrients, but proteins as sole nitrogen source. It only allowed the growth of the proteolytic strains. Three donor strains were selected with distinct proteolytic profiles, in terms of released FAA and peptides, and overall degree of proteolysis. Nine pairs of donor/receiver strains of different species (Enterococcus faecalis, Lactococcus lactis, and Lactobacillus plantarum) were co-cultured in compartment chambers that physically separated the bacteria, and in direct co-cultures. LAB growth and metabolism was compared in co-cultures and mono-cultures in terms of bacterial counts, acidification rates, amount in FAA and peptides, residual sugars, and volatile compounds.

Receiving strains grew differentially depending on the donor strains they were co-cultured with. The results suggest that the changes in growth of the receiving strain may be influenced by the size, nature, and/or concentration of nitrogen compounds produced by the donor strain. The co-cultures presenting positive interactions showed higher acidification rates, decreased final concentrations of peptides, FAA and residual sugars.

This study gives insight into the mechanisms that rule microbial interactions in fermented food products. It also gives strategic knowledge on how to assemble novel bacterial communities that gather multiple targeted functions, leading to better or new food products.

Studying and enlightening food microbial ecosystem and microbiome

O3.6 Predicting virulence of Listeria monocytogenes using whole genome sequencing and machine learning

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Listeria monocytogenes (LM) is among the most concerning food-borne pathogens and poses a substantial public health threat. Hence, many countries have rigorous regulations for LM in food products. These regulations assume that the virulence (i.e., harmfulness) of LM is constant on a species level. In contrast, recent research has found major differences in LM virulence on a sub-species level. Currently, most of the detection methods used in the food industry cannot resolve beyond the species level. However, the emerging shift in the industry to use Whole Genome Sequencing (WGS) enables resolution to sub-species levels. WGS has already become the standard for pathogen surveillance in some countries, and thorough screening networks combining clinical and food industry data have been implemented. Our study aims to harness these networks and unravel LM virulence even further. To do this, we are combining state-of-the-art Machine Learning (ML) techniques with WGS data to predict LM virulence on a sub-species level.

The data used in this study was obtained from two exhaustive surveillance systems of LM conducted by Danish and French authorities. After filtering the data for isolate clones, 115 data points remained. We used the clinical frequency (number of clinical isolates/ (number of clinical isolates + number of food isolates)) as the best estimate for virulence. This study compares the two-layer cross-validation performance of three different genomic levels (i.e., virulence genes, pangenome genes, kmers) and two aligners (i.e., blast, kma). The preliminary results suggest that a broader genomic level (i.e., pangenome) yields better predictive performances (F1-score: 0.88; 95%-CI: 0.87, 0.91).

In conclusion, the study suggests that exhaustive WGS surveillance data can be used to predict LM virulence on a sub-species level. Unfortunately, the findings are limited by the sparsity of well-suited data. Hence, we are exploring the possibility of using other publicly available datasets for our methodology. In the future, the ML algorithm will be part of an online tool to predict virulence and disinfectant resistance in LM.

Studying and enlightening food microbial ecosystem and microbiome

O3.7 Investigation on prevalence of living archaea community in milk and clams

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Archaea are defined as prokaryotic organisms, different from bacteria in structural and molecular aspects. They are mainly known for their living in extreme environments. Culture-independent molecular methods have been already revealed the sequences of archaeal DNA in various types of food matrixes, in particular highly salted/fermented ones. However, few studies focused on isolation of living archaea in the other food products, respect to the difficulties in providing special requirements for growing. The present study aims to investigate living archaea in food samples. Our previous data obtained by 16S rRNA gene NGS screening in different food matrixes (cheese brine, milk, cheese, honey, hamburger, and trout) confirmed the presence of different genera of archaea in the samples. Two matrixes (milk and clams) that showed the higher reads of archaea were selected for investigation of living archaea by microbial culture methods. In total, DNA of 30 samples of cultures obtained from different incubation media and conditions were sequenced by using 16S rRNA NGS sequencing. Taxonomical assignment was obtained by using Kraken software against archaeal genome database. Most of the reads were obtained after 30 days of incubation at 22°C. Despite of obtaining low number of reads for both matrixes, 9 and 24 distinct genera of archaea were identified in milk and clams, respectively. Among the genera, Halostagnicola and Nitrosopumilus were identified with the maximum abundance in milk (41%) and clams (46.25%), respectively. Interestingly, the frequent isolates of archaea (Halorussus and Halostagnicola) in milk were belonged to a family of, which describes as extreme halophiles.
The results of this study proved the survival of archaea in foods other than salted/fermented ones and the growth in different media. Despite of detection of various archaea in human intestinal microbiome, their possible role on human's intestinal health has been underestimated until now. Therefore, further knowledges are required to look for their presence in other food matrix in order to understand their possible vertical transmission associated with human's health.

Studying and enlightening food microbial ecosystem and microbiome

O3.9

Fate of Salmonella enterica in tahini (sesame paste) products

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Sesame seed-based products have been increasingly linked to recalls due to the presence of the pathogenic bacterium Salmonella enterica as well as to salmonellosis outbreaks. The objective of this study was the assessment of the pathogen's survival in different tahini (sesame paste) products. Three commercial tahini products [regular tahini (RT), tahini with honey (HT) and tahini with stevia (ST)] were inoculated with a four-strain composite of S. enterica (5-6 log CFU/g) and stored at 4 and 25°C for a total time period of 90 days. Non-inoculated samples were also stored under the same conditions. At regular time intervals, duplicate samples (i.e. distinct product jars) were analysed, and S. enterica populations were determined on both selective (XLD agar) and non-selective (tryptone soy agar) media for the enumeration of "healthy" and total ("healthy" and "injured") bacterial populations, respectively. The pH and water activity (aw) of the products also were monitored during storage. The survival of S. enterica appeared to be affected by both the product type and the storage temperature. Overall, the organism exhibited a better survival potential at 4°C than at 25°C, with the highest inactivation, however, being observed at both temperatures in RT. The mean (± standard deviation) population reduction (log CFU/g) after 90 days of storage at 4°C was 1.54 (± 0.70), 0.39 (± 0.06) and 0.49 (± 0.01) in RT, HT and ST, respectively, whereas the corresponding reductions at 25°C were 2.23 (± 0.82), 1.69 (± 0.36) and 0.70 (± 0.04). A remarkably higher survival of S. enterica was specifically observed during storage at 25°C in ST as compared to the other two tested products, along with a correspondingly lower level of "injury" as demonstrated by the comparative evaluation of the populations recovered on the selective and non-selective media. The survival behavior of S. enterica could not be explained in terms of pH or aw of the tested products, with the observed differences being potentially associated with parameters such as food matrix composition and structure. Although further research is required, the collected data should be useful in microbiological risk assessment regarding tahini and tahini-based products.

Studying and enlightening food microbial ecosystem and microbiome

O3.10 Exploring associations between metataxonomic profiles and pathogen presence along infant food production chain

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Food industries have not yet ceased the challenge conducted by pathogens that tend to persist after diverse stages of the process line. This critical issue is fundamental for infant food industries since their end users belong to a group of the population that has not yet thoroughly developed its immune system. Therefore, the presence of foodborne pathogens can cause severe illness and death on infants.

This study aimed to investigate presumable correlations among metataxonomic analysis and pathogens prevalence, through enrichment, isolation and molecular approaches.

Hundreds of samples were collected during various stages in the production of infant food in a commercial facility. Samples included environmental swabs, raw material, intermediate and final products. On every sample, 16S rRNA amplicon - based sequencing has been performed and Amplicon Sequence Variants (ASVs) distribution was examined thoroughly. Furthermore, the presence of Listeria monocytogenes, Bacillus cereus, Salmonella spp. Staphylococcus aureus and Clostridium perfringens was inspected prior and subsequent to twenty - four hours of enrichment, whereupon pathogens isolation, species specific PCR and Real - Time PCR were performed.

Heterogeneous bacterial communities were observed between food samples in relation to their composition, while in the case of the environmental samples a clear segregation was not easily discerned. Metataxonomic analysis seems not sufficient to directly detect low abundant microbial taxa, like the cases of pathogens. Nevertheless, identification of pathogens could feasibly be reached in cases of samples with high biodiversity, carrying narrow bacterial concentration. The presence of low abundance microorganisms should not be underestimated; because within them could be found new emerging microorganisms with potentiality in pathogenesis. This does not exclude the possibility to correlate specific metataxonomic profiles with potential pathogen's presence.

Our outcomes suggest that metataxonomic and other multi - omics approaches could be contributing elements in understanding and possibly predicting the distribution of pathogens (in time and space) during food processing.

Studying and enlightening food microbial ecosystem and microbiome

O3.11 Characterisation of a large group of Listeria monocytogenes using whole genome sequencing; assessing their virulence and persistence potential

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Listeria monocytogenes is the etiological agent of Listeriosis, a foodborne illness associated with high hospitalisation and mortality rates. L. monocytogenes can persist in food associated environments for years and persistent strains have been linked to outbreaks of foodborne disease with high mortality. This study aimed to improve food-borne subtyping and identification of persistent and virulent L. monocytogenes in food associated environment through the application of whole genome sequencing (WGS). In total, WGS was applied on 274 L. monocytogenes isolates from a longitudinal study of a small food production facility, collected from 2014 to 2020. Post sequencing, MLST, cgMLST and SNP analysis were performed to characterise the isolates and detect potential persistent strains. The sequences were also screened for biocide and stress resistance genes, plasmids and prophage sequnces to further explore persistence potential. Assessment of the presence and integrity of virulence genes allowed for the determination of virulence profiles and subsequently the isolates were grouped into hypo-virulent, intermediately virulent and hyper-virulent strains. WGS can achieve more than just discrimination between unrelated isolates. An additional benefit of WGS is the opportunity to extract specific information, such as the determination of virulence, antibiotic or biocide resistance status, as well as, the assignment to serotypes. WGS is the ultimate method for characterisation of bacterial isolates as it provides the highest possible resolution in strain typing and represents a new paradigm for outbreak investigation and contamination-source tracking.
Enrolled in the PhD programme in September, 2019.

Studying and enlightening food microbial ecosystem and microbiome

O3.12 Genomic analyses of biofilm-forming and non-biofilm-forming Escherichia coli populations from meat processing environments

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Biofilms are microbial communities embedded in self-produced extracellular substances (e.g., curli and cellulose) and can cause the recalcitrant contamination of meat with pathogenic and generic Escherichia coli. The objectives of this study were to : 1). To determine the phylogenetic relationship of biofilm forming and non-biofilm forming E. Coli from various stages of beef processing; 2). To characterize genetic elements involved in biofilm formation in E. Coli. A total of 114 E. Coli isolates from beef carcasses before hide-on wash (BHW; n=20) and after hide-on wash (AHW; n=20), and from beef carcasses before (CH0H; n=20) and after chilling (CH4H; n=20), fabrication equipment before (EBC; n=20) and after routine sanitation (EAC; n=13) were shotgun whole genome sequenced using an Illumina HiSeqX platform. The isolates from each source had equal number of extremely strong biofilm formers and non-biofilm formers as determined by the crystal violet staining method except for the EAC population where only three non-biofilm formers were available. The sequences were processed and annotated using an in-house workflow. Each genome was in silico typed for phylogroup. A phylogenetic tree was constructed based on core genes. Genome wide association analysis was carried out using Scoary. The 114 isolates belonged to four phylogroups, A (n=33, 28.9%), B1 (n=72, 63.2%), D (n=3, 2.6%), and E (n=6, 5.3%). Phylogroup B1 made up 73.8% and 50.9% of the biofilm formers and non-formers, respectively, followed by the A phylogroup at 23.0% and 35.9%, respectively. Unlike isolates in all other groups, the EAC isolates were exclusively of phylogroup B1. The core genome tree showed intermingle of biofilm formers and non-biofilm formers even though clustering of closely related isolates were spread out the entire tree. No genes were overrepresented in the biofilm formers, compared to the non-biofilm formers, based on Scoary analysis, with the criteria of present in >60% of the biofilm former group, but absent in > 60% of the non-biofilm formers. Interestingly, missing genes in the bcs operon (cellulose synthesis) did not always result in a cellulose negative phenotype, contrary to the csg operon (curli) and curli phenotype, as determined by the commonly used Congo Red method.

Studying and enlightening food microbial ecosystem and microbiome

O3.13 Disentangling the complex pathways between dietary molecular composition, gut microbiome enzymatic bio-transformation and health outcomes

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The principles of a healthy diet - increasingly recognized as promoter of physical and mental health - are nowadays based on population-wide patterns. By following one-size-fits-most recipes to meet the diet requirements of 'average' humans, these principles tend to overlook a key element at the core of the diet/health nexus: the personal traits of each individual, including the highly dynamic and enzymatically rich microbiome communities that inhabit the human gut and convert thousands of nutrients into bioavailable and bioactive compounds.
Aware of the multitude of responses to one same dietary recipe, research is needed to pave the way that will lead humanity to a new era of nutrition, settled on personalized diet guidance informed at the small dietary molecule and microbiome levels. However, this paradigm shift requires a deep understanding of the complex mechanistic links between food molecules, gut microbiome, and health outcomes. In order to shed light on these interactions, we performed a very large-scale analysis of human microbiomes (> 3000 shotgun metagenomic samples) covering 5 continents. We integrated the microbiome functional traits with an in-house molecular level database NutriChem 2.0 containing 18478 pairs of 1772 plant-based foods and 7898 phytochemicals using 9 biochemical reaction databases. During my talk I will show how we exploited the molecular nutrients-microbiome interactions towards scientific breakthroughs in two distinct, yet complementary levels, namely:
1) Mapping the human gut microbiota-diet interactome, providing mechanistic links between food's small molecules, microbial enzymes and main taxonomic drivers, and biomarker-based health status.
2) Based on a deep understanding of microbial metabolism of food compounds, building the scientific basis for the development of a new generation of highly nutritional foods, prebiotics, probiotics and postbiotics.

Scientific Diversity Serving Food Microbiology

O4.1 Impedometric analysis: an alternative way to study different aspects of food microbiology

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In a time when the word of microbiology is searching for even more rapid methods with fast and reliable results, and thus is moving ever closer to molecular biology, we want to propose a new approach, which maintain a foot in the past but with an eye to the future.
Our foot in the past lies in using impedance analysis, that is based on a principle that dates back on '70s, by which, the growth of bacteria in a culture medium produce, as end-products of their metabolism, charged molecules, that cause an impedance variation of the medium. This variation, is proportional to the change in the metabolism and number of bacteria, makes possible the measure of their presence. To date, this technique has been mainly used by industries for a rapid detection of spoilage bacteria in foods.
Considering this, our look at the future allowed us to think about new and smart applications of this principle. The novelty has a double aspect: i) the way in which the impedometric data are elaborated, in fact by using a primary model we were able to obtain objective parameters (Lag, Rate, yEnd), that well describe the microbial kinetic, but also ii) the application of the method to new environment and microorganisms.
In fact, once developed, this new approach was applied to different topics. For instance, the study of acidifying performances of starter lactic acid bacteria (LAB), allows to discriminate the strains trough their ability to adapt to the changing growing conditions (Lag time), their acidification rate (Rate) and acidifying capacity (yEnd). Furthermore, it allowed to quantified the presence of non starter LAB in fermented milk and select them for their potentiality to be used as adjunctive cultures. This method was also successfully applied to the detection of LAB's exopolysaccharides production. Furthermore, the stimulatory or antimicrobial effect on microorganism growth, of natural compound, that could be added as ingredient in food formulation was investigated.
In conclusion, this method could overcome the limit of some in-use traditional analysis, but also it could open new possibilities for application to the study of microbial dynamics in foods.

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O4.2

Shotgun metagenomics as a new, one-step and fast method for strain-level food-borne outbreak investigation

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The management of a foodborne outbreak depends on the rapid and accurate identification of the responsible agent and food source. Conventional methods are specific for each contaminant. For bacterial pathogens, it is based on isolation after stepwise culturing, followed by a characterization by targeting specific genes with qPCR or, more recently, by whole genome sequencing of the isolate. However, the isolation is not always straightforward nor successful. For viruses, where isolation is not possible, a qPCR reaction is often followed by a Sanger sequencing of some regions of interest for characterization.

We have developed a detection and characterization method based on strain-level shotgun metagenomics. This alternative approach based on sequencing of all genetic material in the sample requires no isolation, and can also be applied for the full genomic characterization of foodborne viruses.

Our approach has proven successful to detect, characterize and relate to human cases several STEC strains (co-) spiked on beef meat and goat cheese. The same method has been applied to identify the food source responsible for a Salmonella foodborne outbreak that occurred in Belgium in 2019. This allowed us to gain a better view on the time saved for source tracking with our approach compared to the conventional methods. We also evaluated different sequencing technologies (short versus long read) to estimate if an even faster workflow could be developed. We showed that after only 12 hours of sequencing on a MinION device, we could detect the pathogen, determine its virulence and antimicrobial resistance genes, and perform source tracking to the SNP level using phylogenetic analysis. Finally, we turned our focus to the challenging detection and characterization of foodborne viruses (norovirus, hepatitis A) at very low contamination loads. We could show that it was possible to detect one or even several viruses in the food, but further optimisation of the method is still necessary to make it cost-effective for practical use.

This approach of shotgun metagenomics at strain level, offering a faster alternative compared to conventional methods to resolve foodborne outbreaks for various pathogens, could have an important impact on actors involved in food safety.

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O4.3

qPCR method for identifying highly pathogenic Salmonella serotypes

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Salmonella is one of the leading causes of foodborne gastroenteritis worldwide. Eggs, dairy products, chicken and pork are the primary sources of infection. The economic, social and public health importance of diseases caused by this pathogen has brought many developing and developed countries to implement their monitoring systems. Today Salmonella serotypes are identified by slide-agglutination test and whole genome sequencing analysis. Both methods are expensive, time consuming and require specific skills or animal derived products (rabbit sera). In addition, serotyping does not identify "rough" non-agglutinable strains and the sequencing still generates very large volumes of data that are difficult to manage.

We developed and validated 69 relevant genetic markers able to identify more than 40 Salmonella serotypes of animal and human public health interest. The molecular serotyping method developed is carried out by using the High-throughput real-time PCR in Fluidigm chips. For testing the specificity and sensitivity of the genetic markers, 900 bacterial strains and 10 Salmonella surrogate plasmids were used. Strains were divided into 798 S. enterica subsp. enterica strains corresponding to 64 different serotypes, 38 strains of other S. enterica subspecies and S. bongori and, 64 strains other than Salmonella. Ten Salmonella surrogate plasmids were used when biological material was not available. Strains included in the validation study were isolated within a period of 20 years, from different sources. For each serotype, different phenotypes (different antimicrobial susceptibility) and genotypes (different Pulse-Field gel Electrophoresis (PFGE), Multi Locus VNTR Analysis (MLVA) and Multi Locus Sequence Typing (MLST) profiles) were included.

The molecular serotyping method developed allows distinguishing the different genomic lineages of polyphyletic serotypes. In addition, the detection of a Salmonella serotype in a food matrix has been achieved during a Salmonella Bovismorbificans outbreak where it was possible to identify the specific Salmonella serotype within a few hours thanks to the qPCR tests developed.

Such a PCR approach is easy to use and only needs a basic equipment, such as a thermocycler. Adaptation on a high throughput PCR format, such as Fluidigm chips, is another advantage of the method providing results for multiple samples in a minimum of time.

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O4.4 A text mining approach to enhance knowledge of food microorganisms

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The large and growing number of scientific articles related to food microbiology makes the task of finding relevant information on a specific issue both complex and time-consuming. Text mining has been proposed as a solution: to help the researcher find relevant information, an automatic system can make a first selection of potentially useful articles and information. Without being able to replace a systematic study (that requires time and budget), this type of tool offers a first global approach in a few clicks.
We have developed a semantic search engine for the literature about microbes, giving online access to 2.5 million PubMed abstracts in 2020. This search engine uses text-mining tools to provide relevant abstracts and extract information about microbial biodiversity. It covers all types of microbial habitats, and food in particular.
The text-mining process used consists of three main steps:
1) the detection of terms that designate microorganisms, habitats and phenotypes, as entities in the text;
2) the normalization of entities with shared controlled vocabulary, (the NCBI taxonomy and the OntoBiotope ontology); e.g. "artisanal cheese-making dairy" in text labeled by "cheese factory" term in ontology;
3) and the extraction of links between entities. This semantic search engine thus takes into account the synonyms of taxa (Aspergillus and Petromyces), but also the hierarchy of habitats or phenotypes (for example, a query on "dairy products" includes that on "cheese" and "yogurt"). As a result, the search engine can present abstracts with co-occurring terms, or more precisely only the abstracts where an explicit link between microbes, habitats and/or phenotypes has been detected.
The search engine can be used to quickly analyze the scientific literature for a given use case. Some examples will be presented, such as hazard identification or exposure assessment in microbiological risk assessment process; or the identification and selection of publications investigating the presence of pathogenic microorganisms (human, animal or plant) in food matrix. Since text-mining for microorganism detection is not limited to pathogens, this tool can also be used to extract information on the microbial ecosystems of specific food, and the bio-preservation activity of a microorganism of interest.

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O4.5

Rapid microbiological spoilage assessment of chilled chicken meat using non-invasive spectroscopic sensors

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Over the last two decades, there has been a surge of research interest on the rapid assessment of microbiological quality of poultry meat through the use of different spectroscopic sensors. The objective of this study was to evaluate the potential of Fourier-transform infrared and fluorescence spectroscopy in tandem with machine learning algorithms and multivariate data analysis to quantitatively assess chilled chicken spoilage. Chicken breast fillets were stored aerobically at 4°C and were periodically analyzed: a) microbiologically (n=6, three batches) for the enumeration of total viable counts (TVC), Pseudomonas spp., Brochothrix (B.) thermosphacta, lactic acid bacteria (LAB) and Enterobacteriaceae, and b) spectroscopically (n=18) with a Fourier transform infrared spectrometer (FTIR) and a hand-held fluorescence (FreshDetect) spectrometer for the collection of spectral data from the surface of chicken samples. Spectral analysis, model development and validation were performed through two machine learning algorithms, i.e. partial least squares regression (PLS-R) and support vector machines regression with radial basis function kernel (SVM-R). The developed PLS-R and SVM-R models were externally validated through independent- and intra-batch testing, respectively. The square of the correlation coefficient (R^2) and root mean squared error (RMSE) were employed for performance evaluation. In general, the SVM-R models based on spectral data from FTIR sensor exhibited better performance than the respective PLS-R models on the estimation of the different microbial groups. Moreover, the SVM-R models predicted more satisfactorily TVC (R^2 0.879, RMSE 0.607), followed by B. thermosphacta (R^2 0.873, RMSE 0.654) and Pseudomonas spp. (R^2 0.793, RMSE 0.855) populations than LAB (R^2 0.686, RMSE 0.649) and Enterobacteriaceae (R^2 0.477, RMSE 0.801). Conversely, the developed models based on data from FreshDetect achieved poor prediction performance, irrespective of the algorithm applied and microbial group examined. Results of the current study confirm the potential of FTIR coupled with SVM-R algorithm to be exploited for the fast and accurate assessment of chicken meat quality and suggest the need for further investigating of the suitability of the hand-held fluorescence (FreshDetect) device to predict microbial growth.

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O4.6

Listeria monocytogenes: Identification by high-throughput real-time PCR of 30 Clonal Complexes prevalent circulating in food, animal, environment and human in Europe

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Listeria monocytogenes (Lm) is a bacterium that causes a food-borne illness, the Listeriosis. Typing studies using Multi Locus Sequence Typing (MLST) reveal that most strains are gathered into few major Clonal Complexes (CCs) that account for a majority of human cases worldwide. CC language is harmonized internationally and provides crucial information on strain virulence. To date, the CC identification is based on sequencing of the strain genome and requires between 3 and 5 days depending on labs. Thus here is a need of a front-line typing method for rapid identification of the major circulating CCs. Such a method would aim at screening the wide amount of food strains collected for surveillance and research purposes.

At European level, the EU Reference Laboratory for Lm, Anses is coordinating a network of 40 National Reference Laboratories (NRLs) in charge, amongst other duties, of Lm monitoring in food products. The ANSES Laboratory for food safety in collaboration with its French and EU partners (technical food centers, NRLs) has developed a new high-throughput real-time PCR assay based on a microfluidic system that enables simultaneous analysis of 39 real-time PCR arrays on 44 strains in one test. This test provides accurate identification of 30 CCs, including the most prevalent CCs recently reported from human cases and food in Europe [1], the serogroup of the strains and subdivision among CCs. This one-day method is suitable for routine analysis and developed for multiplex fast and conventional real time PCR. We describe here (i) its design from a wide panel of 2299 strain genomes from human, food, animal and environment (ii) its validation according to the standard EN-ISO16140 on a various panel of 402 strains whole sequenced and collected from 18 European countries and (iii) its assessment for the typing of nearly thousand field strains collected during monitoring activities.

This assay will represent a key tool to assist surveillance laboratories to establish strain relatedness with human clinical strains, during outbreak investigations. Moreover, this test can assist the food sectors in their microbiological management plans to target contamination sources on the production lines.

[1]: Painset et al. 2019 Microb. Genom.

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O4.7 Newly developed system for Listeria monocytogenes detection in dairy products based on a bioelectric cell biosensor

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Human food-borne diseases caused by pathogenic bacteria have been significantly increased in the last decades, causing numerous deaths, as well as money and time loss in the agri-food sector and food supply chain worldwide. The standard analyses that are currently used for bacteria detection have significant limitations regarding cost, special facilities, highly trained staff, and a long procedural time that can be crucial for foodborne pathogens with high hospitalization and mortality rates, such as Listeria monocytogenes. Improved and accurate techniques that provide fast detection are of great importance since it is very crucial to detect pathogenic microorganisms and withdraw the contaminated products from the markets before their distribution to consumers, thus preventing pathogen dispersal and human infection. Aim of this study was to develop a biosensor able to perform robust and accurate detection of L. monocytogenes in various food substrates within 3 minutes. For this purpose, a cell-based biosensor technology (BERA) and a portable device developed by EMBIO Diagnostics called B.EL.D (Bio Electric Diagnostics), were used. Biosensors were created for L. monocytogenes detection using anti-Listeria monocytogenes antibodies and tests were conducted in milk and halloumi cheese samples. Results indicated that the biosensor managed to differentiate samples with and without Listeria species with 88% and 89% accuracy in milk and halloumi samples, respectively, after primary enrichment. Method's sensitivity, specificity, positive predictive and negative predictive values ranged from 80-95%, while the limit of detection was determined to be as low as 10^2 CFU/g in both food substrates.

O4.8 Optimal detection of Campylobacter spp. in swine caecal contents – the impact of selective media, time between sampling and start of analysis and the number of colonies confirmed

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Background: Campylobacteriosis is the most reported zoonosis in the European Union causing approximately 9 million human cases annually. Food-producing animals are a major source of Campylobacteriosis. The aim of the study was to optimise the protocol for detection of Campylobacter jejuni and Campylobacter coli in pig caecal contents. Material and methods: Caecal contents were collected at four time points from 15 clinically healthy slaughter pigs at one abattoir in Sweden. The samples were analysed by direct plating on three selective media, modified charcoal cefoperazone-deoxycholate agar (mCCDA), Preston agar and Butzler agar after 4, 48, 72 and 96 h of storage at 4°C. The plates were incubated for 48 h at 37°C at a microaerophilic atmosphere. After incubation, 5 presumptive colonies of Campylobacter spp. were selected from each plate for confirmation. Species identification was performed using MALDI-TOF. The association between media type, storage time, number of selected colonies and the probability of detection was tested using logistic regression. Results: Campylobacter spp. was detected from 59 of the 60 samples: C. coli from 58 (98.3%), C. jejuni from 31 (51.7%), C. fetus from 18 (30%), C. hyotestinalis from 16 (26.7%) and C. lanienae from 2 (3.3%). Longer storage time reduced the sensitivity of detection of C. coli compared to storage of 4 hours. For C. jejuni, only a storage time of 96 hours was significantly different from 4 hours. The performance of Butzler agar was significantly higher than mCCDA for detection of C. coli while Preston agar performed significantly lower than mCCDA. For detection of C. jejuni, the performance of Butzler agar was significantly lower than mCCDA. Picking more than colony per plate was associated with increased sensitivity for confirmation of C. coli when compared to picking one colony. For confirmation of C. jejuni selecting more than two colonies per plate was associated with increased sensitivity. Conclusions: Time interval between sampling and start of analysis should be shorter than 96 h. For detection of C. coli and C. jejuni in pig caecal samples a combination of mCCDA and Butzler agar is most optimal. Three presumptive colonies should be confirmed per selective medium.

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O4.9 The MilkyBase database - Mapping the human milk composition at molecular level

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Human milk is the first nutrition an infant comes across and one of our most complex foods. It has been studied extensively, still its biochemical complexity is insufficiently explored. Our work has been concentrating on building a database, called MilkyBase, of the biochemical composition of human milk, from scientific publications. The data were selected while focusing on quantitative descriptions of human milk components and entered in a novel structured database. The components are hierarchically classified, providing a tree-structured ontology. We recorded the biochemical components as responses to various qualified and quantified conditions. The purpose of the database is to provide a platform to users to put their own data in the unified format. The database is in MS Excel, supplied with macros helping the input with correct syntax and semantics. The organization of the fields reflects that that the biochemical composition of human milk can be considered as a dynamic response to maternal and infant characteristics, as well as to the measurement methods and other conditions thus to give way to predictive potentials. Beyond providing resources to analyze the role that various conditions play in human milk, our goal is to support researchers from other fields of food science with a source and template to place their own data in this format. Facilitated and encouraged by supporting macros they can analyze their own data by comparing them with published ones. The database can also identify gaps in knowledge and help design what new experiments are needed to reach a critical data mass for decision making in business as well as in science.

O4.10 Applications of machine learning to design novel microbiome-targeted foods: valorisation of vegetable waste and by-products

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Food waste management is a key issue to global food security and friendly environmental governance. There is a general interest to achieve zero waste production schemes and increasing evidence points towards vegetable food waste as a rich source of a wide array of carbohydrate structures and fibres with potential prebiotic activity. Therefore, the aim of this work was to develop a novel computational framework for the rational selection of potentially prebiotic vegetable sources. For this purpose, different machine learning algorithms and other computational tools were applied to publicly available data to establish structure-function relationships of novel vegetable oligosaccharide mixtures. Specifically, associations between fine physico-chemical and structural properties of various prebiotic fibres encountered in vegetable food waste and by-products, and the enzymatic machinery available in gut commensal microbial communities were elucidated. According to the results obtained, combination of artificial neural networks and principal components analysis (PCA) revealed the influence of vegetable source and processing conditions on the structural features of oligosaccharides, resulting in different biological effects. In this sense, partial hydrolysis of artichoke, bergamot and sunflower by-products following enzymatic treatments, leads to the production of pectic oligosaccharide mixtures with high uronic acids contents that enhance the growth of Bacteroides, Enterococcus and Prevotella. Moreover, by-products containing ?-galacto-oligosaccharides and xylo-oligosaccharides are likely to promote Atopobium while high verbascose contents may stimulate Clostridium growth. On the other hand, positive relationships between different genera (Butyrivibrio, Citrobacter, Mogibacterium and Oscillospira) were elucidated, that could indicate a synergistic metabolism in the presence of prebiotic substrates. Finally, comparative genomics of gut bacteria revealed the presence of functional domains from pectin-degrading glycosidases in reference genomes from Bacteroides, Enterococcus and Prevotella species. These sequences involved a wide range of activities such as polygalacturonases, rhamnosidases, rhamnogalacturonases, pectin lyases and pectin methyl- and acetyl esterases. Integration of the body of knowledge within the field of vegetable food waste valorization, from different perspectives, allows a rational selection of carbohydrate-based substrates that selectively stimulate gut commensals. The computational framework presented could be extended to a wide range of commensal microbes and carbohydrate structures in future studies.

Food Safety in 2050 – Is it too early?

O5.1 Estimation of growth, reduction and invasion behavior of Campylobacter jejuni in human gastrointestinal tract

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The current approaches to estimate the infectious risk of low-dose ingestion of pathogenic bacteria rely on model extrapolation, although a dose-response model plays an important role in quantitative microbial risk assessment on food. An alternative approach to evaluating low-dose response relationships has been proposed based on mechanistic processes from intakes to infections of foodborne pathogens. In this study, the behaviors of Campylobacter jejuni in three processes during gastrointestinal tracts were evaluated quantitatively and modeled. The processes included the behavior of C. jejuni of reduction in stomachs, competition with intestinal microflora and invasion into intestinal cells. The reduction of C. jejuni in artificial gastric juice during the model digestion system of standard meals taking into account the changes in pH and the gastric retention times were successfully described by Bayesian Weibull models. The developed model enabled to estimate the bacterial reduction by gastric passage after standard meals. The results demonstrated that most of the C. jejuni entering the stomach with meals pass through stomachs without inactivation (ca. 1.0 log reduction). Afterward, C. jejuni cells were co-cultured with standard intestinal microflora in artificial intestinal juice, and their competitive behaviors were observed. The results showed the Jameson effect on C. jejuni by microbiota and reductions of C. jejuni counts by standard intestinal microbiota. The competitive behaviors of both the C. jejuni and intestinal microflora were successfully described with the Lotka-Volterra Baranyi-Roberts model. Finally, monolayer Caco-2 cells, which are small intestinal epithelial cells, were exposed to C. jejuni populations. The behaviors of invading C. jejuni counts were determined and described with a newly developed mathematical model as a function of invading numbers of C. jejuni and its increasing rate. The results illustrated that even if a few seconds of exposure induced C. jejuni invasion into Caco-2 cells when the bacterial suspensions were more than 6.0 log CFU/mL. From the above results, the developed models would enable to estimate the death, growth and invasion behaviors of C. jejuni in the gastrointestinal tract. Applications of the developed models will reveal the dose-response relationship of low-dose intakes of C. jejuni.

Food Safety in 2050 – Is it too early?

O5.2

Exploring the incorporation of gut microbiome omics data in next-generation risk assessment of xenobiotics in foods

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The human gut microbiome (GM), the microbial community occupying the human gut, encompassing the microorganisms (microbiota) and their structural elements, metabolites, and surrounding environmental conditions has been associated with a range of diseases. In this context, the interactions between the GM and xenobiotics, are of particular interest. Among such exogenous toxicants found in foods, special emphasis has been put on endocrine-disrupting chemicals (EDCs), such as bisphenols, parabens and phthalates, since they have been associated with metabolic disorders, as well as with changes in the GM (dysbiosis).

Risk assessment (RA) is the science-based component of the food safety risk analysis framework. Traditionally, xenobiotic RA relies on data from animal experiments, human trials and/or human observational/epidemiological studies. Importantly, the extrapolation of this data across species or studied populations carries considerable uncertainty, partially due to GM variability and the complexity of MDC/GM interactions. Thus, the use of uncertainty/safety factors is necessary, potentially resulting in overestimations or underestimations of the risk associated with exposure to xenobiotics. Therefore, the need for the incorporation of the GM in food safety RA of xenobiotics and especially EDCs is well-justified. Nevertheless, the road towards this next-generation RA (NGRA) remains challenging for various reasons, including the elusive nature of what constitutes a healthy GM.

This work extends the scientific support for the application of a tiered framework, which aims to set out principles for evaluating the potential of xenobiotics to alter the GM and ultimately human health. An initial, cross-reference tier reviews recently compiled evidence on the impact of several groups of xenobiotics on the GM. This may raise early concerns, depending on the nature and chemical structure of the xenobiotic under RA. The second tier focuses on resilience, therefore, circumventing the need to define a healthy GM. The final tier prioritises microbiome function over composition and focuses on active microbial fractions via omics approaches in combination with single-cell techniques. It aims to identify key species or other biomarkers associated with host health or disease. Such biomarkers are crucial for the development of NGRA approaches for EDCs and other xenobiotics, which would take into account the GM.Beyond providing resources to analyze the role that various conditions play in human milk, our goal is to support researchers from other fields of food science with a source and template to place their own data in this format. Facilitated and encouraged by supporting macros they can analyze their own data by comparing them with published ones. The database can also identify gaps in knowledge and help design what new experiments are needed to reach a critical data mass for decision making in business as well as in science.

Food Safety in 2050 – Is it too early?

O5.3 Sanitiser resistance of South African Listeria monocytogenes isolates to commercial sanitisers: benzalkonium chloride is old news

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With an ever-increasing number of listeriosis outbreaks being linked to quaternary ammonium compound (QAC) resistance, benzalkonium chloride (BAC) which is a first generation QAC, has been studied extensively. Despite the availability of many other generations of QAC-based sanitisers as well as cocktails of mixed QAC generations being available, BAC still dominates the global research pool for QAC resistance. This study investigates newer generation QACs and a promising alternative: a novel sanitising agent based on triethylamine and polyhexamethalene biguanide hydrochloride in eradicating QAC-resistant L. monocytogenes. QAC resistance amongst isolates of L. monocytogenes is encoded by various genes such as bcrABC and emrC which have all been found in clinical isolates from listeriosis outbreaks. A sample set of 50 L. monocytogenes isolates from South African Food Processing environments (FPE) (comprising of drains, food contact surfaces and non-food contact surfaces) were analysed. Conventional PCR and phenotypic disk diffusion methods were utilised to elucidate the prevalence of sanitiser resistance. Twenty eight percent (28%) of the isolates were assigned to lineage I and 72% of the isolates were assigned to lineage II. More than half of the isolates had the bcrABC gene (68%) whilst 62% had the emrC gene. Forty eight percent (48%) of the isolates had both the bcrABC and emrC genes. Phenotypic resistance to BAC (a first generation QAC), a fourth generation QAC and a combination of a first and a fourth generation QAC were encountered with all the drain isolates (100%) expressing resistance towards BAC. When looking at the efficacies of the QAC sanitisers versus the novel QAC-free sanitiser, the novel sanitiser proved to be most effective with a 36% tolerance being encountered amongst drain isolates and more than 50% susceptibility being encountered for isolates from other FPE niches. QACs have historically been used due to their initial strong bactericidal effects and residual activity. This novel QAC-free sanitiser has a strong bactericidal effect against all the isolates as well as an independently confirmed residual action. The question now remains- with BAC resistance being so widely reported, why are investigations into alternatives not receiving more attention?

O5.4 Quantitative microbial risk assessment for foodborne viruses: past, present, and future

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Quantitative microbial risk assessment (QMRA) is a tool used in analysing and managing the risk of foodborne infectious disease. Microbial risk assessment was formally recognised as a distinct field in the 1990s, culminating with the publication of the Codex Alimentarius Commission's guidelines. It was later recognised that food safety standards were not as effective in managing the risk of foodborne virus, with the FAO and WHO first convening an expert panel on the problem in 2008.

This problem was broadly due to two factors: first, the different behaviour of virus as a foodborne hazard compared with bacteria, and second, a lack of data for modelling this behaviour due to difficulties in measuring and detecting viruses in food. Enteric viruses like Norovirus and Hepatitis A now represent over 50% of the overall foodborne disease burden, according to WHO estimates. Zoonotic viruses like Hepatitis E have also emerged as a threat.

In the years since the first FAO/WHO expert panel in 2008, several new technologies have been developed that can tackle this issue, and much more data is now available for virus QMRA. These include developments in molecular detection methods, like viability PCR, and developments in cell culturing for viruses like Norovirus.

This talk will address the progress made in detecting and modelling virus activity in food, and what this means for the future of QMRA. It will also discuss the data now available for risk assessment modelling compared with a decade ago, and the virus QMRA models published to date. The future of methods like whole genome sequencing will also be considered. The increasing availability of data reveals the true scope of virus hazards, which leads to them being understood and controlled. Looking ahead to 2050, many emerging threats will likely be viruses, and viral QMRA will be an important tool for tackling them.

Food Safety in 2050 – Is it too early?

O5.5 Using genomics and transcriptomics technologies to design Hurdle Technology combinations that eliminate microorganisms from food. An example for eliminating L. monocytogenes through oxidative stress-based techniques in foods

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It is well-known that SigB in L. monocytogenes enhances stress resistance towards multiple stresses. However, we have shown that oxidative stress is an exception, and upregulation of SigB results in increased sensitivity to oxidative stress in stationary phase. This phenomenon is due to decreased catalase activity in the presence of SigB. Through transcriptomics we have found that catalase is expressed in peaks, which predominantly occur in exponential and early stationary phase and they are higher in ΔsigB compared to the WT explaining the higher oxidative stress resistance and catalase activity of the first mainly after early stationary phase of growth. Furthermore, catalase activity increases incrementally during growth, suggesting a protein accumulation after each transcription peak. The above suggest that SigB activation through a stress could make cells hypersensitive to oxidative stress with important implications in efforts to eliminate this pathogen in foods. As expected, pre-exposure to sub-lethal acidic environment (pH 4.5) caused a significant decrease in survival to H2O2 or sonication, which supports our observations. This suggests a novel synergistic effect with other stresses that could be used in the food industry to enhance the antimicrobial activity of oxidative-based techniques. We also show that light clearly affects growth only when challenged with H2O2 during growth, and not when challenged after being grown in constant light exposure. Furthermore, we show that light sensor Lmo0799 promotes oxidative stress resistance despite the fact that the opposite was expected since it is a SigB activator. Overall, we show that an active SigB is detrimental for L. monocytogenes survival under oxidative stress due to downregulation of catalase and this could be exploited to eliminate this organism in the food industry.

O5.6 The prevalence and virulence of Clostridioides difficile on farms, in abattoirs and retail foods in Ireland

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Clostridioides difficile infections (CDI), often considered hospital-acquired, have rapidly increased in the community in recent years. This suggests a different route of infection in humans. Several studies have described the presence of these bacteria in farm animals and food products, which suggests a potential foodborne transmission route. The focus of this research was to investigate C. difficile prevalence and virulence on farms, in abattoirs and in retail foods in Ireland. Culture based methods were used to analyse 540 samples along the food chain and molecular biology techniques were applied to confirm presumptive positives and to detect toxin (tcdA, tcdB, cdtA, cdtB) and accessory (tcdC and tcdR) genes. The prevalence on farms (32.3%) and in abattoirs (43.3%) was high, indicating a widespread presence of the bacteria at both stages of the food chain. Retail foods had a lower C. difficile prevalence of 3.3% (9/270), but concentrations were as high as 6.8 log10 cfu/g in cottage cheese. The toxin genes (tcdA, tcdB, cdtA, cdtB) were detected in 41%, 99.2%, 33.6% and 32% of isolates, respectively. Accessory genes were detected in 46.7% (tcdC) and 31.1% (tcdR) of the 122 C. difficile isolates obtained. It was concluded that although the prevalence of C. difficile decreased along the food chain, their presence in retail foods suggests that this pathogen might be foodborne, perhaps necessitating dietary advice for potentially vulnerable patients.

Food Safety in 2050 – Is it too early?

O5.7 Impact of climate change on the spoilage risk of shelf-stable food products

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Climate change threatens food production, having severe implications for primary food production, food safety, and food security. Yet, less attention has been paid to the impact of global warming on food spoilage. This study fills the existing gap in the literature by looking at the microbiological spoilage of shelf-stable food products. These products are considered microbiological stable, given that the spores of thermophilic spoilage bacteria, surviving thermal processing, require relatively high temperatures to germinate and grow to spoilage levels. Their stability is based on the fact that current distribution and storage temperatures do not allow germination and growth of the surviving thermophiles. In this work we assess the potential risk of spoilage of canned milk due to Geobacillus stearothermophilus under different climate change scenarios for 38 European cities. The results indicate that the risk of spoilage is high, leading to the collapse of the shelf-stable food chain when the temperature increase exceeds 2 °C. The current study also discusses the preparedness strategies required by policymakers and the food industry in order to tackle climate change implications on food spoilage of shelf-stable food products.

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O5.8 Transcriptomic analysis of the adaptation of Listeria monocytogenes to growth at low temperature in presence of exogenous unsaturated fatty acids

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Listeria monocytogenes is a food-borne pathogen responsible for listeriosis that causes a real public health issue and a challenge for the food industry. Listeria monocytogenes is ubiquitous and can contaminate many raw materials. Ready-to-eat products which allow Listeria monocytogenes' growth (Regulation 2073/2005, category 1.3) are especially concerned.

Refrigeration is the main measure to control food safety. Low temperature induces a decrease in membrane fluidity which interferes with normal membrane functions. However, Listeria monocytogenes is able to grow at low temperature. To maintain its membrane fluidity, it changes its lipid composition by increasing the proportion of iso and anteiso branched fatty acids and by shortening the fatty acid's length.

Recently, we have shown that several strains of Listeria monocytogenes can grow faster at low temperature when exogenous unsaturated fatty acids (eUFA) are present in its environment and are incorporated in the membrane fatty acid profiles. Contrarily, the growth rate was shown to remain unchanged at 37°C in presence of the same eUFA.

For this study, we select the strain of Listeria monocytogenes with the highest increase of growth rate in presence of eUFA and its genome was sequenced.

RNA-sequencing was performed to understand the molecular mechanisms of eUFA incorporation at gene expression level, on 4 culture conditions with or without oleic acid (representing eUFA) at 5°C or 37°C.

TS broth (tryptone soy broth) was complemented with oleic acid solution (0.9 mM final concentration) and inoculated from an overnight preculture. Total RNA was extracted using the TRIzolTM reagent method and then treated by Dnase I. RNA integrity and concentration were assessed by Nanodrop and Bioanalyzer instruments before sending samples to RNA sequencing (Illumina NextSeq High). Sequencing quality was assessed by using FastQC and Illumina adapters and low-quality base pairs were removed by trimming. Differential gene expression analysis was performed using R-studio package, DESeq2. Multicriterial analysis of over- and under- expressed genes is performed to decipher the molecular mechanisms of eUFA incorporation by Listeria monocytogenes at low temperature.

Food Safety in 2050 – Is it too early?

O5.9 The effect of sigB and cysteine on the oxidative resistance of Listeria monocytogenes

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Listeria monocytogenes is a Gram-positive bacterium that can be found in nature ubiquitously as well as in fresh or processed foods, causing life-threatening foodborne illness listeriosis. L. monocytogenes is frequently exposed to a variety of challenges in its natural habitat, including oxidative stress. Therefore, L. monocytogenes has evolved a stress defence mechanism to cope with these challenges. The alternative sigma factor SigB, a well-studied transcriptional regulator responsible for controlling the expression of over 150 stress genes, is largely attributed to the ability to survive in a wide range of challenging conditions. L. monocytogenes mutants lacking SigB have been shown to be sensitive to a range of stressors, including acid, bile salts, and osmotic conditions. However, the role of SigB in oxidative stress is debatable, as numerous studies have indicated increased sensitivity. When SigB is activated by stress, it is thought that the cells become resistant to oxidative stress. However, we found that activation of SigB could make cells hypersensitive to oxidative stress which has crucial consequences in the inhibition of this pathogen from food. Pre-exposure to sub-lethal acidic acid (pH 4.5) resulted in a significant decrease in survival towards H2O2 exposure. This alludes to a new synergistic effect with other stresses that can be applied in the food industry. On the other hand, it was previously proven that a non-essential amino acid cysteine plays role in the virulence of L. monocytogenes. Due to the availability of this amino acid in various foods and its antioxidant properties, we also investigated the effect of cysteine on the oxidative stress of L. monocytogenes. We used the SigB deleting mutants to discover whether this gene plays a role in the use of cysteine during oxidative stress or shows hypersensitivity. Our results shed light on the role of the SigB factor under oxidative stress and how the presence of cysteine in the environment can affect this mechanism. Therefore, better solutions could be produced to solve food safety-related problems in the food industry.

Controlling and predicting microorganisms in food ecosystems

O6.1 Effect of deheading and storage in modified atmosphere on the shelf life of shell-on-prawns (Pandalus borealis)

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The northern prawn (Pandalus borealis) is of great commercial interest with global annual catches of around 250.000 tonnes. P. borealis is used for production of cooked shell-on-prawns, which is mostly sold as a frozen, ready-to-eat (RTE) product. However, new refreshed (i.e., thawed and refrigerated) RTE products are emerging on the Scandinavian seafood market. The objective of this study was to investigate if deheading extend the shelf life of cooked P. borealis during refrigerated storage with or without modified atmosphere packaging.
Whole, cooked prawns caught off the coast of Greenland, were deheaded or kept whole and packed in air or in modified atmosphere (MAP, 40 % CO2/60 % N2) at 5°C during 21 days. Prawns were analysed for sensory changes (shelf life), aerobic plate counts (APC), total volatile nitrogen (TVN), trimethylamine-oxide (TMAO), trimethylamine (TMA) and composition of the spoilage microbiota by 16S rRNA Sanger sequencing of isolates and 16S rRNA amplicon sequencing of the microbial community. Deheaded MAP prawns had reached end of shelf life at 21 days, followed by whole MAP prawns (18 days), deheaded prawns in air (14 days) and whole prawns in air (11 days). For all four treatments, APC reached >8 log CFU/g at the time of sensory spoilage. The spoilage microbiota was dominated by Carnobacterium sp. for MAP prawns and by Pseudoalteromonas sp. for prawns stored in air. At the onset of sensory spoilage TVN levels reached 32 and 41 mg N/100 g for whole, and deheaded MAP prawns, respectively. Whereas whole prawns in air reached a level of 66 mg N/100 g, and deheaded prawns in air reached 143 mg N/100 g. No increase of TMA was seen for deheaded MAP shrimp, indicating that the spoilage microbiota was not able to convert TMAO to TMA. In contrast, 60-66 mg N TMA/100 g was detected at the end of experiment for prawns in air. In conclusion, MAP changed the dominating spoilage microbiota from Pseudoalteromonas sp. to Carnobacterium sp. coinciding with an increase in sensory shelf life of 7 days at 5°C. Deheading increased shelf life of chilled prawns by 3 days at 5°C.

Controlling and predicting microorganisms in food ecosystems

O6.2

Modes of action of rosemary and Debaryomyces hansenii against Aspergillus westerdijkiae in dry-cured meat matrix

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Aspergillus westerdijkiae is an ochratoxin A (OTA) producer mould in dry-cured meat products. Natural strategies to control ochratoxigenic moulds using biocontrol agents (BCAs) are currently in the spotlight. The aim of this study was to test the potential antiochratoxigenic activity of rosemary leaves (R), rosemary essential oil (REO) and Debaryomyces hansenii FHSCC 253H (Dh) as BCAs against A. westerdijkiae in a dry-cured fermented sausage-based medium. The mechanisms involved in their effect were also analysed by Proteomics, using a Q-Exactive Plus. Three batches were carried out: a control without BCAs, another one with R+REO and one with Dh. R (2 g/kg) and Dh (100 µL of 10^6 cells/mL) were added to the medium and REO was added on the casing, which was put onto the medium surface to simulate the real product. Significant OTA reductions of 73.87 % and 88.26 % were provoked by R+REO and Dh, respectively. Proteomics revealed that the BCAs affected to proteins linked to OTA biosynthesis and the cell wall integrity pathway (CWI). Proteins from PKS ER domain, directly involved in mycotoxin biosynthesis, were diminished in abundance by both treatments (R+REO or Dh). R+REO altered the CWI by decreasing proteins related to the synthesis of cell surface polysaccharides and actin assembly, and increasing the cell wall protein PhiA, involved in conidiogenesis. Dh decreased the NRPS protein, indispensable for the formation of the OTB, an OTA precursor, and affected to the CWI by lowering the abundance of proteins associated with the actin binding, the synthesis of polysaccharides and the response against cell wall stress agents. Therefore, rosemary and D. hansenii FHSCC 253H are potentially useful to minimise the hazard posed by A. westerdijkiae in dry-cured fermented sausages within a HAPPCC framework.

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Controlling and predicting microorganisms in food ecosystems

O6.3 Listeria monocytogenes undetected: inter- and intrastrain variability influences the detection chance of enrichment-based procedures

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Testing of foods for the presence of pathogens is crucial to verify food safety control measures and is done by food industry and governmental authorities. Due to low level contamination and the conceivable presence of preservation-induced damaged cells, the international standard (ISO) procedures for microbiological testing rely on enrichment-based test procedures. Listeria monocytogenes is detected from food using a two-step selective enrichment, starting with enrichment in half Fraser Broth (HFB), followed with enrichment in Fraser Broth (FB). The objective of this study was to quantify the impact of strain variability and single cell heterogeneity on outgrowth of L. monocytogenes in HFB, and to estimate the detection chance using the two-step enrichment-based procedure.
A collection of 23 L. monocytogenes strains of clinical and food origin was tested for their ability to grow out in HFB. Outgrowth kinetics of sub-lethally heat-injured and acid-stressed cells were compared to reference cells with no stress pre-treatment. Monte Carlo (MC) simulations were done using the growth parameters obtained in the enrichments to estimate the detection chance. The MC analyses demonstrated that when starting with one cell, the detection threshold for efficient transfer of at least one cell to the secondary enrichment step in FB, i.e. 2 log10 CFU/ml, was not reached by 11 of 23 strains tested (48%) that were heat stressed. Increasing the incubation time from 24 to 26 hour and the transfer volume from 0.1 to 1.0 mL increased the average probability to transfer at least one cell to the secondary enrichment step from 79.9% to 99.0%. The detection chance was also influenced by single cell heterogeneity. Monitoring of single cell outgrowth kinetics in HFB after fluorescence-activated single-cell sorting demonstrated that single cell heterogeneity is strain dependent, and slow-recovery strains showed higher single cell heterogeneity than fast-recovery strains.
These findings underline that when the current ISO procedure is applied, L. monocytogenes can remain under the detection radar when initial contamination is low for stressed cells. When optimizing enrichment procedures, it is crucial to take strain variability and single cell heterogeneity into account as this can have a significant impact on the detection efficacy.

Controlling and predicting microorganisms in food ecosystems

O6.4 Clostridium sporogenes as surrogate for proteolytic C. botulinum - Development and validation of extensive predictive model using data for growth and time-to-toxin formation

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An extensive cardinal parameter growth and growth boundary model for C. sporogenes, as a surrogate for proteolytic C. botulinum, was developed to include the inhibitory effect of 11 environmental factors. 626 maximum specific growth rates (μmax) in broth were generated to determine cardinal parameter values for the growth inhibiting effect of temperature, pH, NaCl/water activity (aw), organic acids (acetic, benzoic, citric, lactic, sorbic) and phosphate melting salts (ortho-, di- and tri-phosphates). μmax-values for C. sporogenes growing in well-characterized processed cheeses were used for product calibration (n = 10) and for product evaluation of the developed model (n = 23). 95 growth/no-growth responses and including 92 μmax-values from the scientific literature for 58 different isolates of proteolytic and toxigenic C. botulinum (Group I) were used for further model evaluation. The developed model performed better than available models and was acceptable for processed cheese (Bias factor of 1.17) and good for meat products (Bias factor of 0.97). The developed cardinal parameter growth and growth boundary model was expanded to predict time-to-toxin (TTT) as the time required for a 3.3 log-increase in cell concentrations. 509 TTT formation data were extracted from the scientific literature for processed cheese, other dairy products, meat, poultry, vegetables and liquid laboratory media. These data included responses for 85 different proteolytic C. botulinum strains. When used within its range of applicability the new extensive model was able to predict TTT for 235 of the 509 data and with just 0.8 % fail-dangerous predictions. The new and extensive model can predict combinations of environmental factors that prevent growth and toxin formation by proteolytic C. botulinum. These combinations of environmental factors are identified as predictions within the no-growth region and with a solid margin to the growth boundary (□-value > 2). Predictions are expected to facilitate development or re-formulation of processed cheese, meat products and other foods including products with reduced sodium content and storage at ambient temperature. The new model underestimated TTT for the studied food categories and should not be used for more precise prediction of TTT.

Controlling and predicting microorganisms in food ecosystems

O6.5 Comparing effects of pulsed light and UVC (λ =254 nm) radiations on bacterial and fungal spores

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Pulsed light (PL) is a non-thermal technology used in food, pharmaceutical and cosmetic industries. PL decontaminates surfaces and packaging materials and eliminates pathogenic and spoilage agents such as bacterial and fungal spores (conidia). PL inactivates microorganisms by exposing them to intense (> 1000 W/cm²) white light pulses for very short time (250 µs). The xenon-light source emits a broad-spectrum light pulse with wavelengths ranging from 200 to 1100 nm, and containing 24% of UV. The aim of this study was to evaluate how specific are the inactivation effects of PL compared to UVC. We first investigated the germicidal effects of both technologies, and then we evaluated the degradative effects on spore proteins.
We found that the germicidal effects of both PL and UVC technologies are strain-dependent. A high variability in sensitivity was observed. For example, the PL and UVC fluences required for 4 log-reduction of Bacillus weihenstephanensis KBAB4 and Bacillus thuringiensis 407 spores less than 1 J/cm² and 0.09 J/cm², respectively. Two- to four-fold higher fluences were needed to obtain the same reduction with resistant spores of strains B. pumilus SAFR-032 and B. cereus AH187. Interestingly, we highlighted a significant correlation ($P < 0.0001$) between the sensitivity of eight Bacillus spores, indicating that the bacterial spore sensitivity to UVC is a good predictor of the sensitivity to PL. However, UVC-exposure needs to be 10× time longer than PL-exposure to obtain the same inactivation efficiency. Importantly, PL showed a higher efficiency than UVC to inactivate conidia of Aspergillus brasiliensis.
To determine whether PL and UVC degrade proteins of Bacillus pumilus spores and identify the targets of these two treatments, we used a shotgun proteomics approach to compare the proteome of UVC-and PL-treated spores to the proteome of untreated spores from four independently prepared batches. Overall, the results showed poor degradation of proteins (2 to 4% of 1357 spore proteins detected). However, for similar log-reductions, the number of proteins targeted by PL was 10-fold higher than that obtained with UVC, and the PL-targeted proteins are different from UVC-targets. A similar work (results in progress) is performed with A. brasiliensis conidia.

O6.6 Nisin immunity and degradation in Lactococcus lactis: new physiological insights into the production of a known bacteriocin

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The bacteriocin nisin has been used for half a century to contribute to extended shelf life of different food types, including cheese. Nisin is well established as an effective agent against several Gram-positive food-spoilers. Nisin can also be produced in situ during fermentation by some Lactococcus lactis. In this study, 722 strains of L. lactis were screened for their potential to inhibit a nisin-sensitive L. lactis. Of these, 52 strains fully inhibited growth of the sensitive strain in liquid medium. The presence of a nisin biosynthetic cluster and detection of nisin in spare medium in approximately 38 strains concurs with nisin-mediated inhibition of the sensitive indicator strain. To evaluate nisin sensitivity, acidification of milk inoculated with L. lactis was performed. In the presence of a nisin producing strain, three profiles were observed: loss of acidification, delayed acidification and unaffected acidification. Most strains with unaffected acidification possess the immunity genes nisI and/or nisFEG. A nsr gene encoding the nisin resistance protein, is present in the majority of L. lactis strains showing delayed acidification. NSR positive strains were confirmed to degrade nisin to the respective NSR degradation product by a newly developed analytical method. In general, strains unable to acidify milk in the presence of the nisin producing strain possess none of the above genetic features. Our data provide insights on production, immunity and degradation of nisin in L. lactis. This knowledge can be used to guide the design of robust cheese cultures.

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O6.7

Development of anti-biofilm coatings applied by Non-Equilibrium Atmospheric Plasma on stainless steel

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Biofilm mediated microbial persistence of pathogenic and spoilage bacteria is a serious problem in food industries. Due to the difficulty of removing mature biofilms, great efforts are being made to find new strategies to prevent bacterial adherence to surfaces, the first step for biofilm development.

In this study, the anti-biofilm activity was achieved through the modification of surface physicochemical properties. Coatings with different chemical nature and morphology were applied by Non-Equilibrium Atmospheric Plasma on stainless steel (SS) AISI 316, the SS most commonly used in food industry equipment. For the coating optimization, the anti-biofilm activity was assessed for Listeria monocytogenes CECT911 and Escherichia coli CECT515. The biofilm formation was measured after an incubation of 24 hours at 37°C by crystal violet staining, including always a parallel control of uncoated SS.

The best anti-biofilm activities were obtained for L. monocytogenes with two coatings consisting of a base coating of (3-Aminopropyl)triethoxysilane and a functional coating of tetraethyl orthosilicate (APTES + TEOS) or acrylic acid (APTES + AA), achieving relative biofilm productions (when compared with those for the uncoated SS) of 55% and 26%, respectively. The anti-biofilm activity of these coatings was assessed for three strains of L. monocytogenes, two of them isolated from a meat industry, after incubation under the following conditions: 24h/37°C, 48h/37°C, 6 days/12°C and 12 days/12°C. A morphological and chemical characterization of the coatings was also performed by atomic force microscopy (AFM), scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS) and water contact angle (WCA) measurement.

The results obtained showed an increased anti-biofilm activity at 12°C, a temperature commonly found in food processing environments, for the three Listeria strains on the coating APTES + AA. The coatings with the highest anti-biofilm activity showed lower surface roughness (AFM, SEM analysis) and higher hydrophilicity (WCA measurements). This suggests that the formation of a hydration layer prevents the adherence of the bacteria, an effect that seems to be enhanced by low temperature conditions, when the wettability of the strains is increased.

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O6.8 D database of microbial inactivation. An innovative online resource for data analysis and meta-regression modeling in predictive microbiology

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Predictive microbiology is a relatively mature field, with vast knowledge already available in the scientific literature. Indeed, a literature review is a common step to calculate predictions using existing information, or to assess novel experimental results. However, extracting literature data can be time-consuming because most of the data is located in scientific articles in unstructured formats, making it impossible to automate data gathering. Although some databases are already available (e.g. ComBase or Sym'Previus), they only provide a detailed view of each study, partially masking the variability of the microbial response when all studies are being compiled and compared.
In this presentation, we will introduce an innovative resource to analyze and build microbial inactivation models: D database. This application provides a user-friendly interface to the outcome of a systematic review of microbial inactivation models (both linear and nonlinear). The data is stored in the cloud using a modern nonSQL architecture (MongoDB), easing future updates and extensions (e.g. new input data). D database provides search features (by microorganism, media, experimental approach, model…), as well as advanced data analysis tools (e.g. interactive visualizations, meta-regression, comparison with external data). It applies an innovative philosophy, focusing on the variability (distribution) of the data gathered from different studies, not just the particular details of the data from a specific study. Therefore, it eases variability analysis and comparison of novel models against the variability in the scientific data, not just individual entries. Nonetheless, the meta-data of individual data points is also accessible within the application. Furthermore, it includes modules for building different types of meta-regression models (linear, nonlinear, mixed effects) in the cloud.
D database is Open Code and freely available online (https://foodmicrowur.shinyapps.io/Ddatabase/). The back-end of the application is developed in R, and the user interface is implemented in the shiny R package. We believe that it provides an efficient way to exploit the inactivation data already available in the scientific literature, so it will be of great interest for scientists in predictive microbiology. Furthermore, it implements several innovations in terms of approach and technical implementation that can guide the development of future tools.

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O6.9 Predicting growth of Bacillus cereus from phylogroups II, IV, V and VI during storage of foods at temperatures below 12°C

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Minimally processed chilled foods such as soups, cooked meats, stews or pasta salads, which are known to support growth of Bacillus cereus, represent an ever-growing share of Europeans' diet. Chilled storage of these foods is specified as 8°C or below in many countries. However, some food administrations are considering more flexible storage temperatures and encourage use of predictive microbiology to establish safe shelf-lives. Unfortunately, documentation on successfully validated B. cereus growth models in foods is limited. The objective was to identify or develop a model that can accurately predict growth of B. cereus group in ready-to-eat and ready-to-heat foods stored at temperatures below 12°C.

The ability of (A) ComBase and published models (B, Food Microbiol. 2013, 33, 69-76; C, Int. J. Food Microbiol. 1996, 30, 55-70) to predict growth rates of B. cereus in foods at low temperature was evaluated by calculating the bias- and accuracy factors (Bf/Af) using data (n=68 growth responses) collected from 14 published studies. The best performing model was further used in model development and evaluation. Challenge tests were performed to collect experimental growth data (n=137) for B. cereus strains belonging to phylogroups II, IV, V and VI in minimally processed foods with different properties (pH 4.7-7.8 and aw 0.935-0.999) during storage at 5.0-11.7°C for up to 49 days. Fifty-eight of the experimental growth datasets were used to calibrate μopt from model B and to develop an interaction term (□ (□ (T, pH, aw))). Remaining datasets were used to evaluate the performance of the new model by calculating the percentage of correct, fail-safe and fail-dangerous predictions and the Bf/Af.

Using the literature data, Bf/Af-values of 1.7/2.4, 0.9/2.3, 1.5/2.5 were obtained for models A, B and C, respectively. Evaluation with the experimental data resulted in a new model with improved performance compared with model B based on an increase in correct predictions (from 54 to 70%) and a decrease in fail-safe predictions (from 46 to 30%) along with Bf/Af-values going from 0.9/1.6 to 1.1/1.6.

The new model can be used to facilitate the evaluation of safe shelf-life of foods stored at <12°C in relation to B. cereus.

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O6.10 Growth versus no-growth of Listeria monocytogenes in the interfaces between sandwich ingredients

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One of the bacteria, frequently associated with safety of ready-to-eat (RTE) sandwiches, is Listeria monocytogenes which is responsible for one of the most lethal foodborne diseases, listeriosis. There are several ingredients in RTE sandwiches, which make it challenging to assess the growth potential of L. monocytogenes. Combination of ingredients may create interfaces where moisture, aw and acid contents are difficult to predict. The purpose of this project was to investigate whether the growth ability of L. monocytogenes changed when combining ingredients, compared to growth in the ingredients when examined individually. Two sandwiches, consisting of ingredients that individually do not support growth of L. monocytogenes, were chosen for the experiments, i.e. a cheese sandwich consisting of rye bread, cheddar and a slice of fresh tomato and a cold-smoked salmon sandwich consisting of wheat bread, cold-smoked salmon and iceberg lettuce were selected. Growth was studied at 10 °C in single and double layers of each ingredient as well as in pairwise combinations with rye bread + tomato and tomato + cheddar as well as wheat bread + cold-smoked salmon and cold-smoked salmon + iceberg. Samples were prepared as pieces with the same circular shape with 25 mm diameter for all ingredients. They were individually inoculated with L. monocytogenes cultures grown at 10 °C. When double layers of ingredients were tested it was always the bottom slice that was inoculated. Samples were kept in petri dishes with lid during incubation for up to 7 days. As expected, L. monocytogenes did not grow within 7 days at 10 °C in any of the ingredients when assessed separately, neither in single nor in double layers. The same was observed for all pairwise combinations involving bread or cold-smoked salmon. However, an increase of 4 log??-cycles was found when tomato and cheddar were combined. This indicated that the interface, created between tomato and cheddar, has the ability to eliminate the growth-inhibiting characteristics of at least one of these ingredients. Investigating growth in the model-system applied in this study, and combining it with detailed product characterization, may prove helpful for development of predictive models tailored for sandwiches.

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O6.11 On the evaluation of the effect of natural antimicrobials and cold atmospheric plasma on the inactivation of Listeria monocytogenes in novel 3D solid(like) food model systems

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Many studies focus on new natural antimicrobials (NA) and the development of novel hurdle strategies for their application in food industries to replace chemical preservatives. It is known that the bacterial behaviour (growth) and response to processing approaches is very different in a 3D system where bacteria form colonies as opposed to planktonic growth. Grape seed extracts (GSE) are known to have antimicrobial properties however there are very limited studies on their antimicrobial efficacy, most of which focus on specific food products. Therefore, the aim of this study is to obtain a fundamental understanding of the impact of GSE on the microbial dynamics as affected (i) by structure (in solid model systems) and (ii) cold atmospheric plasma (CAP) which is a novel minimal processing approach.

Representative to several food products, multiple food model (FM) systems were developed with different physiological and rheological properties. More specifically, three monophasic polysaccharide FMs (1.5, 2.5 and 5% xanthan gum), a biphasic FM (5% polysaccharide + whey protein) and a triphasic system (biphasic + 10% vegetable oil) with the addition of GSE (1%) and nisin (35 IU/mL) were created. L. monocytogenes (10⍰ CFU/mL) was inoculated on the surface of the FMs, and the microbial dynamics were observed for 24 h. Sequentially, CAP was used as combinatory treatment to investigate possible synergistic effects of NA and CAP.

Overall, the results confirmed the antimicrobial activity of GSE and nisin against L. monocytogenes, as the microbial activity was reduced in all FM systems under study. It is worth noting that the extend of the antimicrobial effect was dependent on the biochemical composition and the stiffness of the 3D FM used. The CAP treatment further reduced the bacterial population, and the combinatory treatment was more effective than the individual treatments.

Generally, our findings show that GSE and nisin have a good potential as NA when incorporated in 3D systems. Nevertheless, efficiencies differ to liquid systems, which highlights the necessity of including 3D solid/solid-like systems in food safety studies. This allows improved application of NA alone or in combination with other mild technologies to develop novel and sustainable hurdle approaches.

Controlling and predicting microorganisms in food ecosystems

O6.12

Control of Salmonella and Cronobacter spp. in powdered infant formula

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Following the 2017 salmonellosis outbreak due to the consumption of powdered infant formula (PIF), ANSES updated the report issued in 2008 on the management of the microbial hazards in PIF.
The objective of the work was to conduct a hazard analysis of PIF production process and to evaluate the effectiveness of control measures and sampling plans implemented by food operators.
The expertise was carried out by a working group, based on scientific and technical literature and the analysis of four sanitary control plans representative of the diversity of manufacturing processes in France. The manufacturing process of PIF was analyzed to identify potential routes of contamination and key control measures. Contamination and sampling procedures were modelled to estimate the probability of detection of contaminated batches and the related risk.
Salmonella spp. and Cronobacter spp. are the main hazards associated with PIF. Contamination may occur after pasteurization originated from contaminated ingredients or the processing environment. Therefore, the safety of PIF is mainly ensured through the strict application of good hygiene practices (prevention of environmental contamination, analysis of ingredients, personal hygiene, cleaning, etc.) and the HACCP principles
The sampling of the finished products cannot be considered as a control measure. Indeed, for statistical reasons, when microbial contamination is low, an unrealistic number of samples need to be tested to detect contaminated batches.
Prevention and monitoring of contamination of the processing environment are essential to ensure product safety. The scientific opinion provides general recommendations and proposes tools to identify the sources and routes of contamination, establish an environmental sampling plan and investigate causes in the event of detection of non-conformities.
This expertise provides both the competent authorities and food operators with recommendations and operational tools to improve the control of hazards in PIF.

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O6.13 Effect of the glass transition of dried bacterial cells on the survival under desiccation environment

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To investigate the cause of adaptation of Cronobacter sakazakii to desiccation stress, the present study focused on the glass transition phenomenon of dried bacterial cells using a thermomechanical technique. The mechanical glass transition temperature (Tg) of dried C. sakazakii cells per se prepared by different drying methods (air-drying and freeze-drying) and with different water activity (aw) levels (0.43, 0.57, 0.75, and 0.87) was determined. In addition, we investigated the survival of two strains of C. sakazakii (JCM 1233 and JCM 2127) prepared by different drying methods under different storage temperatures (4, 25, and 42°C) and aw conditions (0.43 and 0.87). While the Tg of the air-dried C. sakazakii cells increased as the aw decreased, the freeze-dried C. sakazakii cells showed an unclear aw-dependency of the Tg. Air-dried C. sakazakii showed a higher Tg than freeze-dried C. sakazakii at aw < 0.57. Freeze-dried C. sakazakii cells were more rapidly inactivated than air-dried cells regardless of the difference in aw and temperature. The difference between the Tg and storage temperature was used as an index considering the difference in the drying methods and aw levels. As the difference between the Tg and storage temperature increased by > 20°C, the dried C. sakazakii cells regardless of the drying methods survived stably. In contrast, when the difference between the Tg and storage temperature was reduced by < 10°C, the viable cell numbers in dried C. sakazakii cells were quickly decreased. Thus, the Tg is a key factor affecting the desiccation tolerance of C. sakazakii.

O6.14 Prediction of population behavior of Listeria monocytogenes in food using data mining and Combase Database

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In predictive microbiology, statistical models are employed to predict bacterial population behavior in food using environmental factors such as temperature, pH, and water activity. As the amount and complexity of data increase, handling all data with high-dimensional variables becomes a difficult task. We propose a data mining approach to predict bacterial behavior using a database of microbial responses to food environments. Listeria monocytogenes, which is one of pathogens, population growth and inactivation data under 1,007 environmental conditions, including five food categories (beef, culture medium, pork, seafood, and vegetables) and temperatures ranging from 0 to 25 °C, were obtained from the ComBase database (www.combase.cc). We used eXtreme gradient boosting tree, a machine learning algorithm, to predict bacterial population behavior from eight explanatory variables: 'time', 'temperature', 'pH', 'water activity', 'initial cell counts', 'whether the viable count is initial cell number', and two types of categories regarding food. The root mean square error of the observed and predicted values was approximately 1.0 log CFU regardless of food category, and this suggests the possibility of predicting viable bacterial counts in various foods. The data mining approach examined here will enable the prediction of bacterial population behavior in food by identifying hidden patterns within a large amount of data.

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O6.15 Role of substrate, oxygen and competitive microbiota on detection efficacy of Campylobacter

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Campylobacter is the leading cause of zoonotic gastroenteritis, making reliable detection in food important. Low storage temperatures of food can cause sub-lethal cell damage, which is why the ISO 10272-1:2017 procedure includes an enrichment step in Bolton Broth (BB) to repair damage and increase cell concentrations, thereby supporting detection of Campylobacters. Extended-spectrum beta-lactamase-producing (ESBL-) Escherichia coli are known to suppress the growth of Campylobacters during enrichment resulting is false-negative detection outcomes, but the cause for this growth suppression is unknown. The objectives of our study were i) to quantify the growth kinetics of C. jejuni and C. coli in BB to assess whether there is a species difference, and ii) to investigate whether Campylobacters and ESBL-E. Coli compete for the same medium components which could cause growth repression. Monitoring the growth of 13 C. jejuni and 10 C. coli strains in BB demonstrated that the lag phase and growth rate were not species dependent, but the lag phase increased when cells were damaged by freeze-stress. Quantitative scenario analyses revealed that a 'worst-case'-scenario, that took into account the effect of strain variability and the physiological state of the cell, resulted in positive detection outcomes. This confirmed that other factors cause false-negative testing outcomes of food samples. Observed trends in compound utilization during growth in BB were similar for C. jejuni and C. coli, while ESBL-E. Coli was more efficient in utilizing substrates, including serine. Since final cell densities of C. jejuni and C. coli in co-cultures with ESBL-E. Coli were not enhanced by the addition of serine and final cell densities were similar in fresh and spent media, growth suppression of Campylobacter in co-culture with ESBL-E. Coli was not caused by a lack of substrates or the production of inhibitory compounds. Presence of fermentation end-products during co-culture enrichments pointed to a role of oxygen limitation during these enrichments. Indeed, higher oxygen availability during enrichment increased final cell concentrations of Campylobacter in co-culture to those levels without competition. These findings highlight the critical role of oxygen during the growth of Campylobacter and offers potential for improvement of enrichment efficacy.

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O6.16 Tracing Listeria monocytogenes in the production chain of Atlantic salmon (Salmo Salar L.)

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Salmon products are often eaten raw or mildly processed, putting the foodborne pathogen Listeria monocytogenes as one of the salmon industry's most severe food safety concerns. The prevalence of L. monocytogenes in raw salmon products is low, however L. monocytogenes is regularly detected in the salmon slaughterhouses and do represent a potential risk. Furthermore, detecting L. monocytogenes in prepackaged products can lead to costly withdrawals of whole batches. Previous research has stated contamination of L. monocytogenes through several pathways, including personnel, seawater, and the external environment. The potential to grow and survive in biofilms is also significant. However, scant information exists about contamination pathways in the production chain of Atlantic salmon from the feed factories, via production farms to slaughtering/processing. Therefore, the present project was set up to gain more knowledge about these contamination pathways of L. monocytogenes in the production chain of Atlantic salmon.

The experimental design was selected based on an initial qualitative risk assessment of the production chain by using on-site inspections, stakeholder workshops, and literature research as data input. Three feed producers (including feed boats) and three salmon farms (including their feeding barges) were selected and followed through salmon growth. Moreover, when the fish reached commercial size, sampling was performed during well-boat transport, slaughtering, filleting, and packaging. In total, 1727 samples were collected, 339 from the feed/feeding chain, 1022 from production/well-boat transport, and 366 from the processing plant. The average share between environmental and feed/fish samples was 20% and 80%, respectively. Listeria spp. and L. monocytogenes were detected in 8.0 and 2.3% of the samples, respectively.

A total of 425 Listeria-isolates from the experiment (some in duplicates) representing the different parts of the value-chain, and 76 from the company`s own quality department, were selected for ON-rep-seq as a pre-screening method prior to whole-genome sequencing. WGS on selected isolates taxonomically classified as L. monocytogenes revealed 11 different MLST sequence types. Higher diversity was found among feed samples and within the feed environment compared to the filleting department where only ST8, 37 and 637 were detected. This shows a potential link between contaminated feed and the processing environment.

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O6.17 Abiotic elicitation of plant secondary metabolites limits Salmonella enterica in lettuce and kale

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Leafy greens are frequently implicated in foodborne illness and outbreaks that impose a hefty public health burden and steep financial losses. Contamination that occurs at the pre-harvest stage is difficult to detect and eliminate. The principal strategy is to exclude enteric pathogens from fields and controlled environments through Good Agricultural Practices (GAPs). Effective strategies are needed to tackle enteric pathogens that breach GAPs before establishment in the plant niche. Plants produce a multitude of secondary metabolites for plant defense against phytopathogens and herbivores and protection against abiotic stresses. Our objective was to assess the impact of drought-induced secondary metabolite elicitation on Salmonella enterica in lettuce and kale. Lettuce (Lactuca sativa, L. var. longifolia cv. 'Parris Island Cos', a Romaine type, and L. sativa var. crispa cv. 'Mascara' a loose-leaf type) and kale (Brassica oleracea L. var. acephala cv. 'Improved Dwarf Siberian') were greenhouse-grown at 16 h light:8 h photoperiod and 23°C/18°C day/night temperatures to the baby leaf stage then subjected to drought by withholding watering for six days or watered regularly (controls). Salmonella Newport or Typhimurium were inoculated on third true leaves of plants at 106 CFU/plant. After 24 hours of incubation, leaves were cut and processed for Salmonella enumeration. Leaf samples were collected, flash frozen, ground and mixed in methanol/formic acid for phytochemical measurements of total phenolics, flavonoids, anthocyanins (lettuce) and glucosinolates (kale). Data were analyzed for a drought effect using JMP Pro 15.2.0. Drought enhanced accumulation of total phenolics, flavonoids and anthocyanins in loose-leaf but not Romaine lettuce, compared to control plants ($p<0.05$). In kale, total phenolics, flavonoids and glucosinolates were also induced under drought relative to controls ($p<0.05$). Bacterial assays showed that plants subjected to water stress provided a less favourable niche for Salmonella than regularly watered plants ($p<0.05$). Correlation analysis found inverse correlations between Salmonella counts on leaf surfaces and total flavonoids and phenolics in kale and lettuce, and anthocyanins in lettuce ($p<0.05$). Abiotic elicitation of secondary metabolites in plants could be a strategy to augment phytocompounds in kale and lettuce that not only enhance health beneficial quality, but also improve food safety by limiting Salmonella.

Controlling and predicting microorganisms in food ecosystems

O6.18 Bacteriophage control in dairies through non-thermal methods: UV-C treatment of whey for elimination of Lactococcus lactis bacteriophages

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Bacteriophages of lactic acid bacteria may occur in raw milk in titres of up to 104 plaque forming units (pfu) mL-1. Since few of them withstand pasteurization, they can get into the cheese process. In whey, bacteriophages accumulate to titres of up to 109 pfu mL-1. Recycling of contaminated whey leads to fermentation failures and aroma defects. Due to heat sensitivity of whey proteins, thermal treatments >75°C are undesirable. It should be investigated whether ultraviolet-C (UV-C) light, as non-thermal method, is suitable for bacteriophage reduction in sensitive media. The aim of the study was the production of "phage-free" whey by UV-C irradiation.

For the experiments, two representatives (P001, P008) of the most common Lactococcus lactis bacteriophage groups c2 and 936 were chosen. Inactivation was performed in a UV-C chamber (BS-02, Opsytec Dr. Gröbel, Ettlingen, Germany) with low-pressure mercury-vapor lamps at laboratory scale. Water (reference), ideal whey (permeates obtained by membrane filtration from skim milk, using 0.1 µm and 60 nm membranes) and milk (fat contents of 0.3, 1.5 and 3.8% fat) were spiked with bacteriophages (initial titre of 109 pfu mL-1). The spiked media poured in a petri dish (2 mm layer) were treated with UV-C doses varying from 0.025 to 5 J cm-2 at 254 nm wavelength. Before and after treatment, bacteriophage titres were determined using the plaque assay.

In water a UV-C dose of 0.1 J cm-2 was necessary to obtain a complete inactivation of bacteriophages (9-log units). However, in ideal whey samples produced using 60 nm and 0.1 µm membranes, UV-C doses of 2.5 and 5 J cm-2 were required to obtain the same inactivation effect, respectively. Since the absorption coefficient rises with increasing dry matter of the respective media, higher UV-C doses were required in whey samples.

Further work is in progress to investigate the inactivation of other problematic or thermoresistant bacteriophages. Furthermore, UV-C light induced changes (e.g.riboflavin degradation) of the media should be analyzed to define a process window, which guarantees gentle treatment with high bacteriophage inactivation. Moreover, a scale-up should be conducted using a continuous UV-C reactor to show its feasibility for industrial applications.

Controlling and predicting microorganisms in food ecosystems

O6.19 Lemongrass oil-based nanocomposite: An active material of biobased packaging film for food preservation

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Food-borne diseases can be caused after consuming contaminated food carries food-borne pathogens which leads to serious health issues. To avoid pathogen entry and growth with an extended shelf life of food, we aimed to synthesize nanocomposite, an active material that comprises lemongrass nanoemulsion that encapsulates silver nanoparticles (Ag-LNE) to prevent the growth of food pathogens. Silver nanoparticles (AgNPs) were synthesized by a chemical approach while high-energy sonication method was adopted for the synthesis of lemongrass nanoemulsion (LNE). AgNPs and LNE were further characterized by their size and other physico-chemical characterizations. It was found that silver nanoparticles have a size range of 10-30 nm, whereas the droplet size of the nanoemulsion is within 100 nm. Further, it was noted that the contact angle of lemongrass nanoemulsion (61.86 ±1.96) with the substrate was higher than crude lemongrass oil (46.08 ± 1.06) indicating the higher wettable behavior of the nanoemulsion.
A nanocomposite of Ag-LNE was synthesized by encapsulating silver nanoparticles into nanoemulsion and it was evident by the increment in the size of the nanocomposite. Furthermore, characterization of nanocomposite by FT-IR revealed the presence of functional groups which correspond to LNE and AgNPs. Moreover, Thermogravimetric analysis (TGA) and ICP-AES studies also revealed the encapsulation of silver nanoparticles in lemongrass-based nanocomposite. The antimicrobial activity of nanocomposite against Escherichia coli was significantly higher, by 36 % and 9 % than silver and lemongrass nanoemulsion respectively. In addition, the minimum inhibitory concentration of the nanocomposite was 0.75 % to inhibit the growth of E. Coli. Thus, the present study reveals the efficacy of Ag-LNE nanocomposite as an active component of food packaging film, which could be a potential food preservation tool by enhancing the shelf life of food-stuffs sustainably.

Controlling and predicting microorganisms in food ecosystems

O6.20 Microbial food safety in real time by integrating MICROHIBRO prediction capabilities into an event-based food safety system

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MICROHIBRO is an online free-access user-friendly software tool to quantitively assess the fate of microorganisms in foods and their impact in public health. Connecting prediction tools to sensing data along the product supply chain would yield more accurate and precise predictions of microbial behaviour in foods while this information may be available in real time for food players. Hence, specific value chains can benefit from MICROHIBRO prediction capabilities for assessing and managing the microbial safety of their products. This work aimed at integrating IT-standards-driven solutions in MICROHIBRO to improve food traceability and food safety assessment and management over the food logistics distribution chain. MICROHIBRO metadata scheme was adapted to use events generated in Electronic Product Code Information Services (EPCIS), which are GS1 standards that enable sharing visibility event data regarding the physical movement and status of products throughout the food supply chain. MICROHIBRO vocabulary analysis were developed to identify compatibilities in JSON schema with requirements of EPCIS event system. JSON and JSON-LD formats are supported by EPCIS version 2.0. EPCIS data from specific steps of a product supply chain, such as temperature-time profiles and environmental conditions during transport, can be used by MICROHIBRO algorithms to develop fit-to-purpose prediction analysis. The effects of the different stages of the logistics chain on the fate of target microorganisms can be mathematically described in MICROHIBRO risk assessment module, by plugging EPCIS events data from food analysis or sensors obtained from a food supply chain. In addition, an alert system to detect non-conforming products can be derived from the interpretation of EPCIS data by an Expert Algorithm System. Case-studies on the food chain of different products (e.g., cheese, strawberries) have been designed in the framework of the European Projects, MEDIFIT and BiofreshCloud, showing the potential of MICROHIBRO to provide food safety rapid responses in a dynamic food environment. The implementation of EPCIS standards in MICROHIBRO increases food supply chains traceability and provides stakeholders with a prospect of the microbial safety of a given product at a specific food supply stage. Therefore, it helps meeting the demands for accurate and detailed information on product microbial safety.

Controlling and predicting microorganisms in food ecosystems

O6.21 Monitoring Listeria in a dynamic frozen vegetable processing environment

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Listeria (L.) monocytogenes is a gram-positive bacterium that is the causative agent for the foodborne illness listeriosis. Human listeriosis is especially of concern for the elderly, immunocompromised persons, pregnant women, and infants and although listeriosis is a rare disease, the case fatality rate is high. In the previous few years, listeriosis has also been linked to contaminated frozen vegetables in listeriosis outbreaks in the US and the EU.

In the current study, Listeria were monitored in a frozen vegetable processing facility over the course of three years (2019-2021). The aim of the study was to identify Listeria hotspots and transmission routes and to characterize (in-house) Listeria spp. strains.

The company runs a sophisticated sampling system including environmental swab samples and product samples to monitor pathogens. The resulting monitoring data was assessed to identify potential Listeria hotspots and the spatial and temporal distribution of Listeria in certain areas in the facility. In addition, Whole Genome Sequencing (WGS) data of L. monocytogenes isolates were used to identify in-house clones based on MLST, cgMLST and whole genome SNP analysis. The presence of stress, virulence and plasmid genes was analyzed in selected L. monocytogenes isolates.

Listeria were widely distributed across the entire food processing environment as well as on food contact surfaces, indirect food contact surfaces and non food contact surfaces. Three different potential Listeria hotspots were identified based on the criteria of high Listeria prevalence and repeated isolation over time. Intense hot spot sampling suggested conveyer belts as source of contamination. Among all L. monocytogenes isolates, five different in-house clones (ST8-cg1349, ST8-cg6243, ST20-cg3737, ST224-cg5623, ST451-cg4117) were identified. Whole genome SNP analysis enabled an in-depth analysis of representative L. monocytogenes isolates showing that the identification of in-house clones based on cgMLST is not sufficient but a higher resolution analysis is needed.

In conclusion, this study showed that WGS is a strong tool in tracking L. monocytogenes in a food processing environment.

Controlling and predicting microorganisms in food ecosystems

O6.22 Influence of strain variability of Listeria monocytogenes isolates on the estimation of the resistance to NaCl

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Listeria monocytogenes was, in 2020, the fifth mostly reported zoonosis in humans in the European Union, standing out fish, meat, cheese and ready-to-eat products contaminated through the processing chain, among the main implicated foods. A better knowledge on the variability in strains resistance to different environmental factors would provide more accurate estimations for microbial risk assessment. The main objective of this study was to determine the resistance of a collection of strains of L. monocytogenes isolated from ready-to-eat meats, fishery products and industries facilities to salt concentrations. For this, microbial growth of 31 strains of L. monocytogenes was monitored by absorbance measurements in Bioscreen C equipment at different salt ranges (0-10 % v/v). The data obtained were processed in MS Excel 365 and in R v4.1.2 to fit a linear model to the relationship between the absorbance reached after growth as a function of the percentage of NaCl, for each strain. The results showed a high variability in salt resistance ranging from 4- 6% for the strains L1X6, L1X9, CG1-S3, L1L1 and L1CH4, and from 10-13% for the strains L1CH2, L1CH5, GL24, L1X2 and L1X11. The most resistant strains belonged to serotype 4b and were mainly isolated from drainages, sinks, handlers' gloves and trolleys as well as from raw fish and cured meats. Finally, from the results obtained, it can be concluded that the resistance to inhibition by NaCl depends on the L. monocytogenes strain object of study

O6.23 Inactivation of Salmonella Typhimurium in skimmed milk powder by Radio Frequency heating

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Radio Frequency (RF) is a dielectric heating process which provides rapid and volumetric heating, outperforming conventional heating methods. The technology is particularly suited for processing of homogenous, low moisture food products. Pathogenic bacteria such as Salmonella can survive in these products during prolonged storage, leading to numerous outbreaks and product recalls.
This study investigates the inactivation of the foodborne pathogen Salmonella Typhimurium (initial cell density of approximately 6 log (CFU/g)) in skimmed milk powder in a 50 Ω, 4 kW, 27.12 MHz RF set-up. The surrogate microorganism Enterococcus faecium was inoculated in separate samples to establish the RF heating process and uniformity. The temperature was monitored during treatment using an optic fibre measurement system and with a posteriori IR imaging on the product surface. To further establish the efficiency of each process and identify possible deteriorative changes to the milk powder, quality aspects like solubility and colour were monitored.
Initially, 2.5 kg of milk powder were dynamically heated to end temperatures of 85 ?C and 95 ?C at a power of 1 kW, leading to approximate log reductions of 0.8 and 1.6 log, respectively. Aiming for higher inactivation rates, the power-time profile was altered; milk powder was first heated to 55°C at a power of 1 kW and then heated further at a slower rate at a power of 200 W to end temperatures of 85 ?C and 95 ?C which resulted in approximate log reductions of 1.8 and 4.7 log, respectively. The highest sublethal injury was observed at the 1 kW-200 W until 85 ?C profile. Even though the slow rate treatment until 95 ?C resulted in the most changes concerning colour and solubility, the decrease in quality was limited. Overall, a slower heating profile using lower power resulted in higher inactivation rates of Salmonella in skimmed milk powder, while still providing a high-quality end product.

Controlling and predicting microorganisms in food ecosystems

O6.24 Meta-analysis of the microbial inactivation under non-thermal high pressure processing of fruit and vegetable juices and purees

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High pressure processing (HPP) is a non-thermal preservation technology alternative to thermal pasteurisation for fruit and vegetable juices and purees, with an increasing market trend thanks to its minimal effect on nutritional and organoleptic characteristics. The purpose of this study was to collect and meta-analyse available data on HPP inactivation of Listeria spp. and Escherichia coli in fruits and vegetables. From an extensive literature search, 55 articles were selected providing Log10 reduction data (1284 values). Up to 12 articles provided data to estimate the Dp, as the HPP time to obtain a decimal reduction (84 values). The time to achieve 5-Log reduction (D5) as the target performance criterion, was calculated accounting for the inactivation during pressure come-up and shoulder. Principal Component Analysis and Generalised Linear Mixed models were used to identify significant factors impacting on kinetic parameters (Dp and D5), including pressure, pH category (high acid (pH<4), acid (4≤pH≤4.5), low acid (pH>4.5)) and microorganism as fixed effects, while strain (nested to microorganism) and study as random effects. Secondary Bigelow models with the LogDp_ref or LogD5_ref and zP as a function of pH category and/or microorganism were fitted to the entire data set. Pressure level and pH category explained 91-93% of the data variability and the mixed models confirmed the significance of these factors, together with the microorganism. The random effects associated with the study and strain were not statistically significant. Through the global model fitting to LogDp and LogD5 data, a Bigelow-based model was obtained with a LogD5_ref and LogDp_ref parameter depending on pH category and a common zp for Listeria and E. Coli. When compared with the Log10 reduction data collected from literature (not used to build the model), most of the predictions provided by the LogDp model were within the acceptable simulation zone (±1 Log) or fail-safe, while fail-dangerous predictions occurred only with 15% and 21% of the data for Listeria and E. Coli, respectively. The global LogDp model is proposed as a good conservative tool useful for risk assessment, for benchmarking and for setting the HPP conditions to comply with the performance criteria.

O6.25 Using lactic acid bacteria biopreservation as an alternative to nitrite for ensuring "foie gras" microbial safety?

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France is the 1st producer of "foie gras" with half of the production being sold as half-cooked. As several processed meat products, their production is accompanied by the addition of nitrites for their contribution to the pink color and for their antimicrobial effect, in particular against Clostridia (1). Nevertheless, because of the carcinogenic effect of some nitrosamines, resulting from nitrites, the use of nitrites is controversed and alternative solutions to their use are required. Foie gras can be spoiled by psychrotrophic Clostridia and also by lactic acid bacteria. These later can also be used as protective cultures through biopreservation. We investigated the potential of a collection of lactic acid bacteria, isolated from foie gras or from the processing environment, for inhibiting the development of Clostridium algidicarnis, Clostridium frigidicarnis, Latilactobacillus sakei, and Latilactobacillus curvatus, and Listeria monocytogenes. Out of 122 isolates belonging to Carnobacterium, Weissella, and Lactobacillus sensu lato genera, 70 do not produce histamine above 50 ppm. Antibiotic suceptibility was assessed based on the EFSA recommendations (2), and only 19 isolates were sensitive to the 8 tested antibiotics. Tetracycline, kanamycin or chloramphenicol resistance was detected in Leuconostoc isolates, whereas Carnobacteria were resistant to clindamycin, ampicilline or streptomycin. Among the 19 antibiotic sensitive isolates, several tests were performed to assess their ability to inhibit the growth of target strains and to determine the inhibitory mechanism. The data concerning antibiotic resistance and inhibitory potential will be presented.

(1) Majou & Christeans, Meat Sci, 2018, DOI: 10.1016/j.meatsci.2018.06.013
(2) EFSA, EFSA J, 2012, DOI: 10.1016/j.meatsci.2018.06.013

Controlling and predicting microorganisms in food ecosystems

O6.26

An agent-based model to predict SARS-CoV-2 contamination of surfaces and meat cuts in processing plants

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During the Covid-19 pandemic, clusters were observed in the food production sector. The transmission of coronaviruses among humans was reported mostly through aerosol. Contaminated droplets emitted by infected persons (sneezing, coughing…) could remain in aerosol, fall on inert surfaces or food, and possibly be inhaled by other persons.

This work aimed to develop a modular agent-based model (ABM) to simulate SARS-CoV-2 circulation as a case study for predicting respiratory disease transmission and food contamination in multi-room food processing plants. This ABM considers spatio-temporal interactions between workers, air, surfaces, and meat cuts by tracking the circulation of contaminated droplets in the facility. Epidemiological investigations, industrial surveys, and literature data analysis were carried out to define model parameters such as: plant characteristics (dimensions, ventilation…), infection parameters (workers' health status, presence of symptoms, virus persistence on surfaces and meat under different conditions…), management measures (distance between workers, mask wearing, disinfection frequency…), meat processing performance (carcasses and meat cuts weights, quantity per day…) and transfer rate between surfaces and meat products. The model run with five-minute time steps during 42 days for different scenarios. It provides as outputs the number of infected workers, the contamination of the environment (air/surfaces) over time as well as the proportion of contaminated meat cuts in each scenario.

The first results obtained suggested that wearing a mask combined with a high air renewal rate reduced notably surface and food contamination. The presence of symptomatic persons in the facility, with frequent sneezing or coughing events, conducted to an increasing number of contaminated droplets in the air and on surfaces, increasing then the exposition of other workers and food contamination premises.

Simulation results for SARS-CoV-2 diffusion within workers are confronted with epidemiological data for validation purposes. Simulation results for surfaces and meat cuts contamination indicate relevant experimental designs that would be required to demonstrate contamination.

The developed model could constitute a useful safety management tool to illustrate the effects of prevention strategies (mask wearing, distancing…) on the risk of contamination (workers/food) from a perspective of workers health protection, preventing food contamination and supply disruption.

Food Microbiology in the context of One-Health (animal, plant and human)

O7.1 Metagenome-wide analysis of antibiotic resistance genes in microbiota of probiotic products, starter cultures, and cheeses

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Background: Antimicrobials have become one of the foundations of modern medicine, yet the rapid spread of resistance threatens their effectiveness. Probiotic bacteria, starter cultures, and fermented products were recognised as a reservoir of resistance, but the actual risk of these products in terms of resistance has not yet been properly evaluated. Objectives: The aim of our study was to predict antimicrobial resistance genes (ARGs) in shotgun metagenomic sequences of dietary supplements, starter cultures, and cheeses and to compare the abundance of these genes between the sample groups. Methods: Metagenomic DNA was isolated from 75 samples representing different product groups: dietary supplements, starter cultures, cheeses prepared without starter cultures (C-S), and cheeses made with starter cultures from pasteurised (C+SP) or raw milk (C+SR). Pair-end DNA sequencing was performed on the Illumina NovaSeq platform using the Illumina Truseq Nano library. The produced reads were trimmed, filtered, and assembled de novo using publicly available programmes. Coding sequences were mined for the presence of known ARGs. Sequencing and bioinformatic analyses were done by Microsynth AG (Balgach, Switzerland). Results: Sequencing data revealed a total of 539 ARGs conferring resistance to a variety of antimicrobials, including tetracyclines, aminoglycosides, beta-lactams, macrolides, trimethoprim, glycopeptides, fosfomycin, phenicol, and quinolones. ARGs involved in resistance to tetracyclines (e.g. tet(34), tet(M), tet(S), tetU), aminoglycosides (e.g. ANT(6)-Ia, str), beta-lactams (e.g. PBPs, ampH), and macrolides (e.g. mph(D), mdf(A), mre(A)) were most abundant, whereas others were less common. Across all sample sources, only tetracycline ARGs were found. Statistical analysis also indicated that the C-S and C+SR groups were highly contaminated with ARGs compared with dietary supplements and, especially, starter cultures and the C+SP group, which were not rich in ARGs. Correspondingly, ARGs were most diverse in the first two groups. Conclusions: Our study highlights that starter cultures, dietary supplements, and cheeses from pasteurised milk do not represent a substantial reservoir of resistance and thus a risk in terms of resistance compared with cheeses made without starter cultures and cheeses made with starter cultures from raw milk.

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Food Microbiology in the context of One-Health (animal, plant and human)

O7.2 Cereulide production and growth of Bacillus cereus in cereal mixes

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Bacillus cereus is a critical foodborne pathogen for the food industry and once its emetic toxin cereulide is formed, it cannot be destroyed. Cereal matrices support B. cereus development and are used as a basis for a wide range of products. The objective of this study is to analyze the growth of B. cereus and cereulide production in three cereal mixes with varying sugar contents.
Vegetative cells of B. cereus' emetic reference strain F4810/72 were inoculated in irradiated cereal mixes A, B and C at an inoculum level of approximately 2 log CFU/mL. The cereal mixes differ in their glucose and maltose content. Cereal mix A contains high levels of both sugars, cereal mix B contains low amounts of both sugars, and, cereal mix C contains only maltose. Growth of B. cereus and cereulide formation were studied over time at different temperatures, ranging from 9 °C to 37 °C.
Growth of B. cereus and cereulide formation was observed in the range of 12 °C to 37 °C, whilst at 9 °C neither growth nor cereulide were quantifiable for up to 578 hours. The time required for the first cereulide quantification varied with the temperature and the cereal mix. Cereulide was quantified earlier and at higher levels in cereal mix C in comparison to cereal mixes A and B for all the temperatures analyzed. Moreover, the rate at which the toxin is produced is up to four times higher in the former. This difference was independent of B. cereus growth, as similar growth levels were obtained for cereal mixes B and C and a slower growth rate for cereal mix A. Furthermore, there was no cereulide detection for B. cereus concentrations below 6 log CFU/mL for all studied matrices.
The results show that cereulide production is lower in matrices containing monosaccharides than in matrices containing disaccharides. This should be further investigated with different strains and sugar sources.

O7.3 Crosstalk between Fusarium verticillioides and Aspergillus flavus on maize

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In agri-food ecosystems, intensification of crop production promotes spread, virulence and diversity of fungal plant pathogens. An important group of fungal plant pathogens comprise fungi able to produce toxins which can be poisonous to the crop (phytotoxins) or to end-consumers eating the crop or derived products (mycotoxins). Many of these pathogenic fungi comprise species found on maize and maize derived products, such as Fusarium verticillioides (F. verticillioides) and Aspergillus flavus (A. flavus).
The occurrence of mycotoxins in maize fluctuates from year to year, as it is a multifactorial phenomenon influenced by confounding factors such as climate change, geographic region, agro technical measures, plant variety, and so on. With the increase of temperature year by year, the confrontation of fumonisins and aflatoxins becomes a significant problem, which means maize will be contaminated by a potentially more toxic cocktail of fumonisins and aflatoxins. This co-exposure to multiple mycotoxins is a serious concern for consumers, public health authorities and agri-food sector. In the current study, this emerging plant health and food/feed safety problem was studied by the tripartite interaction between F. verticillioides, A. flavus and maize. At first, the in vitro interaction between F. verticillioides and A. flavus was explored by a dual culture method on agar media. On daily basis the mycelium growth of both pathogens was measured. After seven days the fumonisins and aflatoxins were extracted and quantified by LC-MS/MS.
According to the vitro experiment, the growth rate of F. verticillioides was significantly (p-values$<<0.01$) suppressed by the presence of A. flavus, in contrast the A. flavus growth rate that was only slightly suppressed by F. verticillioides. The mycotoxins analysis revealed that when A. flavus is present, the concentrations of fumonisins produced by F. verticillioides were higher, and although less pronounced F. verticillioides promoted A. flavus to produce more aflatoxins. So, it can be concluded that there is a possible antagonistic effect between the growth of F. verticillioides and A. flavus, however there is a synergistic effect between the mycotoxins production of F. verticillioides and A. flavus. Validation of obtained in-vitro data on maize cobs will provide further insights into these interactions.

Food Microbiology in the context of One-Health (animal, plant and human)

O7.4 Immunomodulatory role of Propionibacterium freudenreichii extracellular vesicles

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The role of Propionibacterium freudenreichii in mitigating inflammation has been a subject of study for many years. Lately, it has been shown that immunomodulation properties are strain-specific and that the major responsible for immunomodulation by strain CIRM-BIA129 is the presence of the surface layer protein B (SlpB), which has presented immunomodulation even when expressed in other bacteria. Extracellular vesicles (EVs) are nanosized spherical structures, produced by organisms of all kingdoms, including bacteria. They have been associated with inter-organism communication, pathogenesis, competition, and immunomodulation. Recent studies were aiming to address the role of EVs, in the probiotic effects of bacteria. The properties of P. freudenreichii CIRM-BIA129-derived EVs have been investigated. EVs produced by CIRM-BIA129 cultured in milk ultrafiltrate medium (UF) have been characterized regarding size and morphology. UF-derived EVs displayed a monodisperse pattern with a modal size of 84.80 ± 2.34 nm. They are composed of a wide variety of proteins, mainly involved in metabolic processes, cellular processes and signaling, and storage and processing of information. Among these proteins, SlpB was found in high abundance. P. freudenreichii EVs were able to inhibit, in a dose-dependent manner, the increase of IL-8 production in HT-29 cells induced with LPS, due to NF-KB pathway inhibition, without causing cell cytotoxicity. EVs produced by a CIRM-BIA129 mutant strain with a knockout for SlpB showed a less efficient reduction in IL-8 production. Results have shown that the environmental conditions are able to modify EVs content and, consequently, their immunomodulatory effects. A change in the growth medium, from UF to YEL (yeast extract-lactate) showed a lower production of EVs, with a slightly larger size. EVs produced in YEL did not perform as well in the inhibition of the NF-KB pathway and had no effect on IL-8 production. Recent results have shown that P. freudenreichii EVs were able to protect Caco-2 cells from inflammation-induced excessive permeability. Altogether, these results show that Evs produced by beneficial propionibacteria are able to trigger immunomodulation, similar to the parental strain.

Food Microbiology in the context of One-Health (animal, plant and human)

O7.5 Biocontrol of shiga toxin-producing Escherichia coli in foods and food contact surface using bacteriophage cocktail

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Shiga toxin-producing Escherichia coli; STEC remains a global public health and food safety challenge. Despite the various control measures available, there are still reports of outbreak and illness caused by this pathogen. It is therefore important to reduce this pathogen along the food chain. In this study, seven STEC phages were isolated from vegetables irrigation ponds. The host range and lytic activity of phages were characterized using 114 STEC strains previously isolated from fresh and ready-to-eat vegetables. P1, P4 and P7 showed wide host range and broad lytic activity. A phage cocktail designated CCD1 containing the three lytic phages for STEC was tested for its ability to reduce contamination of hard surfaces (gypsum boards), fresh vegetables (lettuce, cabbage, cucumber and carrot). The samples were experimentally contaminated using a mixture of five STEC strains and were treated with CCD1(test biocontrol) and sterile SM buffer (control samples) at 4°C and 25°C. After 10 minutes of treatment with the CCD1 cocktail using different phage concentration (10^{1}, $10^{}$, and $10^{}$ PFU/ml), statistically significant reductions ($P = <0.05$) of 99.5%, 97%, and 95%, respectively were observed in the number of STEC recovered from the gypsum board surface. Also, reduction was observed in the fresh vegetables samples ranging from 95% (at 30 mins) to 99.99%(at 12 hours). Findings from this study suggest that phages can be used to decontaminate vegetables and food surfaces. Its application as biocontrol could be useful in the prevention of food borne illnesses and outbreaks.

Dear Organizers,
I am interested in entering into the developing scientist competition.
I am a doctoral student. Start date: 04/2017The results show that cereulide production is lower in matrices containing monosaccharides than in matrices containing disaccharides. This should be further investigated with different strains and sugar sources.

Food Microbiology in the context of One-Health (animal, plant and human)

O7.6 Metagenomic characterization of bacterial populations and antimicrobial resistance genes during the production chain of a fermented meat sausage

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Introduction: The transmission of antimicrobial resistance genes (ARGs) is a biological mechanism determinant in the increasing number of antibiotic-resistant infections over the world. The extensive use of antibiotics in farm has been recognized as the major contributor into the resistome of food-producing animal. Hence, the food chain may be considered an important way of ARGs transmission from animal to humans. However, each specific food chain and food processing operation can contribute to a different manner as result of the process parameters, environment and food composition.
Purpose: The objective of this work was to investigate the dynamics of the microbiome as well as the incidence of ARGs during the production chain of a spontaneous meat fermentation using a metagenomic approach.
Methods: Swine feces, feed, pork carcasses, and meat batter before and after ripening were collected. Samples were subjected to shotgun sequencing metagenomics.
Results: The metagenomic analysis showed the predominance of Pantoea in feed samples, an ubiquitous microorganism in food environments and also used in bioremediation. Pork carcasses displayed the abundance of Anoxybacillus, a thermophilic bacterium frequently associated with scalding, defeathering, and plucking procedures. Latilactobacillus sakei was the predominant microorganism in meat batter as it is often used as starter culture in the production of fermented sausages. Interestingly, the Bayesian analysis used to identify gene sources estimated feed to be the biggest contributor of ARGs (30%) of cephalosporin and carbapenem classes to the meat batter.
Significance: The metagenomic sequence data generated in this study allowed to identify the food stages and sources with the greatest impact on the persistence and/or reduction of resistant populations in the production chain of fermented meat sausages.
Keywords: Shotgun-sequence, pork, microbiome, salchichón, resistome, feed.

Food Microbiology in the context of One-Health (animal, plant and human)

O7.7 Effect of food-related stress on staphylococcal enterotoxin C expression and its regulatory control

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Staphylococcal enterotoxin C (SEC) is formed by Staphylococcoccus aureus during growth in the food matrix. It exists in six host specific variants and causes staphylococcal food poisoning, the most prevalent foodborne intoxication worldwide. While growth of Staphylococcus aureus is easily inhibited in many foods by the surrounding microflora, it can outgrow the competitive flora under stress conditions such as the ones encountered in food matrices low in pH or high in NaCl, nitrite, or sugar. We aimed to decipher the influence of stressors encountered during food production and preservation on sec expression and its regulatory control. To this end, seven S. aureus strains from different sources and representing different SEC variants were grown under conditions simulating stress during food production and preservation. Growth media were adjusted to 150 mg/L nitrite, 30% glucose, lactic acid (pH 6.0), and 4.5% NaCl. Quantitative Real-Time PCR experiments and ELISA assays were conducted to assess the temporal expression of sec. Regulatory knockout mutants (Δagr, ΔsarA, ΔsigB) were investigated at mRNA level under nitrite stress and control conditions. In addition, whole genome sequences were generated by Illumina sequencing. The effect of the different stressors on sec transcription and translation was time and strain dependent. Overall, a trend towards decreased sec expression under stress was observed on transcriptional and translational level. However, nitrite stress can increase SEC protein levels. While Δagr mutants exhibited lower sec mRNA transcription levels than wt strains, this response was not stress specific. ΔsigB mutants displayed the opposite behaviour under stress conditions compared to control conditions. WGS analysis of the strains revealed a defective agr element in one strain that did however not influence sec transcription or SEC protein synthesis. Our results suggest that glucose, lactic acid and NaCl can be used to control SEC formation in foods.

O7.8 Impact of a multi-strain probiotic on microbiome of free-range chickens

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The inclusion of alternative ingredients in poultry feed is foreseen to impact poultry gut microbiota. Therefore, the current study aimed to use metagenomics approaches to determine how dietary inclusion of prebiotic (inulin) plus a multi-strain probiotic mixture of Lactiplantibacillus plantarum and Lactiplantibacillus pentosus affected microbiota composition and functions of the GI tract of the broilers during breeding.
Fecal samples were collected at the beginning of the trial and after 11 and 32 days for metataxonomic analysis. At the end of the trial broilers were submitted to anatomo-pathological investigations and caecal content was subjected to volatilome analysis and DNAseq.
Probiotic inclusion did not significantly influence bird performance and did not produce histopathological alterations, which indicates that the probiotic did not impair the overall health status of the birds. The multi-strain probiotic inclusion in broilers clearly increased the abundance of several taxa (e.g. Blautia, Faecalibacterium, Lachnospiraceae) and as a consequence an increased level of butanoic acid was observed. The metagenomic analysis showed in probiotic fed broilers a higher number of genes required for branched-chain amino acid biosynthesis, which is crucial in increasing immune function resistance to pathogens and is recognized to improve growth performance. In the presence of the probiotic mix we also observed in the broilers a reduction in the occurrence of ARGs belonging to aminoglycoside, beta-lactamase and lincosamide family. The positive microbiome modulation observed is particularly relevant, since the use of these alternative ingredients could promote a healthier status of the broiler's gut.

Food Microbiology in the context of One-Health (animal, plant and human)

O7.9 Impact of chicken derived ESBL Escherichia coli and VRE Enterococcus faecium strains on complex in vitro chicken and human microbiota and on resistome development

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The microbiota of the gastrointestinal tract may provide a favorable environment for the transmission of AMR genes among commensals and between commensal and food-derived AMR bacteria.
Here, we investigated the ability of chicken-derived ESBL Escherichia coli HV292.1 (blaCTX-M-1, IncI1 plasmid) and VRE Enterococcus faecium CCUG59168 (vanA, Tn1546-mediated) strains to colonise and transfer these genes to other bacteria in the chicken and human gut, in a One-Health vision. We used advanced PolyFermS in vitro gut models combined with molecular methods (qPCR, 16SrRNA metabarcoding and metagenomics) to elucidate the impact of AMR bacteria and antimicrobial treatments (cefotaxime-CTX and vancomycin-VAN) on complex gut microbiota and on resistome profile and to evaluate the ability of AMR strains to colonize the gut models, with and without selective pressure. Moreover, we tested the ability of reuterin, an antimicrobial system produced by Limosilactobacillus reuteri, to inhibit the growth of AMR strains in batch fermentations.
We successfully designed and validated a new in vitro model of chicken cecum fermentation inoculated with immobilized cecal microbiota, operated stably for up to 70 days. On chicken and human in vitro models, antimicrobial pressure was required for the colonization of HV292.1 and CCUG59168. Supplementation of individual AMR strains without antimicrobial pressure had no effect on the complex microbial communities, thought the spiking of HV292.1 during CTX treatment reduced the detrimental effects of the antibiotic on the microbiota in both models. VAN treatment strongly affected chicken and human microbiota composition and activity, and the abundance of Enterobacteriaceae appeared to drive the overall chicken resistome in vitro. On the other hand, a dose-dependent increase in human resistome was observed in VAN-treated reactors indicating a potential co-selection of multidrug-resistant strains in response to VAN. Reuterin system promoted microbiota metabolism, with a very large effect on butyrate, and inhibited the growth of AMR strains in complex chicken microbiota.
Altogether, this study provides insights in strain-level dynamics, changes in the resistome structure, evolution of resistance genes and factors associated with variations of dissemination potential.Keywords: Shotgun-sequence, pork, microbiome, salchichón, resistome, feed.

Food Microbiology in the context of One-Health (animal, plant and human)

O7.10 The dairy bacterium Propionibacterium freudenreichii against colitis and mucositis: a key role of the surface layer protein SlpB

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Context
Gut inflammation constitutes a growing health concern in developed countries. It may consist in spontaneous ailments of the gut, involving both the host immune system and microbiota, such as IBD, including ulcerative colitis and Crohn's disease. It may be caused by a medical treatment, such as mucositis induced by cancer chemotherapy and/or radiotherapy. It coincides with a dysbiosis including a lack of anti-inflammatory bacteria. As an example, propionibacteria are lacking in the microbiota of newborns that develop necrotizing enterocolitis.
Methods and results
We thus focused on the immunomodulatory properties of GRAS propionibacteria. Selected strains of Propionibacterium freudenreichii induced the regulatory IL-10 cytokine in human immune cells (Foligné et al., 2010, 2013), depending on surface proteins (Le Marechal et al., 2015). Mutation of the slpB gene suppressed this immunomodulatory effect and the resulting DslpB mutant induced a rather proinflammatory response (Deutsch et al., 2017). Consumption of wild-type P. freudenreichii protected from colitis induced by both TNBS and by DSS. It alleviated severity of symptoms, modulated local and systemic inflammation, as well as colonic oxidative stress and epithelial cell damages (Plé et al., 2015, 2016; Rabah et al., 2020). Accordingly, consumption of Lactococcus lactis NCDO 2118 harboring pXIES-SEC:slpB and expressing the propionibacterial SlpB reduced severity of colitis, lowered weight loss, disease activity index, shortening of the colon length, and histopathological score, compared with mice treated with L. lactis NCDO 2118 wild-type strain.
In the context of mucositis induced by the chemotherapy 5-FU, P. freudenreichii prevented weight loss, reduced inflammation and consequently intestinal damages. It regulated key markers, including Claudin-1 and IL-17a genes, as well as IL-12 and IL-1 cytokines levels (Cordeiro et al., 2018). Mutant strain DslpB displayed opposite regulatory effect on cld1 expression and on IL-12 levels, and failed to afford protection towards 5-FU-mucositis (do Carmo et al., 2019).
Conclusion
This work emphasizes the importance of SlpB in P. freudenreichii ability to reduce both mucositis and colitis inflammation. It opens perspectives for the development of probiotic products aimed at decreasing side effects of chemotherapy and at helping treatment of colitis, thanks to GRAS bacteria.

Food Microbiology in the context of One-Health (animal, plant and human)

O7.11 Paradigm shifts in our perception of the Bacillus cereus group and its relevance to food safety

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While the relevance of Bacillus cereus as a major cause of gastroenteritis is undisputed, our perception of closely related members of the B. cereus group is rapidly changing. Bacillus thuringiensis has been widely regarded as safe biopesticide, whereas the handful of Bacillus cytotoxicus strains known to date gained notoriety for leading to fatal cases of diarrheal disease. A recent EFSA scientific opinion stresses the urgent need for further data allowing for improved risk assessment. We aimed to gain further insights into the ecology and hazardous potential of B. thuringiensis and B. cytotoxicus by using Vero cell cytotoxicity assays, various strain profiling and fingerprinting approaches including amongst others whole genome sequencing, as well as a food matrix model. Our results show that the relevance of B. thuringiensis and B. cytotoxicus as aetiological agent of foodborne disease needs to be reassessed. We were able to show that B. thuringiensis can multiply in a food matrix at prolonged temperature abuse. In addition, the majority of B. thuringiensis isolates exhibited enterotoxicity, with one strain isolated from rosemary being classified as highly enterotoxic. This strain was surpassing the cytotoxic activity of a high-level B. cereus sensu strictu reference strain by a factor of 1.5. We also demonstrated that some biopesticide strains cannot be differentiated from isolates collected from foods or linked to outbreaks. We therefore present evidence suggesting that the use of B. thuringiensis strains as biopesticides can represent a food safety risk, underpinning the importance of assessing the hazardous potential of each strain used.
Furthermore, our studies revealed that only a small number of strains belonging to B. cytotoxicus, which is regarded as fatal foodborne pathogen, is highly enterocytotoxic while the large majority of B. cytotoxicus strains do not produce detectable levels of enterotoxins. In summary, our work suggests that a paradigm shift in the perception of the Bacillus cereus group species in the field of food safety will be necessary.

Food Microbiology in the context of One-Health (animal, plant and human)

O7.12 Characterisation of the nature and distribution of virulence-associated factors and pathogenicity islands in a collection of Shiga toxin-producing Escherichia coli (STEC)

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Shiga toxin-producing Escherichia coli (STEC) are zoonotic bacteria which colonise the gastro-intestinal tract of sheep. While all STEC can cause disease, the severity of infection can be variable and is determined by the array of virulence determinants harboured by each induvial strain. In this study, a collection of STEC genomes (N=178) previously isolated from the recto-anal junction of Irish slaughter-age sheep were analysed in silico for virulence determinants frequently associated with disease-causing STEC strains.

Thirty-five different serotypes were identified, fifteen of which were not yet reported in sheep. Serotype O91:H14 was the most frequently reported. Eight Shiga toxin gene variants were reported, two stx1 and six stx2, and three novel Shiga toxin subunit combinations were observed. Variant stx1c was the most prevalent, while many strains also harboured stx2b. Adhesion factors were highly prevalent among strains and factors associated with iron uptake and haem utilisation were also widely distributed. Strains harbouring a LEE locus carried a significantly higher number of virulence-associated genes than LEE-negative strains. Many of these factors were encoded on pathogenicity islands including the LEE locus and OI-122. A closer inspection of the structure of the LEE locus revealed that the core region is highly conserved between strains, with some discrete differences observed between islands associated with different tRNA insertion loci. However, the flanking regions showed much greater variability in both the structure, composition and assembly of the surrounding genomic elements.

In conclusion, there is a high prevalence of STEC circulating within sheep, many of which are non-O157 STEC, whose contribution to human disease has been under investigated for many years. The circulating STEC harbour a broad array of factors which have been identified as contributing to STEC pathogenesis, including a variety of Shiga toxin variants, some of which are of high clinical importance. Four strains were determined to harbour a LEE locus. The impact of the flanking regions of the LEE locus on the virulence of these strains is currently unknown and further investigations into their contribution to STEC pathogenesis is necessary.

Food Microbiology in the context of One-Health (animal, plant and human)

O7.13 Antimicrobial resistance of Escherichia coli at single cell level

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Resistance of pathogenic bacteria to antimicrobials poses a worldwide threat to food safety and human health, due to their widespread and often inappropriate use. Until now the majority of the studies dealing with the efficacy of antibiotics on pathogens or the estimation of Minimum Inhibitory Concentration (MIC) of various antimicrobials is conducted with high population levels and by considering microbial populations as a whole. However, single cells of isogenic populations exhibit behavioral heterogeneity of phenotypic origin under various environmental conditions, thus characterizing single cell behavior heterogeneous at the presence of antibiotics. The objective of this study was to assess single cell behavior at the presence of antibiotics and the implications for the population growth dynamics. For this, the colonial growth of Escherichia coli single cells on solid media with different concentrations of antibiotics was studied using (phase-contrast) time-lapse microscopy. Images of the field of view were acquired every 5 min for 24h. The quality of the videos obtained allowed us to analyze the behavior of individual cells through monitoring characteristics, such as the division times, and estimate the kinetic parameters, lag time and maximum specific growth rate, for each microcolony originating from a single cell. In the absence of antibiotics, a highly heterogeneous behavior of single cells was observed. Some cells did not grow showing filamentation or lysis. Cells that were able to form microcolonies showed highly diverse growth dynamics. The presence of antibiotics affected the probability of growth and the growth dynamics of growing cells. The most interesting observation was the effect of antibiotics on the maximum population of colonies originating from a single cell. To interpret the observations, the variability of the kinetic parameters was characterized using appropriate probability distributions and introduced to a stochastic model which takes into account heterogeneity using Monte Carlo simulation. The developed stochastic model was further validated at the population level. The findings of this study can outline the resultant of the population behavior at different initial population levels under exposure to various antibiotic concentrations and provide a new perspective to MIC estimation methodologies through a probabilistic approach.
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Food Microbiology in the context of One-Health (animal, plant and human)

O7.14

Protein enrichment of gluten free bakery products by Arthrospira platensis (spirulina): in vitro study of the effect of formulation and sourdough process on colon microbiota through MICODE gut model

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The production of Glute free (GF) foods is challenging for organoleptic and nutritional aspects. GF products are nutritionally less adequate than standard products because have low protein and high fat, content. Target audience for GF foods stretches beyond coeliac sufferers. In 2015, only 9% of US GF consumers followed a GF diet due to celiac disease, while others just did it believing it is healthier. Thus, it is necessary to know the impact of such food products on healthy subjects.

In this work we compared two formulations of GF bread, with and without Arhtrospira platensis powder (AP) for protein enrichment, obtained by sourdough and plain (control) fermentations. Comparison was made after gastro-duodenal digestion and proximal colonic fermentation performed for a short-term experiment on MICODE (Multi-Unit In vitro Colon Model), employing donations from healthy individuals. The aim is to highlight and compare the impact of formulation and process of the breads on the human colon microbiota, throughout microbiomics (qPCR and 16S MiSeq) and metabolomics (SPME GC-MS).

Firstly, we have to consider that an aggravation of the Proteobacteria loads was observed during any fermentation, suggesting that there are less beneficial impact of GF breads on a healthy colon than what supposed. Notwithstanding, the outcomes from sourdoughs are generally better, and the bread enriched with AP has even superior performances than the standard. Actually, the results indicated that the GF sourdough containing AP could improve some general indicators of healthy condition of the healthy human colon in comparison to the not enriched GF sourdough bread.

More specifically, at taxa level, the GF sourdough with AP in comparison to the standard one is able to; i) preserve microbial eubiosis; ii) increase the abundance of beneficial bacteria (Bifidobacterium, Akkermansia, Roseburia, Faecalibacterium and Ruminococcus); iii) limit opportunistics, sulfurate producers, and proteolytic bacteria (Escherichia, Bilophila, and Clostridium); iv) produce more bioactive organic fatty acids; v) reduce detrimental compounds (phenol, p-cresol); vi) generate a prebiotic effect. Our results evidence that a sourdough fermentation can mitigate the negative effect of GF foods on healthy condition, particularly when the product is enriched in proteins.

Other

O8.1 Global gene transcriptomic profile of human colorectal adenocarcinoma cells after infection with Bacillus cytotoxicus

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Bacillus cytotoxicus is a thermotolerant member of Bacillus cereus group, identified as the etiological agent of foodborne severe necrotic enteritis. B. cytotoxicus produces the CytK-1 variant of the Cytotoxin K, which is highly toxic to human intestinal colorectal adenocarcinoma cells (Caco-2). To determine the global transcriptomic profile of infected Caco-2 cells, strain NVH 391-98 was inoculated directly on a Caco-2 cell monolayer. After incubation, isolated RNA was subjected to linear amplification and subsequent microarray analysis. The signal intensities were scanned using the Affymetrix GeneAtlas system (Affymetrix/Thermo Fisher Scientific Waltham, MA, USA). Based on the analysis of 48226 genes, B. cytotoxicus altered 6500 genes in Caco-2 cells, from which upregulation was noted in 50.3% and downregulation for 49.7% of tested genes. Genes belonging to the mucin family responsible for the formation of the protective mucous barrier were induced, from which mucin 16 (MUC16) was the highest upregulated gene. Myosin ID (MYO1D), required for normal planar cell polarity of some cells, as well as fibrillin-1 (FBN1), a structural component of microfibrils of the extracellular matrix, epithelial membrane protein 3 (EMP3), were significantly upregulated. On the other hand, genes encoding epidermal growth factors epiregulin (EREG) and amphiregulin (AREG), and fibrinogen beta chain (FGB) were repressed. Transcriptomic analysis showed activation of apoptosis-inhibition and cellular defence mechanism. Inhibitor 1 (CAAP1) was upregulated, indicating that Caco-2 cells were able to activate survival mechanisms and innate immune sensors. Also, activation of the TNFRSF21 gene was noted, which promotes apoptosis mediated by BAX and by releasing cytochrome c from the mitochondria into the cytoplasm. The most upregulated gene was macrophage receptor MARCO-like, which has an important role in the removal of pathogens from the body. Significant alterations in the expression levels of APO family members APOA4, APOC3, APOD, and APOH were observed. The present study demonstrates B. cytotoxicus pathogenesis providing insights into molecular events by gene expression analysis of infected intestinal epithelial Caco-2 cells.

Other

O8.2 Cold shock proteins promote nisin tolerance in Listeria monocytogenes through modulation of cell envelope modification responses

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Listeria monocytogenes (Lm) continues to be a food safety challenge owing to its stress tolerance and virulence traits. Several listeriosis outbreaks have been linked to the consumption of contaminated ready-to-eat food products. Hurdle procedures, including nisin application, are employed to mitigate against Lm risk in food. In response, Lm deploys several anti-nisin defense mechanisms that are not yet fully understood. Cold shock proteins (Csps) are small, highly conserved nucleic acid-binding proteins involved in gene regulatory processes to mediate various stress responses in bacteria. Lm possesses three csp gene paralogs; cspA, cspB, and cspD. Using a panel of single, double, and triple csp gene deletion mutants (n=7), the role of Csps in Lm nisin tolerance was examined, demonstrating their importance in nisin stress responses of this bacterium. Without csp genes, a Lm ΔcspABD mutant displayed severely compromised ($P<0.05$) growth under nisin stress (155-fold growth rate reduction). Characterising single (ΔcspA, ΔcspB, and ΔcspD) and double (ΔcspBD, ΔcspAD, and ΔcspAB) csp gene deletion mutants revealed a hierarchy (cspD >cspB >cspA) of importance in csp gene contributions toward the Lm nisin tolerance phenotype. Individual eliminations of either cspA or cspB significantly ($P<0.05$) increased nisin stress tolerance (2.4- and 1.5-fold, respectively), suggesting that their expression has inhibitory effects on the expression of CspD mediated nisin resistance. Gene expression analysis revealed that Csp deficiency significantly altered the expression of genes encoding proteins involved in cell envelope modification such as DltA, MprF, RmlT and penicillin-binding proteins. Furthermore, the ΔcspABD mutation induced an overall more electronegative cell surface, enhancing sensitivity to nisin and other cationic antimicrobials such as daptomycin, polymyxin B, and the disinfectant benzalkonium chloride. These observations demonstrate that the molecular functions of Csps regulate systems important for enabling the constitution and maintenance of an optimally composed cell envelope that protects against cell-envelope-targeting stressors, including nisin. Overall, our data show an important contribution of Csps for Lm stress protection in food environments where antimicrobial peptides are used. Such knowledge can be harnessed to develop better Lm control strategies. Additionally, the potential that Csps have in inducing cross-protection must be considered when combining hurdle techniques.

POSTER PRESENTATIONS Food Microbial Ecology

P1.1 Microbial diversity of kid yoghurt desserts

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Yoghurt is defined by the Codex Alimentarius (FAO-WHO) as the product of milk fermentation by Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus. Except these bacteria, the co-fermentation with other microorganisms, mainly probiotics, produce a wide range of metabolic products that might benefit consumers' health, especially that of kids. The present study is dealing with the microbiological analysis of commercially available kid yoghurt desserts in Greece. A total of 13 different samples of branded kid yoghurt desserts from eight different dairy companies were collected from retail shops and analyzed on the date of manufacturing and the stated expiry date. Classical microbiological analysis showed that lactic acid bacteria (LAB) counts ranged from 3.0 to 9.0 log CFU g^{-1} during the entire storage period, while no coliforms, yeasts and micrococci were detected in any of the samples. In total, 41 LAB isolates were grouped using rep-PCR and identified by 16S rRNA gene sequencing. Although the yoghurt starters Lb. bulgaricus and S. thermophilus should have been present in all samples according to their labeling, interestingly enough, S. thermophilus was found in 69.2% of the samples while Lb. bulgaricus in only 23.1%. Additionally, Lactobacillus rhamnosus was identified in 38.5%, while Lactobacillus plantarum in 15.4%, and Lactococcus lactis subsp. lactis and Lactobacillus spp. in 7.7% of the samples analyzed. Regarding post-acidification, the pH value of 73% of samples remained constant during the storage period till the expiry date. These results showed that 61,5% of kid yoghurt desserts were supplemented with adjunct cultures that might contribute to enhanced organoleptic characteristics and potential health benefits as well.

P1.2 Probiotic characterization of Bacillus species isolated from Dawadawa

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Dawadawa is a fermented condiment produced from the seeds of the African locus bean and it is consumed predominantly by the people of the West African Sub-region. It is an affordable source of protein and flavor enhancer in foods.
Research has established that dawadawa is a product of alkaline fermentation with Bacillus species being the predominant microorganisms involved.
In our study, we isolated presumptive Bacillus strains on Nutrient agar based on their colony and cell morphology. Bacillus cereus selective agar was used to eliminate strains of the Bacillus cereus group species.
Using phenotypic and biochemical tests, the strains tentatively identified as B. subtilis and B. pumilus groups were investigated for their antimicrobial and hemolytic activities, antibiotic susceptibility and acid and bile salt tolerance.
The strains were seen to grow well at pH 3 and below and were tolerant of bile salts with OD of up to 0.53 after 18 hours of growth. They showed various susceptibilities to antibiotics tested with only one strain being susceptible to all antibiotics tested. Some strains were also seen to inhibit growth of Klebsiella pneumoniae, Pseudomonas aeruginosa and E. Coli. None of the strains exhibited complete hemolysis while 45% of the strains exhibited □-hemolysis. 55% were non hemolytic.

Food Microbial Ecology

P1.3 Microbial biogeography of Greek olive cultivars using metataxonomic analysis

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Microbial biogeography, i.e., the study of microbial diversity over time and space, links the geographical origin and environmental conditions to quality aspects and identity of fermented foods. The present study, part of a bigger project the first of its kind in Greece, is exploring the microbial terroir of olives cultivars in Greece with the ultimate goal to develop new tools towards Greek olives authentication. Towards this, 61 samples from 38 different olive cultivars were collected at the final stage of ripening from 13 well spread geographical regions in Greece and analyzed using amplicon-based metagenomics approach. Total DNA was extracted from the olive samples and the 16S rRNA gene and ITS DNA region were sequenced and analyzed for the identification of bacteria and yeasts/fungi diversity, respectively. Furthermore, PCA was also performed for data clustering based on the average microbiota of all samples from each region of origin. Based on the composition results obtained, the majority of both bacteria (e.g., Pantoea and Enterobacter) and yeasts/fungi (e.g., Aureobasidium and Debaromyces) genera identified were found in all 61 samples. Of note, olive samples collected from the same region had similar microbial fingerprint regardless the variety, indicating potential association between the relative abundance of certain taxa and the geographical region. When samples were grouped by region of origin, distinct bacterial profiles per region were observed, which was also evident from the PCA analysis. This was not the case for the yeast/fungi profiles, since 10 out of the 13 regions were grouped together, mainly due to the dominance of the genus Aureobasidium. A second cluster was formed for the islands Crete and Rhodes, both of which are located in the Southeast Aegean Sea, mainly due to the identification of the genus Toxicocladosporium in relatively high abundance. Finally, the Agrinio region, located in Central Greece, was separated from the others as it showed a completely different microbial fingerprinting. Future analysis of a higher number of samples collected during different cultivation periods is expected to unravel the Greek olives cultivars biogeography.

Food Microbial Ecology

P1.4

Enrichment of model-cheeses with blackcurrant (Ribes nigrum) or cornelian cherry (Cornus mas) increases the total amount of polyphenols

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Introduction: Blackcurrant (BC) and cornelian cherry (CC) are rich sources of polyphenols. These berries are currently used to produce a variety of traditional food products, however, they have yet to be used in dairy products. Dairy products only have small amounts of phenolic compounds, and since these molecules have high antioxidant activity, addition of polyphenols to dairy could improve their functional properties. The objective of the present study was to produce fresh model cheeses from pasteurized cow milk, fortified with BC and CC, in order to evaluate the total amount of phenolic compounds as well as their physicochemical properties, antioxidant activities, sensory and microbial characteristics.
Materials & Methods: Model cheese was constructed using pasteurized cow milk with commercial microbial starter and rennet and enriched with varying concentrations of BC or CC (0.3 – 0.6 % wt/milk volume) and matured for 4 weeks. BC and CC were purchased from a certified organic and a conventional producer and were tested as either freeze-dried or not to test if any difference could be associated to the type of production and the preparation of ingredients prior to cheese enrichment. The concentration of total polyphenols in the cheeses was measured as the Gallic acid equivalent using the Folin-Ciocalteu reaction. The experimental cheeses were evaluated for their sensory characteristics by means of a blind untrained panel; the microbial community was examined by plate counts of lactic acid bacteria and coliforms.
Results: The total phenolic content increased with the addition of BC and CC. We observed that the addition of BC and CC lowered the appearance score of the cheese, whereas taste, smell and texture were no different compared to control. The microbial community found in the cheeses did not differ significantly for either lactic acid bacteria or coliforms.
Conclusion: Overall, the addition of BC or CC shows potential as phenolic supplements for dairy products.
Further Research: In progress are analysis on antioxidant activities and testing with additional herbs and berries. The best solutions will be scaled up for a cheese factory production.

Food Microbial Ecology

P1.5 Performance of selected lactic acid bacteria and yeasts with in vitro probiotic potential as starters in fermentation of black olives cv. Konservolia

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The present study examined the use of two lactic acid bacteria (LAB) (L1 and L2) and two yeast (Y1 and Y2) isolates as starter cultures for the fermentation of black olives cv. Konservolia of Fthiotida region. The isolates were previously recovered from spontaneous fermentations of the same olive variety and selected for their in vitro probiotic profile. Olive drupes were placed in brines initially containing 4% (w/v) NaCl and then inoculated, in duplicates, with the LAB isolates alone or in combination with the yeast isolates. The inoculum levels of LAB and yeast isolates were ca. 8.5 log and 5.0 logCFU/mL, respectively, while control cases without inoculation were also included. LAB and yeast population in control cases progressively increased during the process reaching 6.72±0.03 log CFU and 3.99±0.06 log CFU/g on olive drupes, respectively. Enterobacteriaceae on the other hand gradually decreased reaching the enumeration detection limit (1 log CFU/g) after 13 days of fermentation. The pH value of the brine decreased from 5.2 to ca. 4.0 after 40 days and maintained at this level until the end of the process (90 days). In the inoculated fermentations, the levels of Enterobacteriaceae dropped below the detection limit in 8 days. LAB population was maintained at 7.0-7.2 log CFU/g when the starter L1 was used and 6.8-7.0 log CFU/g for the cases where the starter L2 was used. In the cases of single inoculations with the LAB isolates, an accelerated reduction of the pH was observed, which finally reached values of 3.8-4.0, while in the cases where the yeast strains were used, the final pH in the brine was maintained at ca. 4.2. According to the sensory evaluation performed at the end of the fermentations, all samples were well appreciated. However, a preference was expressed for samples inoculated with the L1 alone or in combination with the yeast isolates. These samples gained higher scores in acidity while the perception of bitterness was less intense. Ongoing research includes the confirmation of the inoculated isolates' survival on the final product with the application of molecular tools.

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Food Microbial Ecology

P1.6

Blue mozzarella is back! Pseudomonas fluorescens detection and typing in dairy products

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Introduction: In the past years (2020-2022) blue discolouration of mozzarella cheese increased in northern Italy, worrying the dairy operators still remembering the 2010 episode, in which over 70,000 mozzarellas coloured blue causing a strong economic impact because the product no longer had consumer confidence. This phenomenon is mainly caused by Pseudomonas fluorescens contamination, being Pseudomonas spp. bacteria one of the main psychrotrophic organisms of concern in the dairy industry. Traditional methods for Pseudomonas spp. detection are time-consuming and often not compatible with the dairies' commercial needs.

Aim: A new protocol was developed to detect and type P. fluorescens complex strains in a short time, to prevent and to monitor their presence and persistence in processing environments. This method was tested in a dairy frequently contaminated by chromogenic -, where disinfection strategies were implemented.

Methods: Mozzarella and water samples from 8 points along the processing plant were weekly tested: from the water entering the processing to the finished product. P. fluorescens complex strains were detected using PCR, the sequencing of gyrB gene allowed species identification, and typing was performed by MLST after colony isolation on specific medium. The newly identified sequences were submitted in the PubMLST database for the Sequence Type (ST) assignment.

Results: The dairy was monitored for 27 weeks (n=211 water samples, n=49 mozzarella samples). The 23% (49/211) of water samples were contaminated by P. fluorescens complex strains. Isolation was possible for 12 samples, highlighting the presence mainly of P. mandelii (49%) and P. fluorescens (28%) strains. The 73.5% of mozzarella samples (36/49) were contaminated, with colonies isolation from 12 samples (P. fluorescens was identified in 88% of the isolated strains). Most of the typed isolates were new and not correlated, except for ST44 that was frequently detected in both water (33%) and mozzarella (64%) samples, suggesting a possible correlation.

Conclusions: The variability of the isolates suggested a contamination caused mostly by the introduction of strains from the external environment. The difficulty of strain isolation indicated that the decontamination measures implemented in the production processes are effective in neutralizing contaminations, despite ST44 possibly persists in the dairy plant.

Food Microbial Ecology

P1.7 Oregano essential oil as a disinfectant agent of Salmonella Typhimurium and Escherichia coli O157:H7 biofilms with anticorrosive effect on stainless steel surfaces

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Bacterial biofilms are cellular communities that live attached to a surface, embedded in an extracellular polymer matrix. This association confers bacterial resistance and persistence to disinfection that is associated with an increase in food-borne diseases. Among the recurrent outbreaks are those caused by Salmonella Typhimurium and Escherichia coli O157:H7 biofilms. Another disadvantage of conventional disinfectants is that they can significantly damage food surfaces, including stainless steel. Plant essential oils are among the options for traditional disinfectants, showing antibacterial effectiveness, and recently are being evidenced as anticorrosive agents on stainless steel. Oregano essential oil (OEO) is an option to be explored in the search for these properties. Therefore, the objective of this study was to evaluate the effect of OEO, sodium hypochlorite (NaClO), benzalkonium chloride (Benz), and hydrogen peroxide (H2O2) against the biofilms of S. Typhimurium and E. Coli O157:H7, and on the damage of 304 stainless steel. Benz required concentrations of 0.05 and 0.05 mg/mL, H2O2 of 0.16 and 0.03 mg/mL, OEO of 0.6 and 0.52 mg/mL, and NaClO of 1.2 and 1 mg/mL to inhibit pre-formed biofilms of S. Typhimurium and E. Coli O157:H7, respectively. However, NaClO was the compound that caused the greatest damage to stainless steel (0.29%), while Benz (0.0028%) and H2O2 (0.0025%) also caused damage when applied with brushes. As was hypothesized, OEO did not cause damage to the disinfected surface and even showed a protective effect. Based on the above, it is concluded that OEO is an effective option to inactivate biofilms of S. Typhimurium and E. Coli O157:H7, significantly reducing damage to the disinfected stainless steel surfaces.
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Food Microbial Ecology

P1.8 Evaluation of microbiota during coffee fermentation in an induced anaerobic environment

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Fermentation has become a tool to improve coffee quality and modify the sensory profile. Alterations in the sensory profiles have been achieved due to the different fermentation methods that have emerged, including microbial starter spraying, open batch, and closed batch fermentations, which might change the profile of indigenous microbiota. Self-induced anaerobic fermentation (SIAF) is performed by the microbial metabolism that utilizes the remaining oxygen for their metabolic reactions releasing CO2, volatile and nonvolatile compounds. Our work aimed to evaluate the impact of microbial SIAF on coffee quality. The microbial diversity was determined by plating and next-generation sequencing (NGS), and sensory analysis was conducted by Temporal Dominance of Sensations (TDS). In natural coffee, mesophilic bacteria (7.81 log CFU.g-1) and lactic acid bacteria (6.72 log CFU.g-1) show high populations within 41 hours of fermentation, while the yeast reached a high population within 52 h of fermentation. In pulped coffee, a high population of lactic acid bacteria (5.56 log CFU.g-1) was detected with 41 h of fermentation, mesophilic bacteria (5.53 log CFU.g-1), and yeast (3.96 log CFU.g-1) showed high populations at the beginning drying. Lactiplantibacillus plantarum and Hanseniaspora uvarum were detected only in natural coffee. Bacillus sp, Bacillus cereus, and Pediococcus pentosaceus were detected only in pulped coffee. NGS analysis identified a high yeast species diversity (74) in pulped coffee. Bacterial and fungal richness consisted of 16 genera and 74 species. Most bacterial sequences were assigned to genera Acetobacter (28.77%), Methylobacterium (23.15%), Sphingomonas (9.96%), Gluconobacter (8.12%), Rhizobium (7.12%), and Novos-phingobium (4.96%). Regarding the fungal species, most sequences were assigned to Cystofilobasidium infirmominiatum (55.887%), Wickerhamomyces anomalus (9.322%), Vishniacozyma taibaiensis (7.600%), Udeniomyces pyricola (4.016%), Cladosporium sphaerospermum (3.436%), and Candida quercitrusa (2.454%). In the SIAF method, the natural process showed dominant notes of caramel, wine, and woody, while the conventional process showed notes of herbaceous. In pulped coffee, the dominant notes were fruity, woody, and caramel on the SIAF method, while caramel notes dominated the conventional process. Changes in the microbiota during the SIAF coffee led to sensory profile variations. The fruity attribute is intensified by using bioreactors for fermentation regardless of the processing type.

Food Microbial Ecology

P1.9 Microbial diversity in artisanal minas cheese produced in Brazil (minas gerais)

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Canastra cheese is an artisanal Minas cheese produced from raw cow's milk and a natural whey from the previously produced cheese, known as "pingo". This study aimed to characterize the microbial diversity and the volatile compounds profile present in this Indication of Origin cheese. The bacterial community was identified by culture-independent method (shotgun metagenomic) and the fungal community of the samples was investigated by culture-dependent approaches (culturing, morphological identification, and MALDI-TOF). Moreover, the method of solid-phase microextraction gas-chromatography mass spectrometry (SPME-GC) was applied to determine the characteristic volatile compounds of this cheese. A complex microbial diversity and volatile compound profile were observed. The shotgun metagenomic analysis identified a total of 85.139, 47.623 and 60.045 sequences in sample P1, P2, and P3, respectively. Thus, it was possible to identify 192 different genera of bacteria on the cheese samples. Species in the genera Lactobacillus, Corynebacterium, Streptomyces, Staphylococcus, Serratia, Bifidobacterium, Lactococcus, and Streptococcus were the most frequent in all samples. The fungal diversity revealed 37 different species of yeasts and molds. However, each sample demonstrated a unique fungal diversity composition. Candida catenulata, Geotrichum candidum, and Aspergillus versicolor were the most abundant species in samples P1, P2, and P3, respectively. A total of 66 volatile compounds was detected: 15 acids, 12 alcohols, 23 esters, 9 ketones, 3 aldehydes, and 4 miscellaneous compounds. The most abundant acids detected were hexanoic acid, octanoic acid, and decanoic acid. Considering esters, the most abundant compounds were ethyl hexanoate, ethyl decanoate, and ethyl butanoate. The most prevalent ketones were 2-Nonanone, 2-Heptanone, and 2-Undecanone. These compounds can be mainly associated with the presence of Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus brevis, Staphylococcus equorum, Staphylococcus epidermidis, Staphylococcus xylosus, Lactococcus lactis, Streptococcus lactis, G. candidum, Kluyveromyces lactis, Torulaspora delbrueckii, C. catenulate, and Kodamaea ohmerim, which are responsible for the sensory notes of musty, oily, fruty, malt, burnt cheesy, buttery, sweety, green, floral, waxy, among others. However, future studies should evaluate the role and the ability of those fungi to produce mycotoxin in cheese, as well as control the growth of certain species during ripening, resulting in a safe and high-quality product.

Food Microbial Ecology

P1.10 Microbiota and metabolism studies in spontaneous sourdough fermentations of legumes and wheats

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Sourdoughs carry a complex and diverse community of yeast and bacteria that interacts during fermentation and forms a pH-stable environment through their metabolism. Traditional sourdoughs, made without addition of starter cultures, relies on the activity of autochthonous flour microbiota and continuous backslopping to establish and reach pH stability. The type of flour and the available substrates affect the composition and diversity of the stable microbiota during establishment of sourdoughs. In this study, the microbial community that developed from the autochthonous microbiota of wheat and legume flours was investigated through sourdough fermentation using a backslopping technique.

Traditional sourdoughs were established from regular wheat, emmer, spelt, einkorn, faba beans and yellow peas. The microbiota was analyzed by culture dependent and -independent methods. Autochthonous bacteria and yeast were isolated, characterized and identified by Sanger sequencing. The diversity of the sourdough microbiota and development during establishing and continued backslopping was analyzed with high throughput 16S rRNA and fungal ITS amplicon sequencing. HPLC analysis was also performed to evaluate the changes in metabolites both during establishing of sourdoughs and through 24h-backslopping cycles.

In wheat based sourdoughs, which took 12 days to reach pH stability, a more abundant bacterial microbiota was revealed compared to yeast. The most common species found belonged to the genera Furfurilactobacillus, Latilactobacillus and Lactiplantibacillus. Overall, the different wheat sourdoughs showed a similar microbial community, independent of flour type. However, the quantity of individual species and the metabolite changes did depend on the flour type and the dominating microbiota. Sourdoughs from legume flours reached a stable pH within 12-15 days depending on the incubation temperature. A change in the microbial community was observed during establishing of sourdoughs, and the resulting stable sourdoughs were dominated by bacteria over yeast. Sourdoughs fermented at low temperature showed a more diverse microbial community compared to high temperature sourdoughs.

This study found that the autochthonous microbiota was able to establish itself in sourdough fermentations of different flour types. The collection of strains gathered here emphasize the potential of utilizing autochthonous microbiota as starter cultures in the food industry and in novel food fermentations.

PhD enrollment October 2021

Food Microbial Ecology

P1.11 Study the composition of the microbiome of emblematic vine Greek varieties and the factors shaping it: implications for the vinification process

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Grape cultivation covers globally 7.4 million hectares with an associated market of 321 billion euros (OIV, FAO 2018). Wine production is a process strongly mediated by the activity of microorganisms with commercial vinification relying largely on the inoculation with a limited number of Saccharomyces cerevisiae strains. These ensure high quality wine production but hinder the unique local identity of wines produced globally. Recent studies have shown that the native vine microbiome (the sum of microorganisms colonizing the inner and outer parts of vines) is shaped by plant genotype, geography, microclimate and human practices, all contributing to the establishment of a microbial terroir. The exploitation of the vine microbiome to produce high quality local wines instead of relying to allochthonous inocula has been proposed years ago, however we are still lacking autochthonous microbial strains with high oenological value. In this frame we aimed (a) to determine the microbiome of two emblematic vine Greek varieties (Agiorgitiko and Vidiano) and identify the factors shaping their eukaryotic and prokaryotic microbiome (phyllosphere and carposhere) and (b) to identify along the vinification process the key players involved and their association with desirable wine characteristics. We collected leaves and grapes from 2-3 terroirs per variety at harvest and we determined via amplicon sequencing the composition of the prokaryotic and eukaryotic vine microbiome. Ongoing bioinformatic and statistical analysis will identify the factors shaping the microbiome in these two varieties and their core microbiome, an invaluable source of microorganisms which could promote plant growth, protect vines from biotic or abiotic stress and participate in the vinification process. The data will be discussed in the conference, while amplicon sequencing, metagenomic and metatranscriptomic analysis along the vinification of Vidiano will determine who is there, who is capable of doing things and who is actually doing things and can contribute to the production of high-quality wines with improved local character.

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P1.12 Extended shelf life milk processing: Effect of cleaning in place (CIP) on the germination and attachment of Bacillus cereus spores

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The effect of simulated cleaning in place (CIP) was determined on the structure, attachment, growth, and viability of Bacillus cereus spores isolated from biofilms in filler nozzles from extended shelf life (ESL) milk processing lines and raw milk. CIP structurally affected ≥ 92% of B. cereus spores, while 0.1% remained intact. B. cereus attached to stainless steel coupons and formed biofilms following simulated CIP treatment. Regarding viability, B. cereus spores were capable of germination and growth under refrigerated conditions over a 28 day period. B. cereus spores may lead to a reduced shelf life and potentially be a safety risk in ESL milk with a prolonged shelf life.

Food Microbial Ecology

P1.13 Modeling the Dynamics of Microbial Population in Milk Kefir

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Kefir is a fermented dairy product based on the fermentation of milk by bacteria and yeasts. It is produced by adding kefir grains, consisting of a consortium of microorganisms, to milk to start a natural fermentation. Kefir is recognized for its potential health value, however, there have been concerns about the potential growth of pathogenic microorganisms in kefir and the potential health hazards associated.

A mathematical model was developed in order to describe the evolution of microbes present during kefir fermentation and the potential growth of Listeria monocytogenes as one example of a potential food safety hazard. For this, equations previously described in the scientific literature were combined and adapted. In order to assess the safety of the product, the growth of L. monocytogenes was predicted considering its interaction with the medium and other species. The drop in pH; generation of yeasts metabolites such as ethanol; and buffer capacity was described and considered when modeling L. monocytogenes' kinetics. Interaction between the pathogenic species and the background microflora was included in the model.

Parameters of some well-described systems were taken from literature. Some other parameters describing specific assets from the system were estimated using experimental data of microbial population kinetics during kefir fermentation. The growth of yeasts, lactic acid bacteria, L. monocytogenes together with the pH were experimentally collected at critical processing times and fitted to the mathematical model by minimizing the residual sum of squares. Confidence intervals of 95% were calculated. For further validation, the model´s output was contrasted with an independent data set.

It was concluded that L. monocytogenes is able to increase its population during the first hours of the kefir fermentation process. Inactivation can be apportioned to the drop of pH as a consequence of the LAB metabolism, once the pH reaches values below 4.5. After this no growth of L. monocytogenes seems to be found in milk kefir as confirmed by the model. However, after inactivation, a residual population of the pathogen is observed. This suggests that by controlling the growth and metabolism of the background microflora, safety can be assured in milk kefir.

Food Microbial Ecology

P1.14 Identification of a potential Specific Spoiler Organism (SSO) in a seafood-based ready-to-eat product using a 16S rRNA gene metabarcoding approach

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A shelf-life study was conducted to determine the acceptability of a novel circular economy ready-to-eat (RTE) seafood product stored under refrigeration conditions (5 ± 0.5 oC). Firstly, freshness was determined by an experienced sensorial panel by the evaluation of odour, flavour and texture. Properties such as "artificial" odour, salty and bitter taste were identified in all sampling days. Also, differences in texture between the crust and the inside of the fillet were detected upon arrival but were not appreciated after 9 days. Remarkably, despite lipid oxidation occurred in the product, it did not affect the preferences of the panel. Overall assessment led to the rejection of the product after 12 days of storage.

In order to identify a specific spoilage organism in the RTE seafood product that could be used as microbiological quality indicator intended to follow the remaining shelf-life, a 16S rRNA gene metabarcoding approach to characterise the bacteriota present in the fillets was carried out. Sampling was performed at 5, 8 and 12 days of storage.

Proteobacteria initially dominated the bacteriota of the product but was surpassed by Firmicutes over time. At a family level, there was a variation of Proteobacteria mainly due to the diminution of the family Moraxellaceae, reduced from 51.35 to 6.8 % from 5 to 12 days of storage, respectively. Conversely, the family Planococcaceae increased from 15.8 to 72.18 %. At a genus level, during the storage there was a clear decrease in Acinetobacter (from 43.8 to 6.27 % of the mapped sequences) that was parallel to an increase in the relative abundance of Sporosarcina (from 15.62 to 71.92 % at 5 and 12 days, respectively). Finally, sequence mapping allowed to identify major species in the bacteriota composition being Acinetobacter radiotensis the main component at the beginning of the storage, whereas Sporosarcina aquimarium was the most abundant at the end of shelf-life.

Summarising, outcomes yielded pointed out S. aquimarium as a the potential major responsible for the alteration of the organoleptic properties and subsequent rejection of the RTE fillets and highlighted metabarcoding as a powerful tool to be used in shelf-life studies.

Food Microbial Ecology

P1.15 Shotgun proteomics of Starmerella bacillaris and S. cerevisiae in sequential fermentation with a focus on glycans biosynthesis and metabolism

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Introduction: The use of Starmerella bacillaris in sequential fermentation with Saccharomyces cerevisiae has been previously linked to improved wine quality due to an increased release of mannoproteins into the wine matrix. However, metabolic pathways involved in this process during sequential fermentation are still underexplored. This study aimed at investigating cellular response by exploring the proteomes of two strains (S. bacillaris FR1751 and S. cerevisiae EC1118) in single and sequential wine fermentation. Material and methods: Three fermentation trials were performed with synthetic must (MS300) at 20°C using single yeast inoculum (2x106 cells/mL), and sequential fermentation starting with the inoculum S. bacillaris FRI751 and adding S. cerevisiae EC1118 after 48h. The yeast pellets were collected after 168 hours for the single fermentation of S. bacillaris FRI751 and sequential fermentation (SEQ), and 120 hours for the S. cerevisiae EC1118. The proteins were prepared by suspension trapping (STrap) method using C18 and micro-quartz fiber paper tip-column, digested with trypsin, and sequenced by Liquid Chromatography with tandem mass Spectrometry. The proteins were identified and quantified by MaxQuant software (Version 1.6.17.0) with the MaxLFQ algorithm against the global proteome database of S. cerevisiae EC1118, and S. bacillaris FRI751. The differentially abundant proteins (DAPs) were analyzed following S. cerevisiae EC1118 single versus SEQ, and FRI751 single versus SEQ by the DEP R package. Results: A total of 1907 proteins were obtained after removing contaminants and reverses, and 277 of these proteins were DAPs. Among them, six proteins involved in the glycan biosynthesis and metabolism in the sequential fermentation group compared to single fermentation of S. cerevisiae EC1118 (reaching up to 5.42 log2 fold change). Conclusion: The presence of S. bacillaris in sequential fermentation with S. cerevisiae up-regulated DAPs involved in glycan biosynthesis and metabolism, indicating a yeast-to-yeast interaction. Glycans are involved in cell wall composition, and they can lead to modifications in yeast cell wall structure, mainly beta-glucans and mannoproteins. These compounds released into the wine matrix can impact improving wine instability and quality.

Food Microbial Ecology

P1.16 To culture or not to culture: careful assessment of metabarcoding data is necessary when evaluating the microbiota of a modified-atmosphere-packaged vegetarian meat alternative throughout its shelf-life period

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As the increased consumption of ready-to-eat meat alternatives is a fairly recent trend, little is known about the composition and dynamics of the microbiota present on such products. Such information is nonetheless valuable in view of spoilage and food safety prevention. Even though refrigeration and modified-atmosphere-packaging (MAP) can extend the shelf-life period, microbial spoilage can still occur in these products. In the present study, the microbiota of a vegetarian alternative to poultry-based charcuterie was investigated during storage, contrasting the use of a culture-dependent method to a culture-independent metagenetic method. The former revealed that lactic acid bacteria (LAB) were the most abundant microbial group, specifically at the end of the shelf-life period, whereby Latilactobacillus sakei was the most abundant species. Metabarcoding analysis, in contrast, revealed that DNA of Xanthomonas was most prominently present, which likely was an artifact due to the presence of xanthan gum as an ingredient, followed by Streptococcus and Weissella. Taken together, these results indicated that Lb. sakei was likely the most prominent specific spoilage organisms (SSO) and, additionally, that the use of metagenetic analysis needs to be interpreted with care in this specific type of product. In order to improve the performance of metagenetics in food samples with a high DNA matrix but a low bacterial DNA load, selective depletion techniques for matrix DNA could be explored.

P1.17 Microbial composition of goat raw milk with elevated bacterial count

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Presence of high bacterial level and SCC in goat raw milk is a big challenge for the dairy industry due to quality problems during production and reduction in quality payment to the farmer. Causes of high bacterial count in raw milk are usually attributed to environmental contaminations, poor hygiene or presence of intramammary infections. However, few studies have investigated the composition of bulk tank milk from goat farms with high bacterial level (over 100 000 bacteria cells/mL). The aim of this study was to investigate the microbial composition of goat raw milk with elevated microbial level collected around Norway. Goat bulks milk were analyzed through routine laboratory analysis for the bacterial level, somatic cells level and composition. Immediately after tests, samples with over 100 000 bacteria cells/mL were collected for microbiota analysis. High-throughput amplicon sequencing of the 16S gene was used to identify taxa and abundance in each sample. Multivariate analysis of the microbiota divided the samples in three groups. Each group was correlated to a specific genus of bacteria. The majority of the samples were present in two groups with high level of Staphylococcus and Pseudomonas while few samples were enriched with Streptococcus genus. Among the Staphylococcus genus, several sequence variants were assigned to known udder pathogens, while Pseudomonas was the genus with the highest number of sequence variants and the most abundant in the entire experiment. Very little correlation was detected between the level of somatic cells, which is a known problem in goat milk, and the level of bacteria in the milk samples. This study highlights some of the causes of high bacterial level in goat milk.

Food Microbial Ecology

P1.18 Prevalence and characterization of Shiga toxin-producing Escherichia coli (STEC) isolated from Chinese beef processing plants

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In order to investigate the prevalence, cross-contamination and biological properties of Shiga toxin-producing Escherichia coli (STEC) in two small beef plants with a processing capacity of 60-70 animals per day, a total of 435 samples collected from eight processing sites (slaughter fence, hide, pre-evisceration carcasses, post-washing carcasses, chilled carcasses, meat, feces and environment samples) were screened for the existence of Shiga toxin-encoding genes by PCR. STEC in positives were further isolated and then characterized for serogroup, antibiotic sensitivity and stx gene subtypes. Molecular typing method Multilocus sequence typing (MLST) was also applied to determine the genetic diversity of STEC strains and subsequently, the cross-contamination among different processing sites.
The results showed that the overall PCR prevalence was 14.00% with the isolation of 3.90%. The PCR prevalence rate (isolation rate) in each sampling site was 36.36% (7.27%), 27.27%(12.72%), 14.55%(3.64%), 8.00%(8.00%), 1.81%(0.00%), 12.72%(0.00%), 10.90%(1.81%) and 2.50%(0.00%). Serotype O157 (15/45) and O121 (19/45) were found to be the most common among the 45 isolated strains. These strains were further divided into ten different MLST types. ST-11 (28.89%) was the dominant type and all O157 strains were identified as this gene type. Cross-contamination between the hides and slaughter fence were found through the MLST traceability analysis. Therefore, pre-slaughter management should be strengthened, and intervention measures such as spraying on the carcass surface after hides should be taken. The virulence gene stx2 was detected in most strains (29/45) and three subtypes of the stx2 virulence gene were detected by PCR. The subtype stx2a which was recognized to have the most ability to develop severe disease was found to have the highest detection rate in the current study. The virulence gene hlyA and eae were also found in 35 and 14 of the 45 isolates, respectively. All of these indicate the high pathogenicity of the isolated strains. Among the overall 45 isolates, 20 strains showed resistance to tetracycline and 18 were resistant to at least three antibiotics, indicating a high antibiotic resistance in STEC strains isolated from the two beef processing plants.

P1.19 Effect of concentration on the microbial quality of short set yoghurt stabilized with okoho (Cissus populnea) extract and powder

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Okoho (Cissus populnea) can be used to replace exotic stabilizers but the stabilizing effect of Okoho on the microbial stability of short set yoghurt is not yet known. In this study, the microbial load of short set yoghurt, stabilized with different concentrations of extracts and powders from the stem and root of Okoho plant was evaluated. Extracts and powders were obtained both from the roots and stems of the plant. The extracts and powders were in the concentrations of 0.0, 0.2, 0.3, 0.4, and 0.5%. The extracts and powders were dozed into the yoghurt mix and allowed to ferment in an incubator at 42 oC for 5 h. The experimental design adopted was a split-split-plot design in randomized completely block design. The microbial load of the yoghurt samples was evaluated. From the results, the total viable count (TVC) and Lactic Acid Bacteria (LAB) count in cfu/ml ranged from 1.0 x 108 to 2.4 x 106 and 2.7 x 107 to 2.0 x 105, respectively. Generally, a significant ($p<0.05$) difference was observed between yoghurt samples stabilized with Okoho stem and yoghurt samples stabilized with the roots, with the TVC and LAB count of the roots higher than that of the stem with the same concentration. The control sample was significantly ($p<0.05$) higher in TVC and LAB count than the other samples. An increase in the concentration of extract from the root led to a decrease in TCV and LAB count while an increase in the concentration of powder from the roots led to an increase in TVC and LAB count. An increase in the concentration of extract and powder from the stem led to an increase in the TVC and LAB count. No significant ($p>0.05$) difference was observed for samples stabilized with the same plant part (root or stem). No mould growth was detected in the yoghurt samples. It can be concluded from the study that stabilizer from Okoho roots were more efficient in stabilizing and maintaining the probiotic effect of LAB in yoghurt samples, with the root extract more efficient than the root powder.

Food Microbial Ecology

P1.20 Multidrug-resistant IncA/C plasmids is the main driver of emerging foodborne Salmonella clone ST45

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Salmonella is one of the most important foodborne pathogens in the world, and the emerging of multidrug-resistant Salmonella clones pose a significant threat for veterinary public health and food safety. However, the genetic and/or evolutionary pressure for the selection of antibiotic-resistant (AR) pathogens in food-animals and foodborne transmission remains poorly understood. This study was a global investigation of the population diversity of Salmonella enterica serovar Newport (S. Newport) by studying MLST of 2,250 isolates. Three clades were identified that correlated with the niches/origins of isolation (human, animal, and environment). Sequence analysis of 1,855 S. Newport genomes identified Sequence Type 45 (ST45) as the predominant clone among the animal isolates (87%), but only in 9% of the isolates from human infections. ST45 isolates carried multiple plasmids, the majority (> 90%) had a unique IncA/C plasmid that ranged in size from 80 to 200 kb. The plasmid(s?) carried genes responsible for multidrug resistance, including floR, tetAR, strAB, sul, mer, and bla. Importantly, three Chinese strains carried the mcr-1 gene that confers plasmid-mediated resistance to colistin, one of the last-resort antibiotics for treating Gram-negative bacterial infections. A genome-wide association study (GWAS) correlated chromosome regions or genetic variations with maintenance of an IncA/C plasmid in ST45 isolates. An additional investigation of MIC of 27 antibiotics in 3,728 isolates isolated from the food-chain (food-animals, retail meats, and humans) suggested that AR S. Newport from humans have multiple, but distinct origins. Animal and retail-meat isolates are distinct from > 92% of the human isolates by their antibiotic-resistance patterns. Taken together, our findings suggest that S. Newport ST45 is the dominant clone in food animals around the world. The GWAS data will serve to investigate genetic determinants that contribute to maintenance of this clone in food-animals.

P1.21 Metataxonomic study to evaluate the impact of formulation and process on the microbiota dynamics of nutritionally improved dry fermented sausages

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Consumer preferences are changing towards nutritionally improved (i.e. salt and nitrite-reduced) processed meat products. This represents a challenge for the food industry in terms of technological and food safety aspects, which has to adapt formulations and processes. The aim of the present study was to evaluate the effect of sodium and nitrite-free formulations and low-temperature process on the bacterial community's dynamics of fuet (a low acid dry fermented sausage) by metataxonomics. Eight baches of fuet were manufactured with standard, sodium reduced, nitrite-free (with/without a liver-based ingredient) formulations and submitted to ripening processes at mild (12.5°C) and low (3°C) temperatures. Fermentation was performed by either spontaneous microbiota or Latilactobacillus sakei CTC494 bioprotective starter culture. Physicochemical characterization and culture dependent (MRS and MSA agar) and independent (16S rDNA sequence based metataxonomics) microbiological analysis were performed at day 0, 4, 12, end of ripening (aw<0.90) and after a 15-day refrigerated storage. Temperature was the most important factor determining the change of pH, aw and LAB levels while the presence of starter culture affected the pH decrease and the time for LAB reaching the stationary phase (ca. 8.5 log cfu/g). Metataxonomic results showed that in the meat batter batches elaborated without starter culture and specially the batch containing liver ingredient showed the highest diversity due to the endogenous microbiota of this ingredient. From day 4, diversity decreased, L. sakei being the most abundant species (>70%) in all the batches except for that formulated without starter culture and with liver ingredient. In batches containing starter culture, L. sakei CTC494 clearly lead the fermentation. Diversity indicated that sodium reduction had a low impact in fuet microbiota. On the contrary, absence of nitrifying agents kept microbial diversity. The most similar microbial communities were those of fuet formulated with starter culture and nitrifying salts. Nutritionally improved formulations and low-temperature processes, usually used to ensure food safety of nitrite-free dry fermented sausages, only caused a minor shift on the physicochemical characteristics and bacterial communities of fuet.

Food Microbial Ecology

P1.22 Effect of microbial strains on hexanal

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The positive effects of plant-based protein consumption have been reported, nonetheless, several disadvantages such grassy or beany off-flavors are encountered (1) and are limiting the consumers acceptance towards these proteins.
In this project, we investigated the potential of selected microorganisms to metabolize compounds responsible for the leguminous off-flavor. Hexanal, well-known as a main contributor to the beany descriptor (2), was chosen for this study.
A selection of 16 strains representative of different fermentative types has been used to treat a liquid model matrix containing hexanal at 2 mg/L. The strains were applied on the medium at the end of their growing phase. The inoculum was between 10⁸ and 10⁹ UFC/mL depending on the microorganism. The amounts of hexanal and hexanol at the end of the fermentation were determined using HS-SPME-GC-MS.
Hexanal was reduced by all the strains but at different levels, and different amounts of hexanol were produced.
Results were discussed regarding the different metabolic pathways of the strains with particular attention to the alcohol dehydrogenase, responsible for the conversion of hexanal into hexanol (3,4,5,6,7). Strains presenting constitutive alcohol dehydrogenase in their catabolism, like obligatory heterofermentative lactic acid bacteria or acetic acid bacteria, were particularly efficient for the reduction of hexanal.

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P1.23 Potential of the yeast species Debaryomyces hansenii isolated from artisanal fermented products for bioaromas synthesis

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The aroma of food is a key factor determining its acceptance and purchase by the consumer. There are three methods for obtaining aromas: chemical synthesis, extraction of nature and biotechnological production (bioaromas). The two first methods raise ecological issues due to the generation of a large volume of polluting effluents, such as organic solvents needed for the process. The biotechnological production of aromas is shown as an alternative that avoids these problems, yielding natural aromas in sustainable way. In this regard, a good yeast species candidate is Debaryomyces hansenii. This species shows multitude of biotechnological applications in fermented food. As a result of its proteolytic and lipolytic activities, D. hansenii has the ability to generate free amino acids and fatty acids, which are precursors of aromas and therefore has a strong impact on the overall aroma. However, the enzymatic activity of the different strains of this species is variable and, as a result, their potential to generate aromas is also. The main objective of this project was to study the potential of a collection of 35 strains of the yeast species D. hansenii isolated from artisanal fermented products to synthesize bioaromas of interest to the food industry that can lead to increase the quality and acceptance of food products. For this purpose, the yeasts were incubated in growing media enriched with different aminoacids as aroma precursors at two different temperatures (15°C and 25°C). The resulting aroma profiles were evaluated by sensory analysis and the most interesting combinations selected for further studies by GC-MS. The results pointed out differences among D. hansenii strains and potential of some of them to be used in aroma industry.

Food Microbial Ecology

P1.24 Occurrence of spoilage yeasts in the production of the Danish, white-brined cheese and their succession in the final cheese product

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The Danish, white-brined cheese is a soft type of cheese, produced from bovine milk, and ripened in brine solution. Yeasts are major spoilage microorganisms in the dairy products causing defects and off-flavors in the final products and, thereby, quality loss and shelf-life reduction. The aim of the present study was to perform taxonomic characterization of yeast contaminants in the Danish, white-brined cheese and define the hot spots areas of yeast contamination throughout the dairy facilities. In total, 99 and 798 yeast isolates were purified from the production environment and the final cheese product, respectively. Yeast isolates were characterized for their cell- and colony morphology, classified genotypically using (GTG)⍰-PCR fingerprinting and identified by sequencing the D1/D2 region of the 26S rRNA gene or the ITS1-5.8S rDNA-TS2 region. The white-brined cheese was incubated at 5°C and 10°C for 52 weeks and the viable yeast counts ranged between 3 and 7 log CFU/g. The predominant yeast species in the dairy facilities were Pichia kudriavzevii, Candida intermedia, and Kluyveromyces marxianus, whereas in the cheese samples belonged to the genera Candida and Debaryomyces, including C. zeylanoides and D. hansenii, respectively. Yeast species such as Kazachstania bulderi, Kluyveromyces lactis, Pichia spp., Rhodotorula mucilaginosa, Tolurospora delbrueckii, and Wickerhamonyces anomalus were rarely identified in the white-brined cheese. Samples incubated at 10°C were characterized by higher diversity of yeasts (4 - 9 species) and surprisingly slightly lower viable counts compared to 5°C (2 – 5 species). The study emphasizes that taxonomic heterogeneity, rather than contamination levels, is an important factor influencing the product quality at storage. The knowledge on taxonomy and occurrence of spoilage yeast in the Danish, white-brined cheese production will allow the dairies to do a knowledge-based control of contaminating yeasts and ensure extended shelf-life of the dairy products.

P1.25 Safety, functionality and genomic assessment of Pediococcus acidilactici strains isolated from traditional Persian fermented products with potential probiotic properties and hypocholesterolemic effect

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Pediococcus acidilactici has a good reputation for its technological properties, particularly in the production of fermented sausages and has also been considered as a potential probiotic species. Since in recent years there is an increasing demand for probiotics of non-dairy origin, assessing bacterial species from non-dairy environments could be pretty advantageous. In this study, different lactic acid bacteria (LAB) were isolated from a traditional Persian food (Kashk Zard), and strains discrimination was carried out by RAPD-PCR. Subsequently, some strains were identified to the species level and evaluated for their safety and functionality as probiotics, including properties such as antimicrobial activity, resistance to simulated human gastrointestinal conditions, and cholesterol-lowering effects. The genome of P. acidilactici strain IRZ12B was sequenced and the in silico analysis revealed that this strain possesses interesting probiotic properties, such as cholesterol-lowering capability and antimicrobial activitiy. Furthermore, genome analysis confirmed the absence of transmissible antibiotic resistance genes, plasmids, and virulence factors inside the genome. The results reported in this study make P. acidilactici IRZ12B a promising potential probiotic strain to be considered for the production of novel non-dairy-based functional food.

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P1.26 Occurrence, antibiotic resistance, and enteroxigenicity of Staphylococcus spp. in tonsils of slaughtered pigs in Greece

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The aim of this study was to estimate the occurrence of Staphylococcus spp. in the tonsils of slaughtered pigs in a regional slaughterhouse in Greece, the antibiotic susceptibility of the Staphylococcus spp. isolates, and the enteroxigenicity of the Staph. aureus isolates. Staphylococcus spp. were isolated in 70 (48.61%) out of the total 144 tonsil samples obtained from slaughtered pigs in a slaughterhouse in Greece. Among coagulase-positive staphylococci (CoPS), the predominant strain was Staphylococcus aureus found in 26 (37.18%) tonsil samples. Among the coagulase-negative staphylococci (CoNS), the predominant strains were Staphylococcus epidermidis and Staphylococcus saprophyticus identified in 14 (20.02%) and 8 (11.44%) tonsil samples, respectively. The Staphylococcus spp. isolates presented high antibiotic resistance (AR) frequencies to tetracycline (97.1%) or clindamycin (80.0%) and low AR frequencies to fusidic acid (14.3%). No methicillin-resistant Staph. aureus (MRSA) strains were identified, and all Staphylococcus spp. isolates were sensitive to vancomycin. Among the 26 S.aureus isolates, 21 (80.76%) possessed staphylococcal enterotoxin genes with 7 different enterotoxin gene profiles. The predominant enterotoxin profile was seg, sei, and sej with 7 S. aureus isolates.

P1.27 Characterization of microbiota diversity of Greek table olives of different varieties and designation of geographical origin by metagenomic analysis

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Next generation sequencing (NGS) is currently successfully applied to assess the microbial communities of several foods. In the present work, NGS analysis was applied to discriminate the microbial diversity of fermented olives of different Greek varieties and designation of geographical origin. A total of 34 olive samples of cv. Halkidiki from Kavala and Halkidiki region and cv. Konservolia, from Magnesia and Fthiotida region were collected during 2019-2020 and 2020-2021 harvesting seasons. DNA was directly extracted from the olives' surface and subjected to NGS for the identification of bacteria and yeast communities. Lactobacillaceae family dominated the bacterial community in both varieties from all regions and harvesting seasons. At species level, for season 2019-2020, Loigolactobacillus coryniformis, Secundilactobacillus paracollinoides, Ligilactobacillus acidipiscis and Schleiferilactobacillus harbinensis were identified in cv. Halkidiki samples from Kavala region while Lentilactobacillus parafarraginis was identified in all samples from Halkidiki region. The species Lentilactobacillus parafarraginis was also identified in samples from both regions from 2020-2021 season. Regarding cv. Konservolia, Ligilactobacillus acidipiscis and Loigolactobacillus coryniformis were identified in all samples from Fthiotida and Magnesia region respectively from season 2019-2020 whereas Loigolactobacillus coryniformis was detected in samples from season 2020-2021. Pichiaceae was the most common yeast family with highest abundance in samples of cv. Halkidiki. Pichia manshurica was the most abundant species in 3 (out of 6) and 6 (out of 9) samples from Kavala and Halkidiki region from season 2019-2020, respectively, while Brettanomyces custersianus was the most abundant species in the rest of the samples from Kavala. For the same season, Wickerhamomyces anomalus and Pichia membranifaciens were the dominant species in cv. Konservolia samples from Magnesia region while the species Pichia manshurica, Brettanomyces custersianus and Pichia membranifaciens dominated the yeast community in samples from Fthiotida region. Regarding season 2020-2021, Pichia membranifaciens and Wickerhamomyces anomalus were the dominant species in cv. Halkidiki while Candida boidinii was the dominant species in all cv. Konservolia samples. In conclusion, the results obtained reveal the complex structure of the microbiota in olive fermentations and the microbial key taxa that may be linked to specific geographic areas. Acknowledgements: Project MICROBIOLIVE: STER1-002695, OPS Code 3130, Sterea Ellada 2014-2020.

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P1.28 Bacterial complexity of Italian Alpine butter and effect of the Malga-farm procedure on microbiota and diacetyl/acetoin accumulation

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Bacteria can play different roles affecting flavors and food characteristics. Few studies have described the bacterial microbiota of butter. In the present paper, next-generation sequencing was used to determine bacterial diversity, together with aromatic characteristics, in raw cow milk butter processed by traditional fermentation, in fourteen small farms called "Malga", located in the Trentino province (Alpine region, North-East of Italy).
The physicochemical and aromatic characterization of traditional mountain butter (TMB) showed a low moisture level depending on the Malga producing the butter. Counts of lactic acid bacteria, Staphylococci, and coliforms, as well as diacetyl/acetoin concentrations exhibited changes according to the geographical origin of Malga and the residual humidity of butter. MiSeq Illumina data analysis revealed that the relative abundance of Lactococcus was higher in TMB samples with the highest values of acetoin (acetoin higher than 10 mg/kg).
The traditional mountain butter bacterial community was characterized by a "core dominance" of psychrotrophic genera, mainly Acinetobacter and Pseudomonas, but according to ANCOM analysis, a complex bacterial population emerged and specific bacterial genera were able to characterize the TMB bacteria community, with their high abundance, based on the Malga producing the butter.

P1.29 Survival of Listeria monocytogenes in modified fermented pepperoni formulations and process parameters

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In fermented meat such as pepperoni, the control of Listeria monocytogenes is supported by the addition of salt, sodium nitrite, acidic pH (after-fermentation), low water activity (after-drying), and often a mild heating step after fermentation. Due to consumer health considerations, there is a drive in the fermented meat sector to lower the concentrations of added salt and sodium nitrite. This may however lead to enhanced survival of L. monocytogenes in this high risk ready to eat product.
The aim of this study was to evaluate the survival of L. monocytogenes in standard and modified pepperoni formulations salt (2.5%, 1.4%), sodium nitrite (150ppm, 50ppm), and process parameters (final pH 4.8), heating (61□C, 40 min / 64°C, 20 min) and target aw (0.91/0.94) at end of drying period, with the aim of developing a healthier formulation without compromising safety.
L. monocytogenes (five strain cocktail) was inoculated (log106.00 CFU g-1) into pepperoni batters of varying formulations / parameters as above. Pepperoni samples (n=3) were taken at pre-fermentation, post-fermentation, post-heating, mid-point (day 3-7), and at end-point of drying (target aw) around day 7-12. At each stage, the pepperoni were examined for weight, pH, aw, and L. monocytogenes by direct plate count (OCLA agar), and enrichment and plating.
In the standard formulation (salt, 2.5%; sodium nitrite, 150ppm; pH4.8; heat step after fermentation, 53.5°C, 61 min; and end point aw, 0.91) L. monocytogenes was reduced from a pre fermentation level of log106.14 CFU g-1 to log10 1.37 CFU g-1 at the drying endpoint The inclusion of a heat step (61□C,40 min) even with a lower salt (1.4%) and sodium nitrite (50 ppm) had a significant impact on L. monocytogenes with a > 6 log reduction from pre-fermentation (log10 6.50 CFU g-1) to drying end-point, when L. monocytogenes was detectable by enrichment only. When salt in this same product was increased to 2.5% there was no detectable L. monocytogenes at the end-point. The inclusion of a higher heat step compensated for the lowering of salt levels (1.4%) and sodium nitrite (50ppm), but may impact on sensory attributes (fat appearance and colour) which would require further investigation.

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P1.30 Growth of Campylobacter spp. and carbon dioxide production in primary containers incubated aerobically

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The ability of Campylobacter to grow in primary containers incubated aerobically was examined. Ten ml of media composed of (g/L) beef extract, 50; tryptose, 10; soluble starch, 10; sodium bicarbonate, 5.0; sodium lactate, 3.0; agar, 0.5 was added to 25 ml, 12.5 cm2 culture flasks. The media was inoculated with 104 cfu/ml of Campylobacter coli, Campylobacter fetus, Campylobacter jejuni, or Campylobacter lari; and the flasks were closed with plug-sealed caps, vented caps, or vented caps covered with Parafilm laboratory film. The flasks were incubated aerobically at 37C for 48 h. After incubation, number of cfu/ml of Campylobacter in the media was enumerated on a Campylobacter selective agar composed of Blood Agar Base #2 supplemented with 7.0% horse blood and Blaser-Wang antibiotic mixture that was incubated microaerobically at 37C for 48 h. Additionally, the concentration of carbon dioxide in these flasks was measured in parts per million (ppm) with a CO2 Sampling Data Logger. Significant differences in data were determined using GraphPad InStat statistical software. Results indicated that there was a 4.0 to 5.0 log increase in the number of C. coli, C. fetus, C. jejuni, and C. lari recovered from media incubated in the plug-sealed flasks or the flasks with vented caps covered with Parafilm laboratory film. However, significantly fewer Campylobacter were recovered from flasks with vented caps that were not covered with Parafilm, and no C. jejuni or C. lari were recovered from these flasks. Furthermore, there was no significant difference in the growth of the different species of Campylobacter when cultured in same type of container. There were also significantly higher concentrations of CO2 detected in flasks with plug-seal caps or with vented caps covered with Parafilm than in flasks with vented caps. Conclusions indicate that the ability of Campylobacter to grow in containers incubated aerobically was related to the ability of the containers to retain CO2 produced in the flasks. By not requiring the generation of microaerobic atmospheres, utilization of this medium will allow laboratories to simplify procedures for culturing this pathogen by using this medium in sealed containers that can retain CO2.

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P1.31 Recovery of UV-C treated Listeria monocytogenes, Salmonella spp. and AMR Escherichia coli in different irrigation water sources

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Microbiologically contaminated river water used for irrigation of fresh produce can lead to food pathogens and antimicrobial resistant (AMR) bacteria being transferred to humans, contributing to severe illness. Reducing the initial microbial load of the water used for irrigation will make further food safety steps from farm to fork more effective. Ultraviolet (UV-C) treatment has been proven to be an effective and economical solution to make river water safer for irrigation purposes. There are, however, natural defense mechanisms present in bacteria to repair UV-C induced DNA damage due to UV rays being natural environmental stressors. The repair mechanisms identified by numerous studies can be divided into two categories: Direct Repair (light dependent) and Nucleotide Excision Repair (light independent). The aim of this study was to investigate microbial recovery of Salmonella spp., L. monocytogenes and AMR E.coli in different water matrices. The findings will ultimately contribute towards dosage recommendations for the use of UV-C irradiation at farm level to disinfect irrigation water for improved food safety of fresh produce. Inoculums of selected AMR E. Coli, L. monocytogenes and Salmonella spp. strains were prepared in three different matrices (distilled and autoclaved river water from two sources). These were subjected to three different UV-C irradiation treatments (1 x 20; 2 x 20; 3 x 20 $mJ.cm^{-2}$) in a collimated beam device. After treatment, samples were left for three hours in either a box with controlled light conditions or in dark conditions to enable recovery. After recovery the colony counts were compared to a control sample that was plated directly after the UV-C treatment, before recovery. The results indicated that UV-C treatment significantly reduced initial microbial numbers, and that only slight recovery was observed. The degree of recovery was, however, influenced by the UV-C dosage, bacterial species, and water matrix. Of the strains tested the Salmonella spp. isolate had the best recovery regardless of the water type in which the UV-C irradiation was applied. This highlights the importance of monitoring irrigation water for Salmonella spp. contamination even though UV-C irradiation can, in general, be considered an effective and environmentally friendly disinfection method.

Food Microbial Ecology

P1.32 The reduction of Campylobacter jejuni biofilm by fungal lectins

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Persistence of the pathogen Campylobacter jejuni in food processing is mainly associated with the biofilm formation and thus represents the main risk for its spread, especially in the meat food chain. Therefore, novel strategies to reduce biofilms focus on bacterial adhesion and the use of alternative sources of antimicrobial agents. Fungal fruiting bodies are often rich in various proteins that form their innate defence system. One subset is a group of carbohydrate-binding proteins that are highly specific for their target carbohydrate groups of other molecules, called lectins.
Our aim was to identify fungal lectins that substantially reduce C. jejuni biofilm formation on a surface commonly found in the food supply chain - polystyrene. Bacteria were inoculated together with different concentrations of lectins and grown in microtiter plates for 24 hours. The surface was then rinsed, the adherent cells were detached by ultrasound and the cells were determined by the CFU method.
The results showed that of 19 lectins tested, MOA (Marasmius oreades agglutinin) and CGL2 (Coprinopsis cinerea galectin) significantly reduced the adhesion of C. jejuni to polystyrene. Competitive experiments showed that preincubation of target sugars (galactose for MOA and lactose for CGL2) with potent lectins abolished the reduction of adhesion effect on C. jejuni, confirming that they target carbohydrate molecules on the bacterial cell surface or in the biofilm matrix.
We can highlight the fungal lectins MOA and CGL2 as anti-biofilm agents of natural origin targeting the biofilm formation of C. jejuni to abiotic surfaces. They represent an alternative to reduce the development/spread of antimicrobial resistance among pathogenic bacteria and improve food safety and human health.
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P1.33 The effect of natural whey starter cultures on sanitary indicator microorganisms during manufacturing of Brazilian Canastra artisanal cheeses

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High levels of Escherichia coli and coliforms, used as indicators in foods, provide evidence of poor hygiene, inadequate processing or post-processing contamination. During aging of artisanal raw milk cheeses a decrease in counts of indicators microorganisms is expected due to the decrease of pH and Aw and the activity of endogenous culture. The main objective of this study was to investigate the influence of five different natural whey starters (NWS), named "pingo", used for the manufacturing of artisanal Canastra cheeses (Minas Gerais, Brazil), on coliforms, E. Coli and lactic acid bacteria counts and physicochemical parameters (Aw and pH) during ripening for 60 days. Cheeses were manufactured with NWSs provided by five local producers. Control cheeses, without added NWS, were prepared for each lot of cheese. Ripening was carried out in a climatic chamber at 20 °C and 60-65 % RH. Results were analyzed by ANOVA and Tukey test (α = 0.05). Coliforms, E. Coli and LAB counts decreased significantly ($p < 0.05$) during ripening. However, only two NWS resulted in a significant effect ($p < 0.05$) on the decrease of coliforms and E. Coli in the cheeses when compared with the correspondent controls. For these two cheeses, the median reductions in the E. Coli populations, from the 10th to the 60th day of ripening, were 1.6 ± 0.8 and 0.7 ± 0.5 log CFU/g higher than in the control cheeses, while counts of LAB were not significant different ($p > 0.05$) from cheeses with and without NWS. Aw decreased from 0.98 to 0.89 during the 60 days of ripening and a linear relationship was obtained for this parameter and coliforms and E. Coli counts. The coefficient of determination (R^2) for coliforms was 0,87 for data for cheeses produced without NWS and 0.77 for cheeses produced with NWS. On the other hand, there was no significant correlation between pH and counts of indicators microorganisms. These results suggest that reductions in coliform and E. Coli populations were dependent on the Aw and also on the characteristics of the NWS used for cheese production and, consequently, on their microbial composition.
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Food Microbial Ecology

P1.34 Assessment of the spoilage microbiota and the growth potential of Listeria monocytogenes in minced free-range chicken meat stored at 4oC

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Chicken meat represents 89% of the total poultry production which in turn constitutes more than 30% of the global meat production. Current diet and nutrition trends in developed countries led the industry to shift to alternative breeding/production methods, such as organic and free-range. In this study, the evolution of the spoilage microbiota in six retail batches of minced free-range chicken meat was assessed during storage at 4□C in vacuum (VP, 4 batches) or modified atmosphere (MAP 30:70 CO□/N□; 2 batches) packages for 0, 3, 5, 7 and 10 days. The growth potential of inoculated (3 log cfu/g) Listeria monocytogenes (3-strain cocktail) was also assessed under VP. Because commercial antilisterial mixtures of organic acid salts may be injected before mincing, all batches were analyzed for L-lactate and acetate at day 0 and 7 of storage. Initially L-lactate ranged from 808 to 1674 mg/100 g and decreased by at least 3-fold in VP batches after 7 days. The initial acetate was 0-38 mg/100 g except of one VP batch which contained 243 mg/100 g, indicating it was pretreated with acetates; thus, it was separated from the other batches - whose data were pooled - because it also retained the lowest pH (< 5.8), had a vinegar smell at opening throughout storage and showed the slowest growth and lowest levels of total spoilage bacteria, LAB, pseudomonad-like bacteria and Enterobacteria amongst all VP batches after 7-10 days at 4□C. Compared with VP, MAP retarded growth of LAB and pseudomonads by 1-2 log units, at final levels below 6.5 and 4.5 logs, respectively. Growth of enterococci, staphylococci, yeasts and VP-inoculated L. monocytogenes was suppressed in all batches. Preliminary characterization of 120 isolates indicated that the terminal spoilage LAB association of free-range chicken meat was dominated by Lactobacillus sakei under VP (62.5%) or MAP (60.0%), followed by members of the genera Carnobacterium (11.3% and 10%) and Leuconostoc (5% and 17.5%), respectively. In particular, all terminally spoiled MAP samples showed excessive in-package swelling and released a characteristic sulfide off-odor at opening that might be attributed to H□S formation by L. sakei or other subdominant spoilage bacteria.

P1.35 MicrobiomeSupport: Towards coordinated microbiome R&I activities in the food system to support (EU and) international bioeconomy goals

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Microbiomes have crucial roles in maintaining life on Earth, and their functions drive human, animal, plant and environmental health. The microbiome research landscape is developing rapidly and is performed in many different science fields using similar concepts but mostly one (eco)system at-a-time. Thus, we are only starting to unravel and understand the interconnectedness of microbiomes across the (eco)systems.
MicrobiomeSupport is a Coordination and Support Action with the overall objective to establish an international network of experts and stakeholders in the field of microbiome food systems research and assess applicability and impact of the microbiomes on the food system.
Key outcomes include
- database containing information on microbiome activities, programmes and facilities along the food chain and beyond in the EU and worldwide
- recommendations for an internationally agreed microbiome definition, best practices and standards, as well as consistent protocols in research
- establishment of a dialogue between multiple stakeholders (i.e. representatives from science, industry, policy, funding and regulatory bodies)
- publications showcasing microbiomes potential and current hurdles for their full exploitation
- educational materials for the general public

Food Microbial Ecology

P1.36 Investigating the prevalence of Campylobacter spp. in raw poultry and fresh packaged salads sold in Greek retail stores, antimicrobial resistance, and biofilm-forming capacities of the isolates

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Campylobacteriosis is the leading cause of foodborne infections and bacterial gastroenteritis in Europe, USA, and many industrialized countries all over the world. Strangely enough, Campylobacter spp. are fastidious slow-growing microaerophilic pathogens, which are sensitive to desiccation and other stresses, but can still survive oxidative stress conditions to reach the human host. Alarmingly, these bacteria may sometimes display multiresistance to clinically relevant antibiotics, with such antimicrobial resistance being nowadays considered a critical public health issue that affects people worldwide. Additionally, it has long been suggested that biofilms should play a key role in the environmental transmission of this pathogen, especially C. jejuni and C. coli species that are responsible for most of the foodborne outbreaks. In this work, one hundred (n = 100) raw poultry and fresh packaged salad samples sold in retail stores of a Greek island town (Myrina, Lemnos, north-eastern Greece) were initially screened for the prevalence of Campylobacter spp. following established ISO protocols and classical microbiological procedures. Recovered isolates (> 100) were identified by mPCR distinguishing them to the two main species of the genus (C. jejuni and C. coli) and subsequently subtyped using a repPCR approach. Representatives from each cluster were then tested for resistance against a panel (n = 10) of clinically relevant antibiotics, following the agar disk diffusion method, and in parallel for biofilm formation on polystyrene microtiter plates, under various environmental conditions, using the crystal violet assay. Results obtained increase our knowledge on a significant foodborne pathogen, shedding light on some of its intra- and extra-intestinal survival capabilities (i.e., antibiotic resistance and biofilm formation), and may ultimately increase the safety of our food supply protecting human health. Future studies are planned to correlate some of the phenotypic properties observed to genome-specific trains through WGS of some selected isolates, trying to unravel the subcellular mechanisms of action.

Food Microbial Ecology

P1.37 Novel misos shape distinct microbial ecologies and flavours

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Demand for new tasty fermented foods, especially plant-based ones, has increased in the last decade. Among these, Japanese traditions and techniques have excited many fermenters and consumers around the world. One example is miso—a fermented paste traditionally made with soybeans, salt, and kōji (Aspergillus oryzae grown on rice, barley, or other grains), used by chefs and cooks as a flavour enhancer in soups, marinades or even desserts. The aim of this study is to encourage food industries to produce foods that are not only safe and nutritious, but also delicious, with desirable and even novel flavour profiles. In this context, we developed 8 novel misos using protein-rich substrates such as yellow peas, lentils and fava beans (with two treatments: boiling and nixtamalisation), rye bread, and soybeans. All misos were made with the same recipe (3 parts proteinous substrate : 2 parts barley kōji + 4% salt), in triplicate, and fermented for 3 months at 28°C. The misos were sampled at the beginning and at the end of fermentation and metagenetic analyses (16S and ITS) were performed.
In general, as expected, the eukaryotic composition in most samples was dominated by A. oryzae. However, some replicates of yellow pea and soybean misos were dominated by Millerozyma farinosa after 3 months of fermentation. Regarding bacteria, we observed three main groups: (i) ryebread misos and samples at the beginning of fermentation were rich in Enterobacteriaceae; (ii) nixtamilisation and fava bean misos were dominated by the Pediococcus pentosaceus group; and (iii) other samples were abundant in the Staphylococcus genus. While some species of this genus are harmless, S. aureus is a major concern in the food industry. Therefore, specific primers will be used to verify the presence of this potential pathogenic species in our misos. Furthermore, preliminary and informal sensory analysis showed that the flavours of the misos vary according to the main substrate. To collect quantitative data on this preliminary conclusion, we will perform volatilome analysis to identify the compounds generated during miso fermentation. We hope these results can contribute to the development and production of novel, healthy, and tasty plant-based fermented foods.

Food Microbial Ecology

P1.38 Goat milk isolates as starters in soft goat cheese production

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EU countries account for 15%, i.e., 2.5 million tons of the goat milk produced worldwide annually, with Greece ranking fourth with 355,760 tons. Most of the goat milk in Greece is used for the production of traditional cheeses, and, thus, it plays an important socio-economic role in the agricultural sector. The aim of this study was the production of two types of goat milk cheese using pasteurized goat milk and a blend of wild strains previously isolated from raw goat milk and selected on the basis of their technological properties. The first cheese type was prepared with the use of solely lactic acid bacteria (LAB), namely strains of Lactococcus lactis, Lacticaseibacillus paracasei, Lactiplantibacillus plantarum and Leuconostoc mesenteroides, as starter cultures. In the second type, Lacticaseibacillus paracasei was replaced by a yeast strain of Debaryomyces hansenii as a ripening culture. Cheese making trials were performed in triplicates. Milk samples (before and after inoculation) and cheese samples during ripening were subjected to physicochemical and classical microbiological analysis, while the final products to sensory analysis as well. Furthermore, total DNA was extracted from all samples and amplicon-based metagenomics analysis was used to determine the evolution of the bacterial and yeasts/fungal diversity. Both cheeses were characterized by low pH and smooth texture. Classical microbiological analysis revealed that total mesophilic counts and LAB initially increased and then remained stable throughout cheese ripening (day 31). As expected, yeast counts were lower in the first cheese type compared to the second one where the ripening yeast culture was added. No coliforms were detected, which is an indicator of the good hygiene practices followed. The complete overview of the microbial diversity of the samples was revealed by the amplicon-based metagenomics analysis. Both final products were characterized by a mild and aromatic taste, while the addition of D. hansenii provided unique sensory characteristics differentiating the second cheese type.

Food Microbial Ecology

P1.39

Bioconversion of seaweed biomass through an artificial fungi consortium into a new alternative protein and functional food ingredient

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The production stages of animal origin proteins for satisfying human nutritional needs require abundant natural resources, also their intake is associated with health problems. It is necessary to search for appropriate substitutes that are more sustainable and healthy. In this research, we obtained for the first time an alternative and functional protein rich in proteins (~48% DW), amino acids (~29% DW), and ß-D-glucans (~22% DW). This product is the result of enhancing the nutritional value of the Chilean brown seaweed Durvillaea spp. through a submerged fermentation process with a unique artificial consortium of marine and terrestrial fungi. This artificial consortium is regarded as part of the solution to debottleneck the physiological limitations of mono-cultures bioprocesses, such as degrading complex carbon sources and efficient substrate utilization. The rationally designed consortium demonstrated properties that exceeded the monoculture properties, including a ~150% increase in productivity, a significant increase in total protein (~336%), amino acids (~245%), and ß-D-glucans (~ 100%). This product has all essential amino acids, low content of fatty acids (8,7%), good content of total dietary fiber (26.8%), high antioxidant activity (TEAC of 34 µM/g DM), no toxic metabolites, no heavy metals, and no pesticides. In this study, an untargeted metabolomics approach combined with multivariate statistical analysis and dereplication techniques aides by the GNPS Molecular Network was employed to screen the differential metabolites and to identify molecules with nutraceutical properties of the monoculture and of three artificial consortia designed. Thus, the results of principal component analysis revealed their distinct secondary (mono-cultive: PC1, 41.5%; co-cultive: PC2, 30.7%) metabolite patterns, and allowed to discriminate the chemical composition between co-culture and mono-culture. Among the screened 110-top differential metabolites, 22 nutraceutical compounds involving unsaturated fatty acids and fatty acid amides such and as linoleic acid, palmitamide, 13-docosenamide, 9-octadecenamide, among others showed higher content in co-cultive. In conclusion, the bioconversion of Durvillaea spp. through an artificial fungi consortium results in a product that has high-quality protein, great nutritional value, with potential prebiotic due to the presence of ß-D-glucans, and potential nutraceutical due to the production of unique bioactive compounds such as fatty acid amides with broad bioactivity.

Food Microbial Ecology

P1.40 Evaluation of the probiotic potential of microorganisms isolated from fermented food products

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Several beneficial biological functions for animals and humans have been suggested for probiotics, and traditional fermented foods have been identified as important sources and carriers of these microorganisms. The objective of this study was the isolation of microorganisms from fermented food products of both animal and plant origin and the assessment of their probiotic potential. The selected food products were milk kefir (industrial and home-made products) and table olives (natural black cv. Kalamata olives). Furthermore, microbial isolation was performed from olive mill waste, given its anticipated association with the autochthonous microbiota of olive drupes. A total of 15 microbial isolates with distinct macroscopic colonial characteristics were recovered: eight isolates from four different kefir products, five isolates from two table olive batches (from both drupes and brines) and two isolates from olive mill waste. The majority of the recovered microorganisms (13 microbial isolates) were identified as presumptive lactic acid bacteria (i.e., Gram-positive and catalase- and oxidase-negative bacilli/cocci), whereas two isolates corresponded to yeasts. The isolates exhibiting a reproducibly robust growth behavior in appropriate culture broth media were evaluated for their probiotic potential based on a set of in vitro assays: resistance to low pH; autoaggregation; biofilm-forming ability; antioxidant activity; and safety assessment including evaluation of haemolytic activity and resistance to antibiotics. Based on the collective evaluation of the results of the aforementioned assays, six isolates (five presumptive lactic acid bacteria and one yeast) were identified as exhibiting the most desirable in vitro probiotic traits and could, thus, be regarded as good candidates for inclusion in further studies. Such studies should include molecular characterization of the identified isolates, in vivo evaluation of their potential health benefits and in situ assessment of their performance as starter/adjunct cultures, with the ultimate goal of their potential utilization in novel functional food products.

P1.41 Quorum sensing inhibition in Pseudomonas aeruginosa by resveratrol

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The expression of many virulence factors in Pseudomonas aeruginosa is regulated by quorum sensing (QS) through four interconnected systems (las, rhl, pqs and iqs), and the inhibition of this mechanism has been widely investigated. The health benefits of resveratrol, a polyphenol found in grapes, peanuts and red wine are reported in the literature, but little is known about this compound's influence on bacterial communication by QS. This study evaluated the potential of resveratrol to inhibit the QS-las system in P. aeruginosa PAO1. Initially, we performed a molecular docking between seven LasR structures of P. aeruginosa with resveratrol, acyl homoserine lactones (AHLs) and furanones in CLC Drug Discovery Workbench 4.0 Software. Resveratrol presented a good score, suggesting its potential to bind to LasR protein in vitro similarly to AHLs and furanones, known inducers and inhibitors of QS. We then performed an assay using the P. aeruginosa lasB-gfp QS reporter strain, in which the expression of gfp gives rise to a burst of fluorescence when lasB is induced, while gfp expression is turned down in the presence of a QS inhibitor. GFP expression was measured at 485/535 nm (excitation/emission). The GFP signal was drastically reduced by resveratrol at 500, 250 and 125 μM and furanone at 50 μM. Finally, we evaluated the expression of virulence factors regulated by the QS-las system. Protease activity was determined with an azocasein solution at 440 nm and elastase was determined by Elastin-Congo red solution at 490 nm. Protease activity decreased 30 and 36% with 500 and 250 μM of resveratrol, respectively ($p<0.05$). In these concentrations, elastase activity also decreased 34 and 30%, respectively ($p<0.05$). Overall, resveratrol showed promising anti-QS activities in P. aeruginosa PAO1 and should be analyzed as a QS inhibitor for foodborne bacteria, targeting future applications in the food and health areas.

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Food Microbial Ecology

P1.42 Development of a mixed strain synbiotic yoghurt with potential antagonistic activity against pathogens

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Currently, over 1.5 million children in South Africa are stunted and this number could reach 1.7 million by 2025. These children usually have weak immune systems and frequent illnesses. Hence, the broader aim of this study is to develop probiotic yoghurt as a meal supplement for school children in South Africa. Incorporation of probiotics in dairy products remains technologically challenging due to viability decrease during storage. The current phase of the study investigated the effect of processing parameters and combinations of probiotic strains on viability and production of bioactive metabolites in multi-strain probiotic yoghurt. In this study, four yoghurt samples containing combinations of probiotic cultures consisting of Lacticaseibacillus rhamnosus strains (ATCC 53103/GG and DSM 8746) and Bifidobacterium bifidum ATCC 11863 inoculated at different processing stages (pre-and post-fermentation) were prepared and stored at 4°C for 28 days. Viability of the starter and probiotic cultures, pH, and titratable acidity (TA) were assessed weekly for a period of 28 days. Under both inoculation stages, B. bifidum viable cell count remained at 8 log10cfu/g in combination with L. rhamnosus GG but declined by 1.0 log in the presence of L. rhamnosus DSM 8746 after 28 days of storage. L. rhamnosus GG counts were 8 log10cfu/g, while L. rhamnosus DSM 8746 counts were lower by 1.0 log comparatively. S. thermophilus had higher counts of 9 log10cfu/g on day 0 but declined by 1.0 log during storage. All yoghurt samples showed partial inhibition of L. bulgaricus during storage. There was an increase in TA (range: 0.54 – 0.86%) and a decrease in pH (range: 4.51 – 3.99) in yoghurt combinations during storage. As a basis for understanding the bacterial interactions in the mixed strain yoghurt, a preliminary screening for capsular exopolysaccharide (EPS) production was done using Congo red negative staining. All the probiotics screened were potential EPS producers as they displayed clear halo surrounding their cells and colony stickiness ability. B. bifidum colonies further displayed slimy and mucoid phenotype. The antimicrobial activity of the probiotic EPS on the starter culture, EPS structural characterization, and bioactive metabolites profiling are part of ongoing studies.

Food Microbial Ecology

P1.43 Bacterial community and biochemical profile of different altitude coffees fermented anaerobically

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Plant genetics, climate, altitude, post-harvest processes, and type of fermentation are drivers of coffee quality and microbial communities. Coffee fermentation can be aerobic, semi aerobic, or anaerobic. Under the close batch, the self-induced anaerobic fermentation (SIAF) offers an anaerobic environment in sealed bioreactors for microorganisms that promote their metabolisms and consequently produce CO2. This work evaluated if coffees from different altitudes of the Caparaó region processed via natural change the dominant bacterial community, organic acids, volatile compounds, total phenolics (TP), and antioxidant capacity. Coffee fruits (20 L) from altitudes 800, 1,000, 1,200, and 1,400 m were fermented under the SIAF method, and 48 h samples were used for analysis. ITS-Illumina next-generation sequencing (NGS) and the dada2 pipeline evaluated the dominant bacterial community. Organic acids were evaluated through high-performance liquid chromatography (HPLC), volatiles with gas chromatography-mass spectrometry (GC-MS), and TP and antioxidant capacity with spectrophotometric assays (Follin—Ciocalteau, DPPH, and ABTS). Different genera were specific for each altitude, and the genera abundance changed with altitude. Out of the total bacterial genera identified, Gluconobacter was the most abundant at 800 (19.8%), Weissella at 1,000 m (32.7%), Sphingomonas at 1,200 (36.2%), and Methylobacterium at 1,400 m (39.4%). Acetic, malic, and citric acid concentrations were higher at 1,400 and 1,200 m. Genus Leuconostoc showed a high positive correlation (0.93) with lactic acid (LA) content. Volatile alcohols characterized low altitudes (1,000 and 800 m), while esters characterized 1,400 m. Total phenolics concentration was higher at 1,200 m (203.90 mg.g-1). Altitude 1,000 and 1,200 m (331.72 and 340.16 mM TE.g-1) had high ABTS activity and 1,000 m had the highest DPPH activity (95.01 mM TE.g-1). Low altitudes favored bacterial richness. Acetic acid significantly predominated coffees from the Caparaó region. The dominant bacterial community, biochemical compounds, and antioxidant activity under SIAF vary depending on altitude.

Food Microbial Ecology

P1.44 Prevalence of Listeria monocytogenes and Campylobacter spp. in retail quail meat purchased in Spain

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Introduction: The increase in foodborne diseases has become a public health problem. Listeriosis, whose etiological agent is Listeria monocytogenes, and Campylobacteriosis with Campylobacter jejuni and C. coli as the main infectious species are considered the relevant foodborne pathogens. Outbreaks of these illness are usually associated to the consume of poorly cooked food, or ready to eat products.
Objective: The aim was to identify the presence of L. monocytogenes and Campylobacter spp. in samples of retail quail meat in La Rioja, Spain.
Material and Methods: During the year 2020, 37 samples of raw quail meat purchased at retail in La Rioja, Spain, were analyzed in order to determine L. monocytogenes and Campylobacter spp. For enumeration of L. monocytogenes, counts were determined by plating in Chromo Listeria Agar. The determination of the presence of this pathogen enrichment was carried out in Fraser broth and after plating in Chromo Listeria Agar. Finally, for the determination of Campylobacter spp. enrichment was carried out in Bolton broth under microaerophilic conditions and plating in Chromo Campylobacter Agar.
Results: of the 37 meat samples analyzed, only in two of them L. monocytogenes was above 2 log cfu/g (5.4%). The counts were 2.15 and 2.94 log cfu/g. In other 6 samples were detected the presence of L. monocytogenes (16.22%), although the counts were below 2 log cfu/g. Only L. monocytogenes was detected, and no other Listeria spp were found. Campylobacter spp. was only detected in two samples with counts of 1.30 and 1.78 log cfu/g, being C. coli and C. jejuni, respectively.
Conclusions: The results indicate that both L. monocytogenes and Campylobacter spp. can be present in quail meat. Therefore, a correct cooking must be carried out in addition to being monitored and controlled by the competent authority.
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Food Microbial Ecology

P1.45 Pied-de-cuve Optimization for grapemust alcoholic fermentation

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Pied-de-cuve can be considered an alternative to inoculation with selected yeasts in cellars. Many cellars, driven by consumer awareness on ecological and organic handling are moving towards minimal intervention protocols. However, the elimination of inoculation with selected yeast eliminates the appropriate microbiological control that is a request for modern winemaking. Thus, the interest to keep the microbiological control has derived in the consideration of spontaneous pied-de-cuve as mechanism to keep the control.
However, the spontaneous pied-de-cuve could repeat the same problems of uncontrolled fermentation unless a thorough knowledge of the microbiological evolution of the population in the different fermentations and conditions that could derive in the pied-de-cuve.
In the present work, the effect of several conditions such as different levels of SO2 and/or alcohol fortification on the microbiota present in the pied-de-cuve has been analyzed. The results have yield very similar fermentation kinetics between optimized pied-de-cuve and the inoculation with selected commercial yeast. However, the diversity of the microorganism population during the fermentation with pied-de-cuve is much higher, which supports the respect toward the microbial terroir.

P1.46 Use of wild strains of various lactic acid bacteria for the manufacture of Feta cheese

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Introduction: The wealth of Greek traditional dairy products, and particularly cheeses, has been highlighted by the number of cheeses that have been awarded the Protected Designated of Origin (PDO) status. Among them, Feta is considered the most popular and the one that has met worldwide commercial success.
Purpose: The objective was to evaluate the manufacture of Feta inoculated with different strains of lactic acid bacteria (LAB) (Lactococcus lactis as primary starter culture; and Lactiplantibacillus plantarum, Enterococcus faecium, Levilactobacillus brevis and Pediococcus pentosaceus as adjunct culture) in various combinations (control and seven experimental trials) that have been previously isolated from raw sheep milk and traditional-made Greek Feta and Kefalograviera cheese.
Methods: Feta was produced following the standard procedure of the company. The final products were stored at 4oC for 120 days. At constant time intervals (48h, 10-12d, and 60d), a random sample from each trial was collected and analyzed for physicochemical (pH, moisture, fat, protein, lactose, and NaCl), microbiological (Enterobacteriaceae, Escherichia coli, Staphylococcus aureus, Listeria monocytogenes, yeasts and moulds, and LAB) and sensory analysis. The above procedure was performed in duplicate (i.e., two separate biological repetitions were conducted).
Results: Feta cheese made from the addition of a combination of three strains (Lactococcus lactis : Lactiplantibacillus plantarum : Pediococcus pentosaceus 60:20:20 or Lactococcus lactis : Levilactobacillus brevis : Pediococcus pentosaceus 60:20:20) displayed the best results at 60d of storage in terms of sensory acceptance as compared to the control (sensory score equal to 94.0 ± 1.0 for control and 92.5 ± 1.5 for the other two experimental trials). Another important common feature was the presence of Pediococcus pentosaceus as adjunct culture. Using the control samples as a reference, slight differences were observed in some physicochemical parameters of the treated Feta samples, but the microbiological quality was similar.
Significance: Wild LAB strains, especially Lactococcus lactis, Lactiplantibacillus plantarum and Pediococcus pentosaceus, can be used for the development of new fermented dairy products.
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Food Microbial Ecology

P1.47

Towards a miniaturized microfluidic system for B. subtilis sporulation investigation in biofilms

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Biofilms are of particular concern for the food industry as they lead to contaminations and persistence of bacteria. Moreover spore-forming bacteria represent a major challenge as bacterial spores are very resistant and sporulation occurs in biofilms. Yet, few studies address the issue of sporulation within biofilms particularly when they are submitted to fluid flow. Studying biofilms under similar conditions than in food industry can be challenging and could require large amount of fluids to reproduce mechanical and shear stresses. Thus, the use of microfluidic systems to investigate this problematic is a promising miniaturization solution.
We first aimed to develop a simple microfluidic system to monitor biofilm formation and sporulation of the model bacterium Bacillus subtilis. The second objective was to study the effects of environmental factors such as temperature, pH and water activity (aw) on the amount of biofilm formed in the microfluidic system and to compare to biofilm grown in a classic millifluidic loop.
We designed and prepared microfluidic chips, using PDMS (polydimethylsiloxane) and glass, that allow biofilm formation under controlled hydrodynamic conditions of a B. subtilis mutant strain. We engineered this strain to produce two different fluorescent reporters: one is expressed constitutively and the second one during the first irreversible step of sporulation, in order to observe total cells and sporulating cells. First, we were able to follow and quantify biofilm growth dynamics of the strain under different environmental conditions (temperature, pH, aw) using transmitted light image analysis. Then, using confocal microscopy, we observed the biofilm shape and the details about the locations of sporulating cells. The results obtained show different effects of temperature and aw on growth and sporulation. Such effects were observed with both milli and microfluidic systems, which confirms the potential interest of the microfluidic technology to study sporulation in biofilms.

Food Microbial Ecology

P1.48 The survival of Listeria monocytogenes during milk kefir fermentation and subsequent chilled storage

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Consumers are becoming increasingly focused on healthier, less processed and sustainable products including fermented foods. Milk kefir is an ancient, health-promoting fermented beverage. It is produced by the fermentation of milk with kefir grains to produce an acidic and carbonated beverage containing probiotic microorganisms. Our research investigated the survival of Listeria monocytogenes during a 48hour milk kefir fermentation and subsequent cold storage.

L. monocytogenes was inoculated to a final concentration of 5 log10 cfu/ml in 1L of pasteurised whole milk to which 20g of activated milk kefir grains had been added. The fermentation was undertaken at 25°C for 48h, with uninoculated fermentations used as controls. Samples were removed periodically and tested for L. monocytogenes, lactic acid bacteria (LAB) and yeasts using selective agars. The pH was also monitored. After 48h, the kefir grains were removed, and the resultant milk kefir was stored at 4°C for 33 days.

An initial mean pH of 6.7 decreased to 4.3 within 24h, to 3.8 by 48h and was maintained at this pH during subsequent storage. During the first 24h of the fermentation L. monocytogenes concentrations increased to 6.5 log10 cfu/ml which then decreased to 1.4 log10 cfu/ml after 48h. Thereafter the concentration of this pathogen further decreased during chilled storage but was still detectable by enrichment after 33 days. LAB and yeast concentrations also increased from 5.6 and 3.3 log10 cfu/ml, respectively to 9.1 and 5.5 log10 cfu/ml after 24h and these levels were then maintained throughout the remainder of the fermentation and during subsequent chilled storage.

Lactic acid, acetic acid and ethanol concentrations from each of the fermentations are currently being established. In the future, RNA analysis will be used to examine the expression of virulence and survival genes during the fermentation process.

It was concluded that a reduction of up to 5 log10 cfu/ml of L. monocytogenes may be achieved during milk kefir fermentation but a residual resistant population may survive the process and throughout the normal shelf-life of these types of products. The survival of the pathogen, while lower than EU legal criteria (<100cfu/ml), highlights food safety concerns.

Food Microbial Ecology

P1.49 Clean room's contamination transferred to final product

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Food environment and personnel could act as sources of contamination of food spoilage microorganisms. This study was focused on the environmental contamination in a clean room where slicing and packaging steps of cooked meat products were performed. The sampling was carried out during three consecutive months while cooked turkey was processed. A total of 87 samples were taken (13 non-food contact surfaces –NFCS–, 7 food contact surfaces –FCS–, 5 food samples –F–, 2 air samples –A– and 2 gloves from personnel –P– each sampling time). Moreover, the shelf-life of the batch produced each time was followed. Total viable counts (TVC), lactic acid bacteria (LAB), Brochothrix thermosphacta, Carnobacterium spp. and Listeria monocytogenes were analysed.
Results showed that regarding TVC, the first sampling showed the less number (4/29 samples) of samples below the quantification limit, while in the three sampling events LAB was quantified in less number of samples. In every sampling time, NFCS and gloves were the more contaminated samples with TVC with some few exceptions. NFCS and P samples were again the most contaminated with LAB and quantification was only possible once in the conveyor belt that introduces the product into the clean room, in the picker that place the slices in the packaging and one stainless still bench. However, Carnobacterium spp. and B. thermosphacta were present (very low concentration) in NFCS and FCSthe latest in the final product in the third sampling and Carnobacterium spp. in every sampling time in gloves samples. Moreover, final product counts ranged from 1.30 – 2.60 log cfu/g of TVC but LAB were not quantified. However, at the end of the shelf-life, Carnobacterium spp. was the predominant microorganism in the first and third sampling batches while B. thermosphacta was in the second batch. L. monocytogenes was not detected. These results prove that personnel gloves and environment microbiota, even in a low concentration, could be the source of Carnobacterium spp. and B. thermosphacta both related with the product spoilage.

P1.50 Antimicrobial activity of silver-containing surfaces on Listeria monocytogenes biofilm

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Context: Listeria monocytogenes (Lm) is a foodborne pathogen that can persist on surfaces in food processing environments. The persistence of Lm could be due to its capacity to form biofilms. The complex, multicellular structure characteristic of biofilms could offer bacterial cells protection during cleaning and disinfection procedures. The objective of this study evaluated the antimicrobial activity of non-nanometric-sized silver ions encapsulated in smooth and hydrophobic surfaces on the formation of monospecie and mixte species Lm biofilm.
Methods: Five surfaces containing non-nanometric-sized silver ions encapsulated or without silver ions were tested to prevent the mono species and mixed-species (with Carnobacterium) biofilms of Lm in conditions close to the seafood environment (culture at 8°C with the conditioning of the surfaces with salmon juice). After 24 hours of incubation, biofilms were observed by epifluorescence microscopy after live/dead staining. Quantification of viable cultivable (VC), viable (VC and viable but non-cultivable (VBNC)) and total (dead and viable) populations were performed by plate count agar, by qPCR coupled with propidium monoazide treatment and by qPCR, respectively.
Results: Microscopic observations showed that biofilms grown on the surfaces containing silver ions with a density and architecture close to theses carried out without silver ions. Quantification data confirmed that the VC, viable (VC and VBNC) and total (dead and viable) populations were in similar amounts on the surfaces containing or not silver ions.
Conclusion: Under culture conditions tested, we didn't observed the effect of surfaces containing silver ions- to prevent the formation of monospecie and mixte Lm biofilms.

Food Microbial Ecology

P1.51 Investigation and growth modelling of Staphylococcus aureus and Salmonella spp in marinated chicken Shawarma under static and dynamic temperature conditions

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The demand for safe street foods is continuously growing. Chicken Shawarma is one of the most popular marinated meat meals in the middle east and Mediterranean countries, consisting of a marinated cone of meat roasted on a vertical spit. The foodborne pathogens mostly associated with Shawarma are Salmonella spp. and Staphylococcus aureus, which might pose a risk to consumers if good food hygiene practices are not in place.
The purpose of this study was to investigate the behavior of Salmonella and S. aureus in Shawarma under static and dynamic temperature conditions. To this end, two strain cocktails of Salmonella and S. aureus were inoculated in Shawarma samples processed at a laboratory scale. Samples were incubated at different static conditions (10, 20, 30, 40 °C) during a period from 1 to 9 days. The effect of distinct time-temperature profiles recorded in the cooking process of two Shawarma cone-of-meats with different diameters was also tested. Observed counts were used to derive kinetic parameters and develop secondary models describing the effect of temperature on microbial growth of both pathogens.
Salmonella and S. aureus were able to grow in the range of 20-40 °C, while at 10 °C, microbial counts remained invariable over storage, showing a slight decrease at the end of the storage period. For the simulation of dynamic temperature profiles, the best practice and risk profiles with large-cone Shawarma exhibited remarkable growth of both pathogens
(1-1.5 log CFU/g) even though owing to high inactivation temperatures were reached during the last cooking phase, microorganisms died off to undetectable levels. Interestingly, low-cone Shawarma presented no growth for both pathogens.
Growth rate ranged from 0.09 to 0.52 log CFU/h and from 0.25 to 0.80 log CFU/h for Salmonella and S. aureus, respectively. These values are comparable to those observed in similar chicken meat products and demonstrate a growth potential of the pathogens in the studied product.
Results from this study can significantly contribute to conducting microbial risk assessments of Salmonella and S. aureus in traditional marinated products. Moreover, this information can be relevant to improve cooking practices and food safety of this category of products.

Food Microbial Ecology

P1.52

Influence of the use of mycotoxin detoxifying agents in dairy cattle feeding on natural whey starter biodiversity

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Natural whey cultures (NWCs) are undefined cheese-starter obtained by the traditional back-slopping procedures. NWCs are currently used in the manufacture of different European long ripened cheeses and their core microbiome is mainly composed by Lactobacillus helveticus, Lb. delbrueckii, Limosilactobacillus fermentum and Streptococcus thermophilus.

In last years it has become common practice to add inert sorbents to the dairy cattle diet to reduce mycotoxins bioavailability. Although the effects of these compounds on raw milk composition and its yield are described, no information is available about their influence on the dairy products microbiota. In this study a multidisciplinary approach was applied to investigate how two mycotoxin detoxifying agents (sodium smectite and lignocellulose-based material (B1), leonardite and betaine (B2)), added to cows' diet, modify the NWCs microbiota in a Grana-like cheese manufacture. Microbiological and flow cytometry assay showed that content and viability of lactic acid bacteria (LAB) were not affected by the detoxifying agents.

Moreover, no significant differences were also observed in any of the kinetic parameters of acidification, highlighting as the addition of detoxifying B1 and B2 agents to the cow diet did not influence the acidifying activity of the whey cultures. RAPD-PCR fingerprinting and metagenomic analysis obtained from the total DNA of whey samples underlined differences in the NWCs bacterial community and in the relative abundance of Bacteroidetes that increased when B1 and B2 were included in the diet.

Considering the LAB biotypes isolated from NWCs, two out six St. thermophilus biotypes were detected only in control samples, conversely none of the Lb. helveticus found in control samples were isolated from B1 and B2. We may conclude that the two mycotoxin detoxifying agents did not affect LAB content and the acidifying activity of the NWCs, but were able to modify their bacterial community. In particular, we showed that the B1 and B2 binders can promote or prevent the growth of different St. thermophilus and Lb. helveticus biotypes.

Food Microbial Ecology

P1.53 Kitchen cleaning utensils: Consumer practices and safety risks

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Kitchen cleaning utensils such as brushes, sponges and cloths are used to maintain high hygiene in kitchens but may at the same time be vectors transferring microorganisms, including pathogenic bacteria. There is a need for more information about how consumer cleaning practices and the use of different cleaning utensils affect risk.
In the present study, use of sponges, brushes and cloths in households were mapped in a survey among 9966 European consumers in ten countries. Drying properties and growth and survival of bacteria in cleaning utensils were tested in laboratory experiments.
Sponges were the preferred hand-cleaning utensils for washing-up in the majority of countries. The water uptake and drying rate varied considerably, where brushes dried faster than sponges. Campylobacter survived one day in all sponges and Salmonella more than seven days in two of three types of sponges, while both pathogens were more rapidly reduced in brushes. Higher survival of Salmonella in sponges than in brushes were confirmed for used sponges and brushes collected from consumers. Besides cleaning the dishes, over a quarter of the dish brush users also use it to clean a chopping board after soilage from chicken meat juices.
Cloths were the preferred cleaning utensils for cleaning food preparation areas and wiping up spills from countertops. Fifty-seven percent of the consumers reportedly hang the cloth to dry after use. Studies revealed a higher growth/survival potential of Salmonella and Campylobacter in cloths stored humid than hanging to dry.
In conclusion, the results indicated that brushes are more hygienically than sponges and promoting increased used of brushes for washing up should be considered. Drying of cleaning utensils will reduce bacterial numbers, and hanging the cloth to dry can be a risk reduction advice for consumer.

Food Microbial Ecology

P1.54 Microbiome profile and functional diversity of commonly consumed Nigerian fermented food condiments

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Introduction: Fermented condiments including locust beans (Parkia biglobosa), oil bean seeds (Pentaclethra macrophylla), castor oil seeds (Ricinus communis); are plant-based ingredients primarily used in flavouring local foods and soups in Nigeria. These seeds are naturally fermented, resulting in products of poor organoleptic and shelf life qualities with various microbial contaminants.
Objective: This study aimed to evaluate the microbiome profile and functional diversity of commonly consumed Nigerian fermented condiments.
Methodology: Fresh fermented food condiments samples (N=486) were randomly collected from eighteen local food markets in Southwest Nigeria, while structured questionnaires were administered to the vendors to obtain socio-demographic information. The condiments were analysed for proximate composition and microbiome profiles of separated and pooled fermented condiments were determined using shotgun metagenomic sequencing and bioinformatics analysis.
Results: Over 80% of the condiment vendors were females aged 26 to 40 years with an income of less than ₦5000 per week. Poor hygiene and lack of food safety awareness were observed in product handling. Moisture, fibre, fat, protein and ash contents were significantly high in fermented P. biglobosa ($p<0.05$). Atopostipes were the most abundant phylum in fermented R. communis (60.0%) and P. macrophylla while Debaryomyces (35.0%) were the highest in P. macrophylla. Vagococcus and Lactobacillus were abundant in fermented P. macrophylla. Antibiotic resistance genes to antibiotics including tetracycline, sulfonamide, glycopeptides, fosfomycin, fluoroquinolones, beta-lactams, and macrolides were significantly high in fermented P. biglobosa ($p<0.05$). Similarly, functional oligosaccharides including glucoside, maltodextrin, lactose, galactose, and fructooligosaccharides were significantly higher in P. biglobosa ($p<0.05$).
Conclusion: Differential relative abundance of Atopostipes suicloacalis, Vagococcus lutre, and Debaryomyces fabriyi indicated nutrient-active processes involving several unculturable microorganisms contrary to previously reported predominant Bacillus subtilis in Nigerian fermented condiments. The antibiotic resistance genes could influence consumers' gut microbiome with public health concerns and prebiotic oligosaccharides suggested carbohydrate pathways targeting several health-promoting benefits, through monitored fermentation under a hygienic environment.

Food Microbial Ecology

P1.55 Looking at the factors shaping vine and vinification microbiome of the Greek vine varieties Sideritis and Roditis

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Wine is a product with an enormous global market supported by the long-standing cultivation of wine-making vine varieties. Traditional vinification relies entirely on the native microbiota of grapes compared to commercial winemaking processes that use a few microbial starters. These guarantee reproducibility of the process leading always to the same quality product but tamper the local character and identity of local wines. Recent methodological advances in microbial ecology provided insights into the role of vine microbiome on the quality of wine and identified factors that shape the vine microbiome. In the current work we aimed to identify the composition of the prokaryotic and eukaryotic microbiome of two of the most representative vine cultivars Roditis and Sideritis. In this quest we sampled grapes, leaves and rhizospheric soil from two well-defined terroirs per variety. Grapes from each of terroir x variety combination were subjected to three different vinification processes: (a) spontaneous carried out by the autochthonous microbiota (b) spontaneous carried out by the autochthonous microbiota but controlled with preservatives added in commercial fermentations and (c) commercial inoculated with allochthonous inocula. Amplicon sequencing of the 16S rRNA gene and the ITS2 region in Illumina Hiseq was employed to identify the composition of the vine and vinification microbiome in the different terroirs and vinification treatments. Parallel analysis of the metabolome and the antioxidant activity of the wine produced will be correlated with microbiome composition to identify microorganisms potentially associated with desirable organoleptic characteristics of the wine. Our findings will be presented in the conference and we expect to identify the factors that shape microbial terroir and point to microorganisms from the vine microbiome that contribute to the local character of the wines produced.

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Acknowledgements: This work was supported by (i) DRAMA TEROIR "Valorization of Drama wine identity through a holistic characterization of the natural and microbial environment, MIS 5131395", under the call RESEARCH–CREATE–INNOVATE II (code: T2EDK- 02974), (ii) RIS3_MICROBE-TERROIR –"The role of microbial terroir in the production of high quality wine products from local varieties (Δ??6-0019343, MIS5045460), «Western Greece 2014-2020» co-funded by the European Fund of Regional Development and national funding.

Food Microbial Ecology

P1.56 Antimicrobial activity of plasma-activated water on E. Coli planktonic cells and biofilms

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Plasma Activated Water (PAW) is a non-thermal decontamination technology which has several applications including within the food industry. The antimicrobial activity of PAW is mainly attributed to reactive oxygen and nitrogen compounds which are generated, creating a high oxidation reduction potential and also contributing to the acidification of the water solution.

PAW was generated at atmospheric pressure in an electrochemical cell equipped with a copper electrode. Each PAW sample was generated following a 3 h of plasma discharge with water circulating, while air was used as gas with a flow rate of 150 L/h. Basic parameters of the produced PAW such as the pH and the conductivity, were determined before each use. Escherichia coli NCTC12900 was used in this study. Planktonic cells of E. Coli were exposed to PAW and enumeration was performed every 5 min and up to 25 min. Stainless steel coupons were used as surfaces for biofilm formation. For the first form of biofilm, a pre-attachment step was performed, and then coupons were immersed in Tryptic Soy Broth (TSB) for 48 h at 37 °C. The second form of biofilm was developed over 12 days with cycles, where hydration with 5% TSB was provided alternating with prolonged dehydration stages at 37 °C. Both forms of biofilms, after development, were exposed to PAW with contact times of 0, 5, 10, 15, 20 min. Biofilm detachment was achieved by means of sonication. Sterile distilled water was used as control throughout the study.

The results obtained showed that the highest log reduction (2.52 log CFU/mL) was achieved after exposure of planktonic cells to PAW for 25 min, which was the maximum contact time studied. The results of the two biofilm forms indicated that the biofilm developed with dehydration stages, exhibited higher resistance to PAW after 20 min of exposure (1.69 log CFU/cm^2 reduction), when compared with the biofilm developed in TSB for 48 h (2.50 log CFU/cm^2 reduction).

The findings of the study reveal the potential of PAW to be used as a disinfecting agent in food contact surfaces, however, longer contact times are required for higher inactivation rates.

Food Microbial Ecology

P1.57 Sensory differentiation for specialty coffees through co-inoculation of yeasts during induced anaerobic fermentation

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Selected yeasts for coffee fermentation are correlated with changes in chemical compounds and beverage sensory characteristics. Many works using a single yeast during fermentation are being developed and have different characteristics. This work aimed to evaluate the chemical and sensory modifications of coffee fermented with co-inoculation (from two to two and the three together) by dry processing during induced anaerobic fermentation. The monitoring of the microbial population was performed Real-time PCR (qPCR), liquid and gas chromatography were used to analyze organic acids and volatile compounds, respectively, and sensory analysis were performed according to the Specialty Coffee Association (SCA) method. Caparaó coffees showed a higher C. parapsilosis (6.14 Log cell/g) population followed by S. cerevisiae (5.85 Log cell/g) and T. delbrueckii (4.64 Log cell/g), differentiating it from other producing regions. At the end of drying, it was detected that the 3SCT treatment had the highest population of S. cerevisiae (8.74 Log cell/g). These results indicate this yeast showed excellent adaptation and competition with the endophytic microbiota. Citric, succinic, and malic acids were found in all treatments and the consumption of citric and malic acid may indicate a change in the metabolism of microorganisms. At the end of drying, all treatments that were in co-inoculation with S. cerevisiae CCMA 0543 (2SC, 3SCT, 2ST) showed a decrease in the acetic acid content with a significant difference. The group of volatile compounds most related to green coffee were alcohols, aldehydes, hydrocarbon, and esters; the roasted coffee were pyrazines, furans, pyrroles. The 3SCT treatment showed the highest increase in esters when compared to coffee before fermenting. Detection of some organic acids and volatile compounds during fermentation may indicate that the starter cultures used different metabolic routes. The control treatment had a lower sensory score (85 points). All co-inoculation treatments presented the best sensory scores (>86 points). In the inoculated fermentation, fruity, citric, molasses, honey and freshness notes appeared. The co-inoculated treatment with S. cerevisiae CCMA 0543, C. parapsilosis CCMA 0544, and T. delbrueckii CCMA 0684 was the best, considering the diversity of sensory notes descriptors and the final concentration of organic acids and volatile compounds.

Food Microbial Ecology

P1.58 Enterobacteriaceae contribute to the carbon dioxide-mediated bloater defect of fermented cucumbers and are not inhibited by an acidic pH

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The microbial production of carbon dioxide (CO2) in cucumber fermentations results in the development of hollow cavities inside the fruits, a processing defect known as bloater. The role of Enterobacteriaceae in bloater defect and their inhibition by an acidic pH in cucumber fermentation was evaluated. Enterobacter cancerogenous was inoculated in a cucumber fermentation model system composed of acidified and pasteurized cucumbers with an adjusted pH of 6.0 ± 0.5 to confirm a role in the defect. The growth of E. cancerogenous and the changes in the biochemistry in fermentation jars was monitored by plating on Violet Red Bile Agar and pH measurements and HPLC analysis, respectively. Bloater defect was quantitated using a weighted scale for injury type and severity. The formation of CO2 and hydrogen was also followed. Inoculation of E. cancerogenus in the acidified and pasteurized cucumbers resulted in the production of 69% CO2, 2% hydrogen and a bloater index of 24.4 ± 11.1, as compared to 37%, 0% and 0.7 ± 1.5, respectively, in the uninoculated controls. The increase in the Enterobacteriaceae colony counts to 9 log of CFU/g produced enough gas to cause ballon shaped hollow cavities. Lactic and acetic acids were minimally produced (<10 mM) by E. cancerogenous in the cucumber jars. Cucumber Juice Media (CJM) was used determine the pH at which the Enterobacteriaceae stop growing and use such condition to inhibit their ability to produce CO2. While Enterobacter spp. decreased the pH of the CJM from 5.7 ± 0.3 to 5.3 ± 0.2, all other Enterobacteriaceae decreased it to 4.4 ± 0.5, with the exception of Leclercia adecarboxylata which dropped the pH to 3.3 ± 0.2. The adjustment of cucumber fermentation pH at its start to 4.7 ± 0.1, the minimum value at which a complete fermentation can proceed, was insufficient to inhibit the metabolic activity of the Enterobacteriaceae and prevent the defect. It is concluded that the respiratory metabolism of the Enterobacteriaceae indigenous to the fruit contributes to the development of bloater defect and that such activity is not prevented by an adjustment of the initial fermentation pH to 4.7 ± 0.1.

Food Microbial Ecology

P1.59 The influence of salt content in fresh cheeses on microbiological, chemical and sensory parameters

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The average salt consumption in the world is almost twice the amount recommended by the WHO. Cheeses are one among foods with a higher salt content. In this experiment, we focused on fresh cheese, which normally has a salt content of about 1.0% NaCl. The microbiological quality of the pasteurized milk as well as the quality of the laboratory-prepared salt baths was assessed before experimental cheese production. The easiest way to influence the salt content is by shortening the salting time. The technological procedure used was chosen to correspond with the classical production. Two series were produced and in each of them 8 batches differing in the salt concentration and salting time were tested.

Microbial, chemical and sensory parameters were evaluated after the production, after 7 and 14 days of storage. Lactococci numbers gradually decreased and after 14 days of storage they dropped by about one order to a count 108 CFU/g. The most common contaminating microorganisms in fresh cheeses are yeasts and there was an apparent increase of this group after 14 days of storage to a density of 103 CFU/g in cheeses from the first series of production. A significant increase was found in non-salted cheeses packed only by sealing in a protective film without air extraction. Another monitored group were halotolerant microorganisms, which increased to the order of 104 CFU/g during storage of cheeses from the first series of production, especially in samples without salting or with a significantly reduced salt content.

In relation to sensory evaluation, the lowest possible salt content in cheeses was set to 0.5-0.6 % of NaCl. The different salt content in the samples did not affect the chemical parameters or the number of lactococci. The economic and technological demands of the production of fresh and reformulated fresh cheeses are practically the same. The results obtained indicate that in the production of fresh cheeses with reduced salt content, high hygiene standards of operation as well as optimized rennet dosing are of importance.

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Food Microbial Ecology

P1.60 Assessing the behaviour of Listeria innocua in a model of artisanal Canastra cheese during manufacturing and ripening

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Canastra Cheese is a product from Brazil, made with raw milk and a natural whey starter (NWS) called "pingo", by backslopping, and ripened for at least 14 days. Artisanal cheese is a dynamic food matrix and is often linked to listeriosis outbreaks. The influence of NWS on the control of Listeria during raw milk artisanal cheesemaking and ripening needs to be studied. The aim of this research was to evaluate the influence of NWS on the behaviour of Listeria innocua, as a surrogate for L. monocytogenes, during manufacturing and ripening of experimental Canastra cheese. Cheeses were made from raw milk contaminated with 10^2 CFU/mL of two L. innocua strains (CLIST 4530 and UFG) isolated from dairy products: A) cheese with good quality "pingo" (CGP); B) cheese with bad quality "pingo" (CBP) that caused swelling of cheese; C) cheese without "pingo" (CWP). The trials were repeated twice. Populations of L. innocua during the cheese making and ripening (56 days at 20 °C and 65% humidity) were determined. Data was fitted to the logistic model using the USDA Integrated Pathogen Modeling Program and the maximum cell concentration (ymax) and the maximum specific growth rate (μmax) were estimated. The maximum specific growth rate of L. innocua did not differ significantly ($p > 0.05$) between cheeses with (0.36 h^{-1}) and without (0.44 h^{-1}) pingo. The maximum cell concentration of L. innocua in CGP (5.2 Log CFU/g) was reached on the 14th day of ripening and was significantly lower ($p < 0.05$) when compared to CWP (6,1 Log CFU/g) and CBP (6.6 Log CFU/g). After ripening for 56 days, L. innocua population was 3.7 log CFU/g in CGP and 5.4 Log CFU/g on the other cheeses. This data showed that L. innocua can reach a high cell concentration during artisanal cheese production and ripening at room temperature, even when "pingo" is added. There was no significant decrease in the growth rate when NWS was used. The period of 14 days was not enough to inactivate L. innocua indicating a potential risk of growth and survival of L. monocytogenes, when present.
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Food Microbial Ecology

P1.61 Lactobacillus plantarum strains show diversity in fluid flow induced biofilm formation

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Microbial biofilms result from the adhesion and growth of bacteria into a multicellular community on a surface embedded in an extracellular matrix. Microbial biofilms grow under fluid flow in many applications of natural, technological, and food processes. Biofilm formation can cause many problems in the food industry, such as product spoilage, food safety problems, and loss of production efficiency. This work studies the biofilm formation of two strains, Lactobacillus plantarum WCFS1 and CIP104448under under static and flows conditions by designing a novel fluidic assay based on a 48-well plate. The total biofilm formation under static and flow conditions with flow rates of 0.8, 1.6, 3.2 and 4.8 ml/h was quantified using crystal violet staining and the number of viable biofilm cells was determined based on plate counts. We show that the flow differently affects the growth and formation of biofilms produced by the two strains of L. plantarum. Our results showed the amount of biofilm in the CIP104448 strain grows by increasing the rate of flow, while it does not change significantly for the biofilm of the WCFS1 strain However, applying flow increase the number of viable cells in both strains. We have also simulated the flow field of the medium to determine the velocity and shear stress fields and compared them to the spatial distribution of formed biofilms. In addition, applying flow in the CIP104448 strain promotes biofilm formation on vertical walls rather than on the bottom of the container. In addition, based on the impact of DNase I and proteinase K enzyme treatments, we found the composition of the mature biofilm formed in static and under flowing conditions is conceivably different in both strains.
For WCFS1 in the static condition, the amount of mature biofilm declined after DNase I and Proteinase K treatment, while in flow conditions, the decline was only observed after adding DNase I. Unlike WCFS1, in the CIP104448 strain, the decrease in the CV staining was observed only after adding Proteinase K in both flow and static conditions.

P1.62 Explore light emit diodes and photocatalytic surfaces to prevent Listeria monocytogenes biofilm formation in food industry

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The development of new disinfection methods of food contact surfaces is essential to help the food industry cope with the high demand for quality and safe food for human consumption due to the increasing human population. Pathogenic microorganisms can contaminate food and food contact surfaces as they can adapt to various environmental conditions and can live in a variety of habitats. Microorganisms form biofilms, aggregates of microorganisms from one or more species that are enclosed in a matrix of extracellular polymeric substances. The removal of biofilms is a very challenging task. Biofilms can be the source of foodborne diseases and contribute to the deterioration of food.
Listeria monocytogenes is a pathogenic bacterium responsible for listeriosis, a serious infection that can lead to gastroenteritis, meningitis, and miscarriage in pregnant women. Ingestion of contaminated food is the primary route of transmission of L. monocytogenes to humans. This work aimed to explore the efficacy of ultraviolet (UV) light-emitting diodes (LEDs), a sustainable alternative to the conventionally used mercury lamps, to inactivate L. monocytogenes biofilms. A strain of L. monocytogenes was used to produce biofilms in chips of stainless steel (with 10 mm diameter), a material highly used in the food industry. The small circular chips were also modified following a solvent-free procedure (Huertas et al, 2019) to develop photocatalytic stainless steel surfaces that could aid inactivation through the production of hydroxyl radicals. Biofilms formed over a week were analyzed before and after the exposure to UV LEDs that emit light at 265 nm. To evaluate the impact of UV light in biofilms, scanning electron microscope images were obtained before and after the exposure to LEDs. This treatment proved to be effective in the inactivation of the bacterial biofilm since we obtained a log reduction of 1.74 and 3.95, after 2.5 and 5 minutes of exposure to three small LEDs, respectively.

Food Microbial Ecology

P1.63 Portuguese traditional PDO cheeses: Main features of the autochthonous microbiota

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Cheese is one of the utmost consumed milk-derived products worldwide, due to specific organoleptic features, such as taste and aroma. Among these, raw milk traditional cheeses harboring the Protected Designation of Origin (PDO) label are highly appreciated, presenting a significant economic impact and a huge social importance, allowing the preservation of a know-how centenarian heritage. In Portugal, a wide range of traditional cheeses of high richness and specificity are manufactured and marketed, many of which with PDO status. The present investigation focused on two of those PDO cheeses: Azeitão and Nisa.
In both cheeses, fermentation depends on the microbiota naturally present in the raw material, or introduced during the manufacturing process. This consortium of microorganisms is poorly characterized, not only in these two types of cheese but in all other traditional Portuguese cheeses, compared to other countries. Aiming to preserve the artisanal production of regional cheeses, an important part of our cultural heritage, this study aimed the comprehensive description of the autochthonous microbiota, regarding pathogenicity, technological and probiotic features.
Briefly, lactic acid bacteria -LAB- have been isolated since 2016 and submitted to a thorough pipeline of characterization based on identification, genomic comparison by RAPD PCR, screening for antibiotic resistance and virulence traits, assessment of technological (e.g., milk acidification ability, growth under various conditions, enzymatic profile) and probiotic (e.g., survival to gastrointestinal simulation assays) potential.
Although results showed low levels of hemolytic ability and antibiotic resistance, cheese LAB were found to be resistant to clinically important antimicrobials, such as β-lactams, aminoglycosides, and glycopeptides. The majority of the bacteria were able to grow under the tested conditions and displayed antimicrobial activity against the foodborne pathogen Listeria monocytogenes. Moreover, eight cheese isolates exhibited putative probiotic features in preliminary assays.
Overall, the present study highlighted the diversity and richness of the autochthonous microbiota present in artisanal cheeses. Pointing towards the importance of attaining a relevant body of knowledge on the microbial ecology of fermented food products of animal origin.

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Food Microbial Ecology

P1.64 Metagenomics highlights specific microbial dynamics during storage of fish fillets in different conditions

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Fish is a highly perishable product and is easily spoiled by microbiological activity and chemical oxidation of lipids. However, microbial spoilage is the main mechanism affecting fresh fish quality. Under particular storage conditions (e.g., atmosphere, temperature), a consortium of bacteria known as Specific Spoilage Organisms (SSOs) produces metabolites responsible for off-flavours and causes the organoleptic rejection of the product.
Proteins are hydrolyzed into peptides and amino acids by microbial protease, leading to changes in physicochemical properties of fish flesh (texture, color, water-holding capability, etc.) and amino acids released by protein degradation can be converted into □-keto acids, sulfides, ammonia, trimethylamine and various kinds of biogenic amines. Visible slime may also appear as a result of the production of extracellular polysaccharides.
'Omics may be successfully implemented within the food industry, for microbial source tracking investigation or for monitoring the product shelf-life, identifying the presence of microbial spoilers and how processing/storage conditions may affect microbial dynamics.
We used shotgun metagenomics to evaluate microbial dynamics and the genomic potential of the microbiome involved in fish fillet spoilage during storage in different condition. Samples were stored at three different temperatures (0, 4, 10°C), under three different packaging conditions (aerobic; vacuum, VP; modified atmosphere, MAP) and analysed at different time points (up to 10 days for aerobic and 16 days for MAP and VP). At each sampling point, shotgun metagenomics was carried out in order to understand microbial dynamics during food spoilage. These data may be implemented in predictive models, helping to predict the product shelf-life, anticipate microbial spoilage and avoid food loss.
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Food Microbial Ecology

P1.65 Selection of indigenous non-Saccharomyces yeast strains for the production of "Sfursat" wine according to their oenological characteristics

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"Sfursat" is a traditional dry red wine produced in Lombardy region (Italy), using partially dehydrated Nebbiolo grapes. The aim of the study was the selection of autochthonous non-Saccharomyces yeasts to employ in the production of "Sfursat" wine. Sixty-two yeasts were isolated from grapes and identified by molecular methods (5.8S-ITS-RFLP and D1/D2 domain sequencing). Physiological characterization (ethanol and SO tolerance, enzymatic activities, HS production, adhesive properties and antimicrobial activity) was followed by laboratory pure micro-fermentations to investigate oenological performance. Mixed fermentations of four selected strains with a commercial Saccharomyces cerevisiae were tested at laboratory scale using co- and sequential inoculation protocols. Further, two strains were tested in winery in co-inoculation with S. cerevisiae. During mixed fermentations, growth kinetics and chemical profile were monitored. Thirteen species were identified: Metschnikowia pulcherrima, Metschnikowia fructicola, Candida californica, Candida apicola, Starmerella bacillaris, Hanseniaspora uvarum, Pichia kluyveri, Rhodotorula graminis, Rhodotorula nothofagi, Zygosaccharomyces bailii, Debaryomyces carsonii, Zygoascus hellenicus and Zygoascus meyerae. Physiological and oenological characterization in laboratory scale fermentations highlighted inter- and intra-species differences. The promising oenological attitude of Starm. bacillaris, Metschnikowia spp., P. kluyveri and Z. bailii species was highlighted. Sugars were completely consumed in all mixed laboratory scale fermentations except for the Z. bailii/S. cerevisiae couple. In all conditions (pure and mixed as well as in co- and sequential inoculation) Starm. bacillaris/S. cerevisiae produced the lowest concentration of acetic acid and ethanol and the highest amount of glycerol, followed by P. kluyveri/S. cerevisiae. These two couples were tested in winery fermentations. The populations of non-Saccharomyces yeasts dropped to undetectable levels after the day 7 both. Molecular fingerprinting highlighted that inoculated strains dominate the fermentation. The only exceptions were the mixed fermentation with Starm. bacillaris in which a different S. cerevisiae profile was identified. Wines produced using Starm. bacillaris/S. cerevisiae couple confirmed the laboratory outcomes. Acetic acid values were similar among different fermentations tested. Results demonstrated the potential applicability of the two yeasts as starter culture for "Sfursat" production.

Food Microbial Ecology

P1.66 Impact of iron on structuration and functioning of cheese microbial communities

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Microbial interactions are decisive factors in cheese quality and safety. Microorganisms require certain crucial nutrients for their functionality, and iron is on top of the nutrients list. Iron is involved in various metabolic, physiological, and biochemical processes such as respiration, gene regulation, enzyme cofactor, DNA, fatty acids, and amino acids syntheses. However, cheese is a highly iron-restricted habitat (1.24mg/Kg) due to its very low iron quantity, presence of iron-chelating proteins (lactoferrin), and low iron diffusion index. Consequently, iron competition is one of the mediators of microbe-microbe interactions in cheese rinds. It has been demonstrated that iron is a limiting factor for the growth of many surface ripening bacteria.
However, the prerequisite challenge is to evaluate the capacity of iron to disturb the cheese ecosystem and identify the concentration thresholds required to observe such disturbance.
A Munster-type model cheese system comprising a reduced microbial community (RMC) composed of nine strains (Lactococcus lactis, Glutamicibacter arilaitensis, Brevibacterium aurantiacum, Staphyloccocus equorum, Corynebacterium casei, Hafnia alvei, Geotrichum candidum, Debaryomyces hansenii, Kluyveromyces lactis) was used to assess the effect of iron supplementation on the microbial succession and production of volatile compounds during cheese ripening. The cheese was sampled on days 0, 14, and 28 and five different iron concentrations in the form of FeCl3 6H2O (0 µM, 4 µM, 8 µM, 16 µM, 30 µM, and 60 µM final iron) were used for supplementation. The growth was evaluated by plate count, and the production of volatile compounds was followed by GC-MS analysis. The results showed that iron supplementation can significantly modify the growth kinetic of some microorganisms used in the RMC and the volatile compounds profile. For example, Glutamicibacter arilaitensis and Brevibacterium aurantiacum growth increased linearly with increased iron concentration. On the contrary, Hafnia alvei and Geotrichum candidum count decreased with high iron concentrations. Meanwhile, other microbial species remained unaffected by iron. Furthermore, a significant difference was observed among the iron-supplemented model cheeses in volatile compound profiles.
This work provides insights into an iron disturbance in the cheese ecosystem. One pronounced effects of iron is on microbial communities as it can significantly influence microbial interactions.

Food Microbial Ecology

P1.67 Biodiversity, occurrence and antibiotic resistance profile of enterococci isolated in bovine raw milk and feces

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Enterococci are ubiquitous lactic acid bacteria that are widely distributed in raw-milk. They are commensals of the gastrointestinal tract of animals, thus, via fecal contamination, could reach raw milk and dairy products. The aims of this study were: 1) to determine the genotypic diversity of enterococci isolated from individual cow milk and bovine fecal samples; 2) to investigate if cow feces are the source of milk enterococcal contamination and 3) to evaluate the antibiotic resistance (AR) pattern of dairy-related enterococci and their ability to transfer resistance genes.

Thirty-one multiparous Italian Holstein dairy cows was considered and 310 presumptive Enterococcus were isolated at three different time points from milk (n. 177) and feces (n. 133) samples. Differences in enterococcal community structure were observed between the milk and feces samples. E. faecalis (59.9%), E. faecium (18.6%) and E. lactis (12.4%) were the most frequently isolated species from milk, while E. faecium (84.2%) and E. hirae (15.0%) were the predominant ones in bovine feces. RAPD-PCR highlighted a high number of Enterococcus biotypes (45 from milk and 37 from cow feces) and none of the strains present in milk exhibited genetic profiles similar to those of strains from cow feces. These results suggest that the bovine feces are not a source of enterococci for milk.

All the strains were susceptible to ampicillin, daptomycin, gentamicin, teicoplanin and vancomycin. Milk biotypes showed high percentages of AR to streptomycin (86.7%), tetracycline (73.3%), erythtomycin (46.6%) and tigecycline (33.3%), while linezolid (45.9%), quinupristin/dalfopristin (36.1%) and tigecycline (32.4%) were the most prevalent AR phenotypes in cow feces.

In both milk and feces biotypes the tetracycline resistance was conferred by the tetM and tetL genes, and the Tn916/Tn1545 family transposons were detected in E. faecalis, E. malodoratus and E. hirae biotypes. Only the E. faecalis biotypes were able to transfer the tetM gene to the recipient strain, and the frequency from donors to Lb. delbrueckii ranged from 103 to 106 transconjugants per recipient. Our results indicated that Enterococcus biotypes from milk and bovine feces belong to different community and the ability of these microorganisms to transfer AR genes is strain-dependent.

Food Microbial Ecology

P1.68 Microbiological quality of raw ewe's milk from native Epirus breeds and basic culture-dependent characterization of its indigenous lactic acid bacteria (LAB) biota

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Raw milk produced by native small ruminant breeds in Epirus may still be a valuable natural source of autochthonous beneficial, potentially novel, lactic acid bacteria (LAB) strains with superior biotechnological and/or probiotic properties. However, raw milk may also contain pathogenic and spoilage bacteria. The aim of this study was to evaluate the microbiological quality of raw ewe's milks (RM1, RM2) from two sheep yards in the area of Arta, intended for traditional Kefalotyri cheese production, and to identify the indigenous LAB by classical and molecular methods. The RM microbiota was dominated by pseudomonad-like bacteria (mean 6.8 log CFU/ml), followed by mesophilic LAB enumerated on MRS/30oC agar (5.3 log CFU/ml), but overgrown by gram-negatives on M17 agar at 22oC and 42oC. Enterococci (3.7 log CFU/ml), staphylococci (4.4 log CFU/ml; ca. 20% were pathogenic) and coliforms (4.1 log CFU/ml) were subdominant in RM. Batch RM1 (pH 6.6) contained ca. 10-fold higher counts of psychrotrophic spoilage bacteria than RM2 (pH 6.7), however RM2 harbored Listeria monocytogenes in 25 ml. Sixty colonies (30/RM batch) were picked from six agar media to eventually collect 42 LAB (RM1/18; RM2/24) isolates identified by 16S rRNA sequencing; moreover, enterococci were identified at the species by IGS. The isolates were Leuconostoc mesenteroides (10), Streptococcus parauberis (7), S.lutetiensis/equinus (3), S. gallolyticus (1) and Lactococcus lactis (1), representative of the dominant mesophilic LAB populations, plus Enterococcus faecium (8), E.faecalis (5), E.durans (5), E. hirae (1) and E. hermanniensis (1). One E. faecium strain biotype (3 isolates) and one E. durans isolate from RM1 displayed strong and moderate antilisterial activity in vitro, possessing enterocin A-B-P and A-P genes, respectively. Three E. faecalis RM2 isolates possessed the gelE and ace virulence genes. All LAB isolates lacked vanA, vanB for vancomycin resistance, and agg, espA, hyl and IS-16 virulence genes. In conclusion, the most prevalent Lc. mesenteroides group, the two safe antilisterial strains of the E. faecium/durans group and the only wild L. lactis K8 strain should be validated further as potential starter or adjunct strains. The prevalence of □-hemolytic pyogenic streptococci in RM, mainly S. parauberis in RM2, requires further consideration.

P1.69 Acidifix® & Sweety® dairy cultures represent 2 radical innovations that meet modern consumer demands

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Dairy products with improved taste and texture, but fewer additives and reduced sugar and fat addition, or overall calorie content are in high demand by modern, health-conscious consumers. As a major player in the global dairy industry, Chr. Hansen A/S is constantly exploring novel and natural ways to improve products to fulfill demanding consumers. This pushes the boundaries of microbial performance and requires the constant development of new dairy cultures with novel properties.
Here we will present the use and the progress of natural methods for selection and improvement of dairy bacteria that today have led to all-natural concepts for dairy products with reduced added sugar due to high natural sweetness "Sweety" and with controlled acidity and post acidification "Acidifix".

Food Microbial Ecology

P1.70 Differentiation of Alicyclobacillus acidoterrestris isolated from commercial orange juice

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One of the main problems in the fruit juice industry is the presence of Alicyclobacillus acidoterrestris spores. Although this microorganism is not pathogenic, it has the ability to form guaiacol that can cause spoilage, which is difficult to be detected before consumption of the juice, due to the lack of acid or gas production. Therefore, the early detection of the microorganism is very important for juice manufacturers. The objective of this work was the isolation and characterization of Alicyclobacillus acidoterrestris strains from orange juice. For this purpose, 71 isolates were recovered from commercial orange juice according to the method described in IFU no. 12 September 2004/March 2007. The isolates were subjected to PCR-RFLP (restriction fragment length polymorphism) in order to discriminate them at strain level. In brief, the PCR products of amplified region V1-V3 of the 16S rRNA gene were digested with 3 different restriction endonucleases HhaI, RSaI and HiNFI. Representative isolates were subjected to PCR-RFLP targeting the vdc gene to distinguish the different A. acidoterrestris types (I and II) based on HaeIII, HphI and HinP1I restriction profiles. The observation of the different restriction patterns of 16S rRNA revealed that the isolates were categorized in different number of groups, depending on the restriction endonuclease. Moreover, only the digestion of vdc gene with the restriction enzyme HphI succeed to distinguish the A. acidoterrestris type I from type II. In conclusion, RFLP succeeded to differentiate the A. acidoterrestris isolates at strain and type level.
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P1.71 Listeria monocytogenes in salmonid slaughter facilities

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Listeria monocytogenes (Lm) is of special concern in the seafood industry as its robustness makes it particularly adapted to the production environment. Lm represents a special challenge for lightly preserved ready-to-eat products with an extended shelf life, such as for instance cold smoked salmon and trout. In seafood produced under temperate and cold climatic conditions, Lm is among the main concerns with respect to food safety.
In 2020 and 2021, a screening program for Lm in salmonid slaughter facilities was conducted in Norway, and 358 samples from 60 slaughter facilities (49 slaughtering plants and 11 slaughtering vessels) were examined. Samples were collected from the production environment (n=108), from the surface of fish entering the facilities (n=47), and from the surface of fish (n=59) and raw material (n=144) at end point at the examined facility.
None of the samples from the slaughtering vessels were positive for Lm, whereas 22 positive samples were detected in nine different slaughtering plants. In five of these plants, several positive samples were found. Six of the slaughtering plants had positive samples at the end of the production line, where a higher prevalence was found when swabbing the fish skin and gills compared to the examined raw material. In all samples of raw materials Lm were absent or contained numbers below the quantification limit (<10 CFU/g).
Further, the 22 isolates were genome sequenced and SNP and cgMLST analyses were performed. These analyses indicated that in some slaughtering plants, the same strain of Lm was present throughout the production line, whereas in other plants different strains were found at different stages during production. These findings might indicate that some plants have established "in-house" strains inhabiting the entire production environments, whereas other plants have several points of contamination throughout the production environment.
Overall, this study shows that Lm can be present in both fish and the production environment, and that in some cases will be present in fish ready for further processing in the salmonid value chain.

Food Microbial Ecology

P1.72

First description of diversity and technological properties of predominant lactic acid bacteria from traditional African cereal-based dairy food "Gappal"

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Gappal is an African fermented food made from millet dough and milk. It is a very interesting food on nutritional consideration. However, there is no available data on its probiotic properties. This study aimed to identify lactic acid bacteria associated with Gappal fermentation and evaluate their probiotic properties. The polyphasic approach was used to determine the diversity of lactic acid bacteria of Gappal. It consisted of a biochemical characteristics of lactic acid bacteria isolates through carbohydrates fermentation profile (API 50 CHL, API STREP), a repetitive extragenic palindromic PCR (rep-PCR) and the sequencing of 16S RNA . The isolates' technological properties such as acidification, exopolysaccharides production (EPS), amylase activity and bacteriocin production were also appreciated. The results indicate that Weissella confusa, Weissella cibaria, Pediococcus acidilactici, Pediococcus pentosaceus, Lactobacterium fermentum, Enterococcus faecium, Enterococcus faecalis, Streptococcus agalactiae and Aerococcus spp were the main lactic acid bacteria of Gappal. The predominant species of lactic acid bacteria in Gappal are Enterococcus (30.36% of the isolates from dried Gappal and 30% of the isolates from liquid Gappal), Weissella confusa (21.43% of the isolates from dried Gappal and 38% of the isolates from liquid Gappal), Lactobacillus fermentum (14% of dried Gappal isolates and 19.64% of liquid Gappal isolates), Weissella cibaria (3.57% of the isolates from dried Gappal and 6% of the isolates from liquid Gappal), Streptococcus agalactiae and Aerococcus spp were found in dried Gappal but not in the liquid one. About 0.23% of strains produced bacteriocin, 3.3% produce exopolysaccharides and 2.14% amylase. A better use of them is in co-culture for controlled production.

Food Microbial Ecology

P1.73 Microbial safety aspects of traditional wind-dried Faroese lamb or ewe leg, skerpikjøt

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Skerpikjøt is a traditional meat product from the Faroe Islands that comprise wind-dried lamb or ewe leg, stored for several months with no added salt. The product is usually consumed with no further preparation after the removal of the outer layer. As a local product based on empirical and variable production schemes, it is unclear to which degree it meets the contemporary safety expectations of mainstream European meat products. This study describes the composition and levels of the microbiota of nine skerpikjøt products from three producers. The average load of the aerobic mesophilic microbiota was 7.53 ±0.62 log (CFU/g) on the product surface and 4.75 ±1.45 log (CFU/g) in the core, measured on plate count agar (PCA). Gram-positive, catalase-positive cocci constituted the most prevalent bacterial group. Identification of isolates to species level was done by MALDI-TOF mass spectrometry and/or 16S rRNA gene sequencing. Staphylococcus equorum was the predominant species, followed by Staphylococcus saprophyticus and Mammaliicoccus vitulinus. All species are common and benign members of the microbiota of meat products. Lactic acid bacteria were present in some cases, comprising Carnobacterium divergens, Carnobacterium maltaromaticum, Latilactobacillus curvatus, and Leuconostoc mesenteroides. Serratia liquefaciens was the most common Enterobacterales species, and pseudomonads were also found. A few yeast isolates were identified by 26S rRNA gene sequencing. They all belonged to the Debaryomycetaceae family. Yeast and mould were found in high numbers on DG18 agar, measuring about one log lower than the PCA counts. Growth of moulds was observed in cavities and blood vessels inside the meat, thus representing a potential presence of mycotoxins and allergens. Quantities of sulphite-reducing clostridia and Listeria monocytogenes were below detection levels. Levels of biogenic amines were low except for one product with a relatively high amount of cadaverine (54.07 µg/g). The same product also showed the highest levels of histamine, putrescine, and spermine. This study did not demonstrate major concerns for safety issues regarding skerpikjøt.

Food Microbial Ecology

P1.74 Mixed Pseudomonas spp. biofilm aids Listeria monocytogenes to survive disinfection

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The genus Pseudomonas is known to be the dominant genus in many food processing environments. Members of this genus are also known to be important spoilage bacteria in various food products, they are non-fastidious and exhibit high tolerance to many antibiotics and disinfectants. In addition, many of them are psychrotrophic and notorious biofilm producers, resulting in excellent survival in food processing environments.
In this study our aim was to see if a mixed biofilm of psychrotrophic Pseudomonas isolates, with good biofilm forming capacity and high tolerance to a Peracetic Acid (PAA) based disinfectant, would affect the survival of Listeria monocytogenes cells in the biofilm after disinfection. A set of Pseudomonas isolates from a salmon processing plant was screened for biofilm forming capability at 12 °C and tested for resistance towards a disinfectant commonly used in the salmon industry. A high variation in biofilm formation between the isolates was observed and, most isolates showed a much higher tolerance towards the disinfectant in biofilm state than in planktonic state. Further, five selected isolates were grown in multispecies biofilm on stainless steel coupons together with the pathogen Listeria monocytogenes, and the survival after disinfection was investigated. Multispecies biofilm of only the Pseudomonas isolates and L. monocytogenes alone was studied for comparison. No survival was detected in any of the cultures immediately after disinfection but over time a visual regrowth was observed. Three days after disinfection the regrowth was quantified by plate counting. In 18 parallel tests L. monocytogenes alone did not survive the disinfection while the mixed Pseudomonas biofilm survived in 72 % of the cases. In the biofilm with Pseudomonas and L. monocytogenes together, survival of Pseudomonas was registered in 83 % of the cases and survival of L. monocytogenes was registered in 56 % of the cases.
Our results indicate that the Pseudomonas multi-species biofilm served as a shelter for L. monocytogenes resulting in high survival of the pathogen after disinfection. This underlines the importance of controlling the bacterial contamination in food processing environments, also the contamination by the very common but often overlooked Pseudomonas genus.

P1.75 Non-starter lactic acid bacteria characterization of Cretan Staka using MALDI-TOF MS

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This study concerns the exploration of microbial diversity of Staka, which is an artisanal dairy product produced in the island Crete of Greece. Staka is a fermented cream made from sheep and goat milk, usually consumed as spread cheese. To our knowledge, so far, the microbiota of Staka has not been explored. In this work, high-throughput proteomic technology of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was applied as a novel method for microbial downstream characterization. Towards this, a total of 101 lactic acid bacterial isolates from homemade Staka were subjected to the above analysis. Identification was performed using an in-house built reference database of LMG (Laboratory of Microbiology Gent, Belgium). The identified isolates revealed that Staka microbiota disclosed the dominance of L. plantarum (24), E. durans (21), L. paracasei (20), E. faecalis (11), L. paraplantaruum (10), L. brevis (4), S. thermophilus (8), E. faecium (2), and Aerococcus sp. (1). Isolates, which were not clearly sorted by MALDI-TOF MS, were further identified through molecular analysis. In particular, pheS sequence analysis led to the identification of L. paraplantarum and Aerococcus sp. isolates. S. thermophilus as well as Enterococcus isolates were also identified through16S rRNA sequence analysis. The application of MALDI-TOF MS resulted in the identification of > 90% of the bacterial isolates, indicating that this approach is a fast and reliable tool, especially when considering the isolates number, speed and resolution. While the current use of MALDI-TOF MS remains relatively limited, recent reports are particularly encouraging for food microbiota characterization, and, at the same time, stress the need for expanding available MALDI-TOF MS spectra databases. This is the first report on the Greek Staka microbiota and it reveals its rich biodiversity.

Food Microbial Ecology

P1.76 Bacterial diversity of the rare traditional Halitzia cheese produced in Cyprus

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Halitzia is a rare white brined cheese produced in a few traditional dairies in Cyprus. It is a semihard cheese with a subacidic and slightly salty taste which is made from goat and/or sheep milk. The aim of this study was to characterize the bacterial ecosystem of six different Halitzia cheese samples, focusing on lactic acid bacteria (LAB). Isolated LAB strains were phenotypically assessed for their biochemical and technological properties. The cheese samples were analyzed for total viable counts (TVC), LAB, cocci, enterococci and coliform populations. 63 LAB strains were isolated from three randomly selected samples. After DNA extraction, rep-PCR was carried out and the fingerprints were used to cluster LAB strains. Selected strains were analyzed by 16S rDNA sequencing. Moreover, these isolates were tested for their ability to acidify milk and to produce antimicrobial substances against foodborne pathogens like Listeria monocytogenes and Staphylococcus aureus or the food spoilage bacterium Bacillus coagulans. Their proteolytic and lipolytic activities were also investigated. The pH and aw values of the Halitzia cheese samples ranged between 4.18 to 5.80 and 0.88 to 0.93, respectively. The TVC population was 7-8 log CFU/g while LAB ranged to 5 to 8 log CFU/g. Cocci and enterococci populations were 6.5-7.5 and 5.8-7.2 log CFU/g, respectively. Coliforms were detected only in two samples (<4.4 log CFU/g). A total of 16 strains were identified as Lactiplantibacillus plantarum, Lacticaseibacillus paracasei, Lacticaseibacillus casei, Levilactobacillus brevis, Enterococcus faecium and Enterococcus faecalis species. Some strains presented limited lipolytic activity. Most strains exhibited an antimicrobial potential against the exanimated pathogenic and spoilage species. Only E. faecalis strains caused rapid milk acidification accompanied by strong proteolytic and lipolytic activities. For this reason, the genome of a representative E. faecalis strain was sequenced and analyzed. Our findings suggest an important level of complexity of the bacterial population of Halitzia cheese. Strains isolated and characterized during this study could be candidates to be used as adjunct cultures in the production of Halitzia cheese in the future.

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Food Microbial Ecology

P1.77 A primary investigation of the microbial ecosystem of the Greek PDO cheese Sfela and Sfela touloumotiri

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The traditional Greek PDO Sfela cheese was selected to be analyzed in this study. Sfela is a semi-hard white brined cheese produced from ovine or mixture of ovine and caprine milk exclusively in the provinces of South Peloponnese. Amplicon sequencing and metagenomic analysis were selected to characterize the total microbial population of Sfela cheese. Apart from the PDO cheese samples we also analyzed one sample of Sfela touloumotiri, i.e. Sfela cheese ripened in a touloumi, a bag made of skin specially processed to receive the cheese mass. The 16S rDNA amplicon sequencing analysis allowed the identification of bacterial populations at the genus level. Although differences were observed between the microbial abundance of the samples, the predominant genera were Streptococcus, Lactococcus and other members of Lactobacillaceae family. The microflora of the samples was identified at the species level by shotgun metagenomics. Among the species identified in the PDO Sfela samples were Streptococcus thermophilus, Lactococcus lactis, Levilactobacillus brevis, Latilactobacillus curvatus, Lactobacillus delbrueckii, Lactiplantibacillus plantarum, Lacticaseibacillus paracasei, etc. In the Sfela touloumotiri sample, Tetragenococcus halophilus and Lactococcus lactis shared the bacterial population, while yeasts were present with Debaryomyces hansenii showing the highest abundance. We also looked for Metagenome-assembled genomes (MAGs) in our dataset and we performed functional analysis to identify technological properties of the microbial ecosystems. Our findings along with culture-based analysis could contribute to the collection of bacterial and yeast isolates deriving from Sfela cheese to be used as starters or adjunct cultures in its production.
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P1.78 Seasonal variations in microbial communities of European Plaice (Pleuronectes platessa) as affected by packaging and cold storage

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Plaice is one of the most widespread flatfish species in the North Sea. Although regularly consumed in many North-Western European countries, no research has been conducted to characterise the seasonal variations in the initial plaice microbiome nor how the microbiota develops during cold storage under different atmospheres. This knowledge is crucial in developing more consumer-friendly retail products of fresh plaice to increase the utilisation of this species. Thus, we aimed to characterise the initial microbiome and succession of the microbial communities in plaice as a function of packaging and storage conditions. Plaice caught in different seasons (September and April) were filleted and packaged in vacuum and modified atmosphere (70% CO□, 20% N□, 10% O□; gas:product ratio of 1:2) at 4□C and stored for 20 days. Whole fish on ice (0°C) was used as a control. The microbial diversity was characterised by sequencing of the V3-V9 amplified region of the 16S rRNA gene of purified colonies picked from countable Long and Hammer plates. Additionally, fish muscle samples were used for the direct extraction of total DNA and the bacterial diversity was assessed by sequencing of the variable regions V3-V4 of the 16S rRNA gene. A higher microbial diversity of the raw fish was found in April than September. Photobacterium spp. constituted 73% (September) and 55% (April) of the initial microbiome. Packaging had a significant impact on the plaice microbiota during cold storage demonstrated by a higher microbial diversity for whole fish on ice than the other groups. Regardless of the storage conditions and fishing season, the Photobacterium spp. was found to be the dominant bacteria at the end of storage time. The species diversity of Photobacterium was assessed by a multi locus sequencing approach (16S rRNA, gyrB, gapA and recA) and suggested that P. iliopiscarium (53%), P. phosphoreum (41%) and P. piscicola (6%) were presented. However, further research is needed to investigate the possible differences in the spoilage potential between Photobacterium strains that have isolated from different seasons and packaging atmospheres.

Food Microbial Ecology

P1.79

Investigation of pistachio's (Pistacia vera) fresh kernel mycological quality under vacuum and air packaging

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HPistachios have an important role in human diet due to their content in useful vitamins and essential unsaturated fatty acids. Nevertheless, pistachio's kernel is an ideal substrate for fungal colonization which results not only to the deterioration of their organoleptic characteristics, but also promotes the growth of fungi which can produce toxic metabolites for humans. The aim of this study was to investigate the mycobiota of pistachio's fresh kernel (without any process apart from removing the hull) and evaluate its mycological stability, after preservation under aerobic and vacuum packaging at 4°C during a period of 3 months. Two different ways of sampling were applied, namely the direct plating method and the serial dilution method in order to assess moulds and yeasts in terms of colony forming units. Fungal species isolated from the direct plating method were further subjected to identification by cultivation in 3 different culture media (CYA, MEA, G25N), at appropriate temperatures, and characterized at genus or species level by means of microscopic observations. Results showed that the representative fungal species isolated belonged to Aspergillus spp. (A. flavus/A. parasiticus, A. niger aggregate and A. terreus), Penicillium spp. and other miscellaneous fungi such as Cladosporium, Alternaria, Fusarium, Mucor and Geotrichum spp. Besides the investigation of the fungal microbiota, evaluation of the mycological stability of the fresh nut was also performed. From the first month of vacuum packaging, reduced or no fungal growth could be observed on DRBC medium, except a considerable number of yeasts. In the next two months, growth of yeasts and fungi remained stable or even reduced. On the contrary, the samples preserved under aerobic conditions presented a rapid growth from 2 to 5 log CFU/g and 2 to 3 log CFU/g for yeasts and fungi, respectively, during storage for 3 months. In this study, the health risk, posed by the fungal species and especially those with mycotoxigenic potential, highlights the importance of pistachio's fresh kernel storage in a most effective way. Vacuum packaging contributes to both a mycological stability and an extended shelf life.

Food Microbial Ecology

P1.80 Effect of natural compounds in Salmonella sp. biofilm formation

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One cause of contamination in the food industry can be attributed to biofilms which persist in food processing equipment. The use of natural compounds to inhibit biofilm formation has been an active area of research, since alternatives to commonly used biocides are desired. This study aimed to evaluate the effect of curcumin, naringenin, quercetin, baicalein, resveratrol, cinnamaldehyde, phytol, farnesol, vanillic acid, rosmarinic acid and eugenol on Salmonella sp. biofilm formation. Methods. A biofilm producing strain of Salmonella sp. was selected from the bank of the Food Microbiology Laboratory of the Faculty of Pharmaceutical Sciences-USP by using crystal violet technique (CV) for biofilm quantification, with Pseudomonas aeruginosa PA01 as reference of a robust biofilm. Minimum inhibitory concentration (MIC) and growth curves in the presence of each compound were determined using the micro dilution method, testing concentrations based on the literature. CV was used as the screening method to quantify biofilm formation in the compound's presence, compared with growth control. The five compounds with the best inhibitory effect using fixed concentrations of 50 and 500 µg/mL were chosen for further testing, and then compared using a fixed concentration of 50 µM to observe if in this low concentration an inhibition tendency was observed. The compound with the best inhibitory effect was chosen to be evaluated in the future steps of this study. Results. Salmonella enterica serovar Montevideo was selected as the best biofilm-forming strain among 28 tested serotypes. The MICs of each compound were determined and sub-MIC concentrations were used to determine the effect on biofilm formation. The five compounds that showed biofilm inhibitory effect using fixed concentrations of 50 to 500 µg/mL were quercetin, naringenin, eugenol, farnesol, resveratrol and cinnamaldehyde. However, among these, it was evident that the compound with the best inhibitory effect on biofilm formation using a low concentration of 50 µM was cinnamaldehyde, without affecting microbial growth. Conclusions. These results showed that cinnamaldehyde has an inhibitory effect on the formation of Salmonella Montevideo biofilms. More experiments will be carried out to determine how this compound affects the strain's biofilm morphology as well as combinations with commonly used biocides.

Food Microbial Ecology

P1.81 Microbiome mapping in minimally processed vegetables facilities reveals a complex community with spoilage and pathogenic potential

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Minimally processed vegetables have a leading role in the food market, since they can be considered as "commodities", besides being associated to a healthy lifestyle. However, their consumption is not free of risks due to the presence of potential pathogens. In addition, several spoilage or pathogenic microbes might adhere and form biofilms on industrial surfaces, thus enhancing the spread of microbes on the final products and to consumers. Therefore, the aim of this study was to characterize the resident microbiome in the environment of three facilities producing minimally processed vegetables in Southern Italy. Overall, 32 samples were collected, including vegetables at process start and end points, as well as surfaces swabs from equipment, tools, operators' hands, and food processing environment (walls, floors). Whole metagenome sequencing was carried out. Each facility was visited after the routinary cleaning procedures.
Our results show that a core group of microorganisms can be identified throughout the facilities. Among these, members belonging to potentially spoilage genera such as Pseudomonas were widespread, reaching high relative abundances on food contact surfaces. Moreover, we attempted to estimate the contribution of surfaces and ingredients on the microbiome composition of the final product, highlighting that food contact surfaces contribute significantly, besides ingredients.
In addition, the screening of antimicrobial resistance genes reported a wide range of these, widespread on food contact surfaces and assigned to genera Pseudomonas, Bacillus and Acinetobacter.
Finally, we reconstructed Metagenome Assembled Genomes (MAGs) of the human pathogen Bacillus cereus on food contact surfaces, highlighting its ability to form persistent biofilms on food industry surfaces.
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Food Microbial Ecology

P1.82 Metagenomic and metabolomic analysis of Korean rice vinegar productions shows large variability among different producers

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Korean traditional fermented rice vinegar is part of the nation's food culture. Rice vinegar fermentation processes start with saccharification and alcoholic fermentation of rice by using nuruk, a traditional Korean fermentation starter, to make rice wine, followed by its conversion into vinegar through an acetic fermentation. At two production facilities, producers A and B, two fermentation processes were regularly sampled for the entire duration, namely 55 days for producer A and 90 days for producer B.
For producer A, the nuruk used contained species of Lactobacillus, Pichia, and Weissella. During vinegar production, Saccharomyces and Lactobacillus species prevailed during the alcoholic fermentation phase, whereas Acetobacter and Lactobacillus species prevailed during the acetic fermentation phase. The latter phase was initiated by the addition of vinegar with a very low occurrence of Acetobacter species, nevertheless able to lower the pH from 4.2 to 3.6. The duplicate fermentation processes showed little divergence, most likely because the fermenting liquids were stirred regularly, and due to the backslopping step to initiate the acetic fermentation phase. Acetic acid and esters were only produced after backslopping, simultaneously with the consumption of ethanol, until ethanol depletion.
For producer B, the fermenting liquid was less well stirred compared to that of producer A. In one of the two vinegar production processes, no Acetobacter species were found and consequently ethanol was not converted into acetic acid. In the other process, at day 0, Lactobacillus and Leuconostoc species were present, and around day 20, a mixture of different microorganisms was found, encompassing species of Acetobacter, Enterococcus, Lactobacillus, Leuconostoc, Pediococcus, and Saccharomyces. From day 20 onwards, Acetobacter species were found with an increasing relative abundance, together with Lactobacillus and Leuconostoc. Ethanol and lactic acid were converted into acetic acid and acetoin, respectively, and esters were produced from the beginning of the production. At producer B, a less clear separation between the alcoholic and vinegar fermentation phases occurred.
The comparison of two traditional rice vinegar production facilities showed that the fermentation practices applied can result in large differences of not only the end-products but also the reproducibility of the processes.

P1.83 Microbial quality of filtered water samples intended for human consumption

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Water is an essential element in the maintenance of all forms of life on the planet and therefore needs to have its microbiological quality assured. In Brazil, the regulation no. 05/2017 establishes the absence of total coliforms and Escherichia coli in 100 mL of analyzed water, as parameters to consider that water is proper for consumption. The objective of this study was to water samples intended for human consumption, after filtration process by purifiers. For this purpose, 585 water samples were analyzed using the filter membrane method. These samples were categorized into 13 paired and dependent groups, with 45 samples in each and, after descriptive analysis of the data, it has observed that 3.1% (18 samples) presented contamination by total coliforms, while Escherichia coli was not detected in any of the samples. The water samples that were positive to total coliforms were submitted to analysis of variance of the double classification by posts using the non-parametric Friedman and Student-Newman-Keuls (SNK) tests at 5% level. The results showed that the differences in the median values between the treatment groups were greater than would have been expected at random. Therefore, a statistically significant difference amongst the analyzed samples was detected, with the value of $p = 0.028$. The results of microbiological analyses showed that drinking water distribution and purification systems are susceptible to contamination, caused by the presence of pipes with cross connections, dead spaces, cracks or through the passage of reservoirs that are in an inadequate state of hygiene and structure. In purification systems, the spread of contamination may occur due to the high microbial load present in the water or due to lack of hygiene and/or replacement of equipment consumables at the appropriate periodicity. Therefore, low incidence of total coliforms, when considering the amount of samples analyzed, can be attributed to the efficiency of the purification systems used.

Food Microbial Ecology

P1.84 Biofilms in the meat processing environment: Water hoses as an under-investigated route for bacterial transmission

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Biofilms, being microorganisms embedded in a self-produced matrix, pose manifold problems in diverse environments. In the food processing environment biofilms are often mentioned as contamination source for food production in scientific literature, yet research on these biofilms is limited. Using a sampling technique (scraper flocked-swab method) allowing analysis of microbial communities and matrix composition, we showed that biofilms exist in the meat processing environment during production and even after regular cleaning and disinfection. Biofilms were present on food contact surfaces and non-food contact surfaces. Among these non-food contact surfaces, predominately water hoses were identified to harbor biofilms. From these water hoses characteristic microorganisms could be cultivated, including known meat spoilage organisms.
Until now, the evaluation of biofilm presence in water hoses has been performed in critical settings (household, hospitals), yet in the food environment the confirmation of water hoses as biofilm reservoirs was still lacking. It has been previously described that biofilms alter the appearance and microbial quality of water along the distribution chain. This aspect is especially important in the food processing environment, as here safe and hygienic water distribution is required for maintaining product quality and safety.
A detailed investigation of the microbial communities of water hoses, the source water, and environmental sites in contact with water at a meat processing plant was performed. Biofilms were present in all water hoses, as determined by the presence of bacterial DNA and biofilm matrix components (carbohydrates, extracellular DNA, and proteins). The microbial community of biofilms was less diverse than the water microbial community. The source water harbored the richest microbial community and different rooms showed different microbial community patterns, especially with respect to certain abundant genera. Overall, the microbial communities of biofilms, water, and the environment were distinct from each other. Within biofilms, genera that are associated with an intracellular lifestyle (e.g. Neochlamydia and Legionella) were present. On surfaces in the meat processing plant, genera associated with food spoilage were identified (e.g. Pseudomonas, Acinetobacter, Psychrobacter). This study provides first insights into the complex microbial communities of water hose biofilms in the food processing environment.

Food Microbial Ecology

P1.85 Valorization of cheese-whey to an innovative added-value dairy product

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Whey was always considered a high-impact to the environment waste by-product of cheesemaking but nowadays there is a growing appreciation as its benefits for human health and the intrinsic value of its components are numerous and are becoming widely known. In connection to the constantly increasing amount of whey produced in the dairy industry, our aim is to explore new methods for whey utilization.

The sweet (pH 6.2) whey samples of mixed milk whey (i.e. cow 80% and 20% sheep/goat milk), were obtained immediately after the production of Halloumi cheese. The probiotic commercial cultures namely; Bifidobacterium animalis subsp. lactis (BB-12), Lactobacillus acidophilus (LA-5), Lacticaseibacillus rhamnosus (LGG), Lacticaseibacillus casei (L.CASEI 431) and Lactobacillus helveticus (R0052) were examined for their ability to grow in whey. Fruit juices could be an ideal medium for probiotic growth and survival due to their content of essential nutrients such as, vitamins, antioxidants and polyphenols. This will provide consumers with an outstanding nutritional quality product, while creating innovation opportunities for the dairy industry.

Fermentations at 37°C with 2% probiotic were carried out. From the five species of probiotic bacteria which were evaluated for their growth, the highest growth rate was recorded by Lacticaseibacillus rhamnosus (LGG), Lacticaseibacillus casei (L.CASEI 431) and Lactobacillus helveticus (R0052). The scope of the project is the development of a fruit-flavoured whey drink with addition of probiotic cultures (2%), prebiotic-inulin (4%) and Whey Protein Isolate (WPI) (5%) and to assess its physicochemical characteristics, microbiological quality, nutritional/health characteristics and sensory consumer preference.

Preliminary work illustrated that it is possible to develop a probiotic fruit-flavoured whey dairy drink with suitable chemical composition, sensory acceptance and probiotic viability (>7 log cfu/ml) during refrigerated storage (28 d/5°C).

P1.86

Biofilm formation and in vitro testing of commercial disinfectants against Salmonella Infantis strains

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Salmonella Infantis is the most prevalent serovar in broilers and broiler meat in the European Union. In this aspect, the eradication of S. Infantis in poultry flocks is considered as extremely difficult. Moreover, due to its frequency of isolation from humans S. Infantis is also an important public health concern. The aim of our study was to test the biofilm formation and antimicrobial effect of commercial disinfectants on genetically characterized S. Infantis broiler and human isolates (n = 15). For the biofilm formation under various temperature conditions (8 °C; 20 °C in 28 °C) and incubation times (72 and 168 h) the crystal violet (CV) staining method was used. The evaluation of the in vitro antimicrobial effect of ethanol, hydrogen peroxide and Ecocid S® was determined using the broth microdilution method. The antibiofilm effect of subinhibitory concentration (1/8 MIC) of disinfectants was then tested on S. Infantis 323/19 strain that had the highest biofilm formation potential. The results showed that the biofilm formation was strain specific, however significantly higher at 20 °C and prolonged incubation time (168 h). Moreover, strains carrying a pESI plasmid showed the higher biofilm formation potential. Regarding antimicrobial resistance the MIC values for ethanol (MIC = 9 %) and Ecocid S® (MIC = 0.75 %) were the same for all strain tested. The subinhibitory concentration of disinfectants against biofilm formation of S. Infantis 323/19 strain at 20 °C was effective after shorter incubation time (72 h).
Despite stringent cleaning and disinfection measures between the placement of flocks, recurrent infections are often reported. As shown in our study higher precautionary measures should be invested in biofilm prevention and removal in order to control the S. Infantis occurrence in flocks. However, the specific biofilm formation ability and differences in antibiofilm properties of disinfectants tested, showed the urge for further investigations and support the need to apply standardized test protocols

Food Microbial Ecology

P1.87 Exploring the technological properties of indigenous yeasts isolated from Greek wine

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Yeast consortium is evolving rapidly throughout the alcoholic fermentation process while only the more adaptive species could survive in wine environment. Climate change leads to even more hostile and stressful for the wine microorganism conditions and consequently fermentation problems are more frequently observed. The objective of the current research is to investigate the population diversity of yeast species from spontaneously fermented wines and also to evaluate the important technological properties of the isolated yeast under real winemaking conditions. Fourteen spontaneously fermented wines all over Greece were collected. The yeast isolates were subjected in molecular analyses and identification for both species and strain level. In terms of species identification RAPD (Random Amplified Polymorphic DNA) genomic fingerprinting with the oligo-nucleotide primer M13 combined with matrix assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) techniques were applied. Additionally, S. cerevisiae isolates were characterized at strain level by interdelta-PCR genomic fingerprinting. Yeasts strains were examined for their fermentative capacity in Assyrtiko grape must. After alcoholic fermentation sensory assessment was conducted by 8 trained panelists. A free sorting task was applied to categorize the samples according to their possible similarities. Overall, 190 indigenous yeasts were isolated and S. cerevisiae was the most dominant species with the isolation frequency exceeding 83.5%. Moreover, Trigonopsis californica, Brettanomyces bruxellensis, Zygosaccharomyces bailii, Priceomyces carsonii and Pichia manshurica were also identified in minor abundancies. S. cerevisiae prevailed in all wine samples and strain level typing of S. cerevisiae revealed distribution based on geographical origin. Furthermore, after 214 hours of laboratory-scale fermentation only 35% of the inoculated strains had lower ability in catabolizing sugars and couldn't lead to dry wines. Wines were clearly grouped in four clusters based on sensory evaluation results. It is noteworthy that almost all the isolated native strains exhibited interesting enological properties with technological potential while further industrial application experiments will be performed.
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Microbiome; factors affecting microbial diversity and physiology

P2.1 Modelling and comparing the effect of osmotic stress on Salmonella enterica serotypes: a study of the Phoenix Phenomenon

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Salmonella spp. is responsible for several infections in humans, as well as one of the main causes of foodborne outbreaks. The genus Salmonella presents a variable resistance to the osmotic stress promoted by low water activity (aw), and some serotypes present an inactivation followed by growth recovery in this stressful environment, which is so-called Phoenix Phenomenon. However, differences between serotypes or strains can lead to substantial changes in their stress tolerance. This work aimed to model and compare the behavior of four Salmonella enterica serotypes (Salmonella Typhimurium – ATCC 14028, Salmonella Enteritidis – ATCC 13046, and two obtained from a poultry industry: Salmonella Heidelberg and Salmonella Minnesota) exposed to osmotic stress condition leading to Phoenix Phenomenon. The inoculum cells of S. enterica at the exponential phase at 25 °C (previously tested conditions) were diluted to about 105 CFU/mL and inoculated in brain heart infusion broth (BHI) added of 7% NaCl in mass to adjust the aw to 0.950. The serotypes were inoculated individually and incubated at 25 °C. The growth curve was measured by viable counts over time. The Baranyi and Roberts primary model was adapted and fitted to the experimental data based on the assumption that the total microbial concentration is given by the sum of the concentrations of the two subpopulations (dying and surviving-then-growing subpopulations). The predictive ability of the model was assessed through statistical indexes. All tested serotypes presented the Phoenix Phenomenon; however, it was observed differences in the resistance among them. By the statistical indexes, one can conclude that the model proposed presented safe predictions to describe the Phoenix Phenomenon. The results indicated that both serotypes obtained from the industrial plant, S. Heidelberg and S. Minnesota, presented a higher growth rate and reached the stationary phase of growth faster than other tested strains.

P2.2 Microbiome and mycobiome comparison between traditional and modern cheese-making process of Taleggio PDO

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Taleggio PDO is a cheese produced with whole raw or pasteurized cow milk. Ripening takes place on wood axes and the cheese is weekly turned and sponged with salt water allowing the development of a specific surface microbiota which guarantees the color of the rind required by the Production Regulations and the centripetal ripening necessary for typicality. The monitoring of the different techniques and of the diverse production environments therefore represents an essential tool to guarantee the development of microorganisms capable of giving to the product its typical characteristics. With this regard, microbiome and mycobiome of Taleggio PDO cheese produced by two dairies in both ripening rooms, modern and traditional, were analyzed to compare the influence of the ripening conditions on the surface microbiota. Cheese rind of two different processes were sampled for each dairy, at 35 and 60 days of ripening. The V3-V4 hypervariable regions of the bacterial 16S gene and ITS1-ITS4 regions for fungal DNAs were sequenced in two different MiSeq (Illumina) runs with 2×250-base paired-end reads.
In all sampling times, for bacteria analysis, the most prevalent phyla were Firmicutes, Proteobacteria and Actinobacteria. For mycobiota analysis, Penicillium chrysogenum, Candida spp and Ascomycota spp were the most prevalent specie in all the samples analyzed.
Within each dairy, both the alpha and beta diversity of the microbiome and mycobiome did not differ significantly between the two ripening conditions. Cheese metagenomic analysis showed a peculiar distinctive microbiota and mycobiota composition for the two dairies, regardless of the place of ripening.
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Microbiome; factors affecting microbial diversity and physiology

P2.3 Early prediction of food safety & spoilage incidents using microbiome profile

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Early detection of spoilage microorganisms and foodborne pathogens is important step in controlling food safety, avoiding food recalls and foodborne outbreaks. Monitoring the microbiome profile of food products, will provide the required database and knowledge about proper and normal vs. invalid profiles of food products.
Since most foodborne pathogens have low infection dose, the regulation challenge is to identify up to one colony forming unit (CFU) in food products. To reach the required sensitivity, pathogen detection in food sample usually relies on culture-based techniques that are time-consuming. Thus, it is necessary to find ways to detect food spoilage microorganisms and pathogens at early stages.
Although many new technologies are already available for assessment of food products, they are not sensitive, fast and accurate enough to determine microbiological hazards in foods. Culture-independent techniques, based on the analysis of DNA or RNA extracted from food products, can help in overcoming these limitations.
Our research hypothesis is based on the idea that under inappropriate conditions, there will be a change in the microbial profile of the food product and thus it will be possible to detect the changes in the profile efficiently and at near real time. Genomic indicators may also detect the specific reason of the improper food production.
Hear we describe calibration of a reliable and reproducible microbiome profiling protocols of the food products that distinct between live and dead bacteria. Based on the microbiome profile we have developed epidemiologic tools to identify the sources of contaminating bacteria and identify bacteria that serve as indicators of food spoilage or foodborne pathogens. The developed q-PCR tests for these bacterial indictors will serve as rapid early detection system for possible events of foodborne pathogens and food spoilage bacteria.

P2.4 Evaluation of the influence of Lactococcus lactis subp. cremoris bacteriocins used for pre- and post- milking treatment on bovine milk microbiota

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In dairy cattle, pre-and post-milking teat disinfections are widely used to reduce or avoid contamination of the milk with pathogens, limit new intra-mammary infections, and prevent the incidence of mastitis. However, these procedures, routinely performed with conventional chemical disinfectants, could unbalance the milk microbial population, affecting also the cheese making properties.
The aim of this study was to investigate the use of bacteriocins produced by the Lactococcus lactis subsp. cremoris as evaluable alternative of disinfectants. The experiment involved 20 multiparous dairy cows from the same herd with low somatic cell counts (< 100000 SCC/ml). They were divided into two groups of 10 lactating cows each: (1) control group – a commercial pre-milking and a post-milking disinfectant were applied; (2) treated group –a natural formulation with Lc. lactis subsp. cremoris was used. Milk samples were collected before (T1) and after one (T2), two (T3) and three months (T4) of treatment from two out of the four healthy quarters. The V3-V4 hypervariable regions of the bacterial 16S gene was sequenced in a MiSeq (Illumina) run with 2×250-base paired-end reads.
The alpha diversity of the milk microbiome increased significantly (p-values in the range 0.0051 – 0.048) in the treatment group at T2, T3 and T4. Regarding beta-diversity, the milk microbiome showed a statistically significant (p-value = 0.00823) separation both between treatments, as well as timepoints (p-value = 0.0085441) and combining treatments x timepoints (p-value = 0.00464).
The bacterial community was dominated by Bacteroidetes (~20%) and Firmicutes (~54%); their levels showed a decrease from T1 to T4, followed by a significant increase of Actinobacteria (~14%) and Proteobacteria (~16%) phyla. Among the genera, 78 resulted significantly different (p-value < 0.05) between the two groups. In conclusion, the use of Lc. lactis subsp. cremoris bacteriocins for pre- and post-dipping seems to have a sizable effect on biodiversity in dairy cow milk microbiome composition.
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Microbiome; factors affecting microbial diversity and physiology

P2.5

Directed evolution of Oenococcus oeni to improve acido-tolerance reveals fixed beneficial mutations in the citrate locus

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Oenococcus oeni is a Lactic Acid Bacteria responsible for the malolactic fermentation converting malic acid into lactic acid and carbon dioxide in wines. This second fermentation remains essential for the deacidification, the introduction of aromatic notes and the improvement of microbial stability in many wines. Nevertheless, wine represents a harsh environment for microbial growth due to the lack of nutrients, low pH, high ethanol amounts and the presence of sulfites. Therefore, a better understanding of the cellular response mechanisms to these abiotic stresses is needed in order to improve bacterial tolerance. The directed evolution approach was used to identify target genes which are likely to be involved in acid-tolerance.

For this purpose, the Oenococcus oeni ATCC-BAA-1163 strain was propagated in a medium with a progressive pH decrease (from 5.3 to 2.9). This experiment was carried out continuously for 20 months, representing 550 successive generations. The evolved populations obtained underwent fixed mutations located in five genes, including the mae gene which is part of the citrate locus. It appears that these evolved populations, deficient in the mae gene, consume citrate much more slowly than the ancestral strain. Indeed, a transcriptomic analysis showed that the genes related to citrate metabolism are strongly down-regulated in the evolved populations. The citrate consumption altered rate resulting from these mutations seems to confer to the bacteria a better resistance under low pH conditions. Indeed, while the addition of citrate in the medium affects the growth of the ancestral population at pH lower than 3.2, there is no change in growth for the evolved populations.

Microbiome; factors affecting microbial diversity and physiology

P2.6 Exploring different culture media for the isolation of next-generation probiotic strains from the human gut

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Probiotics currently available on the market generally belong to a narrow range of microbial species. However, recent studies about the importance of the gut microbial commensals on human health highlighted that the gut microbiome is an unexplored reservoir of potentially beneficial microbes. For this reason, academic and industrial research is focused on identifying and testing novel microbial strains of gut origin for the development of next-generation probiotics (NGPs). Next-generation probiotics are microbial taxa that conform to the traditional definition of probiotics, but do not have an history of use for health promotion. They also fit well within the US Food and Drug Administration (FDA) definition of a LBP: "a biological product that: (1) contains live organisms, such as bacteria; (2) is applicable to the prevention, treatment, or cure of a disease or condition of human beings; and (3) is not a vaccine". One of the main issues in NGP isolation and culturing is the identification of the best growth medium.

The main issue of this research was the selection of suitable culture medium that allowed the anaerobes growth. For this reason, we tested 9 culture media with different formulations in terms of vitamins, minerals and fatty acids for the study of the culturable fraction of the gut microbiome. We collected 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 give a more reliable picture of the gut microbiome. Moreover, 18 bacterial strains were isolated from each sample and identified.

Results obtained highlighted that none of the media used give the same result. However, YCFAGSC medium is able to grow the largest number of anaerobes species. In particular, it was able to isolate the promising NGP candidate Faecalibacterium prausnitzii.

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Microbiome; factors affecting microbial diversity and physiology

P2.7 Diversity and stress resistome of Listeria monocytogenes from Norway

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Listeria monocytogenes is known to be able to persist for months to decades in food processing environments and can spread through food chains by for example contaminated raw materials. Knowledge about the distribution and diversity of L. monocytogenes is of importance to effectively track and control it in the food chain. In the current study, whole genome sequencing was performed for 512 L. monocytogenes isolates from Norwegian food industry environments. These were subjected to wgMLST, SNP, and comparative genomic analyses along with an additional 257 previously sequenced isolates from the same environment, comprising a dataset of genomes originating from 15 meat or salmon processing facilities in Norway collected over a period of three decades. The clonal complex detected in the greatest number of processing plants was CC121 (found in 10 factories), followed by CC7, CC8, and CC9 (7 factories each). Overall, 56% of isolates showed 20 or fewer wgMLST allelic differences towards an isolate found in a different factory. The prevalence of stress response genes, including resistance determinants and genes associated with biofilm formation, was significantly higher among isolates that shared close genetic links towards isolates in other factories compared with those not sharing such links. Furthermore, comparison with isolates from natural and rural environments (n=218) and clinical isolates (n=111) from Norway showed a significantly higher prevalence of stress response genes in isolates from food processing environments compared with those from other sources. The results show that there is extensive spread of highly similar strains throughout Norwegian food chains, and that the spread of such clones is associated with an overall increase in the prevalence of plasmids and determinants of heavy metal and QAC resistance as well as other genetic elements associated with stress survival mechanisms.

P2.8 Novel ways of high hydrostatic pressure resistance development in Escherichia coli

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High hydrostatic pressure (HHP) is one of the most widely accepted non-thermal food processing methods, however preservation of HHP-treated foods might be compromised because of the emergence of HHP-resistance in pathogenic or spoilage bacteria. Therefore, identification of the possible routes and mechanisms of HHP resistance development in foodborne bacteria is essential to anticipate or prevent the appearance of resistant variants. While upregulation of the RpoS-governed general stress response is a well-established strategy to acquire HHP resistance in Escherichia coli, previous work revealed that mutations causing attenuated cAMP/CRP activity or aggregation-prone TnaA variants can overcome the HHP-hypersensitivity of an E. Coli ΔrpoS mutant. In this study, we explain the apparent co-existence of cAMP/CRP and TnaA mutants during directed evolution towards increased HHP resistance, and furthermore reveal novel evolutionary HHP resistance pathways that are independent of RpoS, cAMP/CRP or TnaA.

Microbiome; factors affecting microbial diversity and physiology

P2.9 Genotypic and phenotypic characterization of a Salmonella Typhimurium strain resistant to Pulsed Electric Fields

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Pulsed Electric Fields (PEF) technology is regarded as one of the most interesting alternatives to current food preservation methods. However, many aspects regarding the mechanisms of bacterial inactivation by PEF are still not fully understood. The aim of this work was to characterize the genotype and phenotype of a PEF resistant Salmonella Typhimurium strain (SL1344-RS), which was previously isolated by applying successive PEF inactivation-growth cycles to the laboratory strain SL1344. This was done in an attempt to determine the mechanisms responsible for its increased PEF resistance but also to quantify the impact that the acquisition of PEF resistance has on other aspects of Salmonella physiology, such as growth fitness, biofilm formation ability, virulence and antibiotic resistance. Results obtained indicate that the increased PEF resistance of the SL1344-RS variant was due to a higher RpoS activity, caused by a mutation in the hnr gene. This increased RpoS activity also resulted in an increased resistance to other stresses including acid, NaCl and UV-C (but not to heat and HHP) and in a decreased growth rate in M9-gluconate. It also resulted in an increased ability to adhere but not to invade Caco-2 cells. This work demonstrates the crucial role of RpoS in the mechanisms of stress resistance development in Salmonellae. However, further studies are needed to determine what are the precise changes (molecules or structures) responsible for this increase in PEF resistance as well as to determine if the relative risk (as compared to the parental strain) that this PEF-resistant would represent from the food safety point of view.

P2.10 Molecular responses of the Campylobacter jejuni pathogen to stresses inspired by the chicken slaughter process

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For more than a decade, Campylobacter has remained the leading cause of zoonosis in Europe, inducing Campylobacteriosis. Chicken meat is considered as the main food responsible for this bacterial enteritis. The control of this pathogen is a public health issue and can lead to measures envisaged from the farm to the processing of poultry products. During the first processing of chicken, Campylobacter may encounter thermal stresses that may induce adaptation mechanisms resulting from modifications in gene expression. The objective of this work was to evaluate the influence of stresses inspired by the stages of the poultry slaughtering process on the behavior of Campylobacter jejuni.

First, conditions mimicking certain stages of the broiler slaughter process were chosen with regard to their potential capacity to generate stress in Campylobacter. Two steps were selected: (i) scalding (soaking poultry in a hot water bath) transposed in the laboratory at 3 different temperatures (54°C, 51°C, and 46°C), and (ii) chilling (accelerated cooling) carried out in the laboratory at -4°C for 2 hours. The expression of a selection of 44 genes from 3 strains of C. jejuni was quantified by transcriptional analysis (RT-qPCR) after the application of the selected stresses. The results obtained were analyzed by statistical analyses.

The main results indicated that the expression of 26 genes varied significantly according to the successive thermal stresses applied, according to three different expression profiles depending on the strains and the stress conditions. Among these genes, some overexpressed ones corresponded mainly to genes involved in the heat shock response, while under-expressed genes belonged to lipid and amino acid metabolism. Four genes, whose overexpression was similar for all three strains, could represent indicators of the response to heat stress at the species level.

Advances in the molecular understanding of the stress response of pathogenic bacteria, such as Campylobacter, under real process conditions will allow progress in the identification of appropriate control methods.

Microbiome; factors affecting microbial diversity and physiology

P2.11 Identification and characterization of Lacticaseibacillus rhamnosus strains isolated from breast milk and infant faeces

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Lactic acid bacteria, especially genera Lactobacillus and Lacticaseibacillus, are frequently isolated from human gastrointestinal tract, dairy products or breast milk for their remarkable functional and technological properties. In the present study, five strains of Lacticaseibacillus rhamnosus were isolated from infant faeces and breast milk and identified based on their 16S rDNA sequences. Furthermore, isolated strains were tested for aspects of safety, such as antibiotic susceptibility, haemolytic and enzymatic activities. Subsequently, their antimicrobial activity, GIT condition tolerance (low pH, bile salt tolerance), hydrophobicity, antioxidant activity, aggregation ability, and adhesion to Caco-2 and HT-29 were evaluated. Isolates were sensitive to the most of the clinically important antibiotics and none of them showed haemolytic activity. Acidic supernatants of tested strains inhibited the growth of the tested pathogens, such as E.coli, E. faecalis or S. aureus. All isolates were able to survive at pH 2.0 but only two of five Lcb. rhamnosus (21S1E, 21S4F) showed high survival rate in the presence of 3.0 % bile salts (> 80 %), indicating good potential for application as probiotics. Adherence properties varied between strains.Values of hydrophobicity were in the range 12 -50 %, the ability to adhere to Caco2 and HT29 cells made 61-78 % and aggregation between 40-53% after 24 h of cultivation. The results of this characterization indicate that Lacticaseibacillus rhamnosus strains isolated from infant faeces and breast milk have a good potential for use as probiotics in dairy products or functional foods, especially the strain Lcb. rhamnosus 21S1E. This research was funded by the Ministry of Agriculture of the Czech Republic, Institutional support, No. MZE-RO1422 and project no. QK 1910024.

P2.12 Functional properties of microalgae Chlorella vulgaris and bifidobacteria, and their potential for development of functional foods

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The market for new functional foods and dietary supplements with probiotics is rapidly evolving, with a current emphasis on using natural sources. Chlorella vulgaris is a promising source of nutritionally valuable substances, including not only proteins or carbohydrates, but also vitamins, pigments, antioxidants, unsaturated fatty acids, etc. Therefore, the presented study assessed different properties of C. vulgaris and its combination with bifidobacteria (Bifidobacterium animalis subsp. lactis BB-12®, Bifidobacterium animalis subsp. lactis CCDM 93, Bifidobacterium faeces CCDM 486, Bifidobacterium breve CCDM 562) with the goal to develop new functional foods. The growth ability of four bifidobacteria was evaluated in basal medium, whey and milk enriched by 1.0 % (w/v) chlorella powder. The evaluation was based on production of lactic and acetic acids, viable cell (CFU/ml) and change of pH of the cultivation media. Subsequently, the effect on lipid metabolism of Prague hereditary hypercholesterolemic rats fed a high-fat diet with chlorella and probiotic B. animalis subsp. lactis BB-12® were determined. After 8 weeks, the significant synergistic effects of chlorella and BB-12® on triglyceride levels in rat heart, liver and serum were detected ($P<0.05$). All the four strains tested produced a significantly higher concentrations of lactic and acetic acids ($P < 0.05$) in comparison with basal medium, whey and milk without supplementation of 1 % (w/v) Chlorella vulgaris. The observed trend also correlated with the pH values. The presented results demonstrate that Chlorella vulgaris has a positive growth effect of all tested Bifidobacterium strains and their combination influence favourably lipid metabolisms, therefore the combination of probiotics and algae might have a significant potential in the development of new functional foods and dietary supplements with health benefits. This study was supported by the Ministry of Agriculture of the Czech Republic, project no. QK 1910130 and Institutional support, No. MZE-RO1422.

Microbiome; factors affecting microbial diversity and physiology

P2.13 Human milk microbiome as a dynamic response to the characteristics of the mother–infant pair: a Big Data strategy

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Background: In the first 1000 days of the newborn, nutrition plays a central role in the maturation of the gastrointestinal tract and the immune system, and this would affect the short and long-term health of the infant. Human milk is the gold standard of the infant nutrition, through its species-specific and unique mother-infant bond that provides essential benefits for the child. Breast milk plays a key role in the formation of the infant's gut microbiome due to both its nutritive and non-nutritive components. Linking the dynamically changing microbiota composition of the human milk to the characteristics of the mother-infant pair is key to understand the maturation of gastrointestinal immune system. A tool to this goal is a systematic collection of available data in an organized database. This strategy, enriched by statistical and computational methods, can help identify patterns in this dynamic system.
Method: After a systematic search strategy and selection process, data focusing on quantitative observations on the human milk microbiome, are entered in a database structured in a novel way. Microbiota is hierarchically arranged following the tree-structured ontology suggested by the NCBI/GenBank* taxonomy database. This gives a framework to record the dynamically changing diversity and relative abundance of microbiota as responses to selected qualified/quantified conditions, e.g.: mode of delivery, use of antibiotic, mother's BMI, etc. Using automated methods for syntax and semantics checks help to clean the data and keep the integrity of the database, while statistical and computational methods help to identify the main sources of variation regarding the effects of conditions on human milk microbiome.
Result: Preliminary analysis show patterns of microbiome composition found in human milk that may contribute to the validation of current hypotheses on its optimal microbiome composition and its role in the development of the gastrointestinal tract and the gastrointestinal associated lymphoid tissue. Furthermore, the database supports the analysis of the factors that has an impact on this composition.
Conclusion: Our database could provide a resource for the scientific community to analyze the role that various conditions play in human milk microbiota.
*Sayers EW et al. GenBank. Nucleic Acids Res. 2021; doi:10.1093/nar/gkab1135

Microbiome; factors affecting microbial diversity and physiology

P2.14 Structural properties of apple pomace and pectins derived therefrom influence its prebiotic potential and modulatory effects on key target commensal microbial populations

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Pectin is a group of structurally diverse dietary fibers, very abundant in agri-food waste and by-products such as those generated during apple cider manufacturing. In recent years, pectin and pectinoligosaccharides have demonstrated good fermentation properties, as well as prominent health promoting traits particularly ameliorating certain inflammatory conditions. In previous investigations apple pomace derived from production of monovarietal Asturian ciders was demonstrated to represent a good source of pectin with varied structural characteristics. In this work we investigated in vitro the modulatory effect of pectin and pomace fractions derived from the production of selected monovarietal Asturian ciders on human microbiota from healthy subjects and inflammatory bowel disease patients through fecal batch fermentations and 16S sequencing. Overall, these fractions selectively promoted the growth of Akkermansia, Lachnospiraceae UCG-010, Prevotella, Sucinivibrio and Turicibacter on samples from healthy donors, while Blautia, Rumicoccaceae CAG-56, Dialister, E. eligens and Intestinimonas were stimulated in fermentations from IBD patients. The growth of Akkermansia, Blautia, E. eligens group, Intestinimonas and Succinivibrio was exclusively associated to pomace and pectin derived from the tested by-products, and did not occur with other non-pectic prebiotics/substrates. Gal content and (Ara+Gal)/Rha ratio was positively associated to the promotion of most of these genera. This work provides a comprehensive characterisation of gut microbiota modulation effects of apple pectin and pomace fractions derived from cider by-products, demonstrating diverse gut microbiota modulatory capacity of structurally distinct pectin and apple pomace fractions, diversifying the opportunities to achieve cider by-products valorisation through formulation of novel prebiotics for particular population groups.

P2.15 Evaluation of the impact of food product with antifungal properties on Candida yeasts in mice organism

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Yeasts are abundant in nature and can be found in foods and drinks, however, they can also cause significant losses in food industry due to spoilage. When the conditions of the human body change (the microbiota changes due to use of antibiotics or the immune response of the host because of big stress, during chemotherapy, or an infection), Candida yeasts can cause health issues in humans (e.g. candidosis).
Based on antifungal activity of Lactobacillus plantarum MI-LPI (collection of KTU Food Institute) and functional additives of plant origin, a prototype of nutritional bar was developed and used for mice feeding. The nutritional bars were studied to determine the survivability of L. plantarum after manufacturing and during 90 days of storage. The number of L. plantarum lowered by 0,3 log10 during the storage. The nutritional bars were fed to mice in an in vivo study to determine the effect of the food prototype in live warm-blooded organisms. The BALB/c mice were divided into four groups fed for 7 days: control group fed with plain food, the second group fed with nutritional bars including Candida albicans (104 cfu/g), the third group fed with nutritional bars including C. albicans for 4 days, 3 days by nutritional bars with L. plantarum (109 cfu/g), and the fourth group fed with nutritional bars with L. plantarum for 7 days. After 7 days samples of contents of the appendix were collected and counts of L. plantarum and C. albicans were determined. Results showed that food matrix enriched with L. plantarum MI-LPI effectively ($P < 0.05$) reduced the number of Candida yeasts in contents of the appendix of mice and reduced the inflammatory process in the gastrointestinal tract.

Microbiome; factors affecting microbial diversity and physiology

P2.16 Association between the physiological heterogeneity and surface properties of biofilm-forming Listeria monocytogenes

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Listeria monocytogenes is a foodborne pathogen that has been isolated from various foods and also reported to isolate from food-processing facilities. L. monocytogenes can adapt and survive in different environments in part due to its ability to form biofilms. Biofilm formation is correlated with the heterogeneity of the bacterial cell population, as this ability is related to environmental adaptability. This study aimed to assess the association between the physiological heterogeneity and surface properties of biofilm-forming L. monocytogenes cells.
Seven L. monocytogenes (serotype 1/2a) strains were grown in TSBYE medium and then inoculated into Hsiang-Ning Tsai (HTM) medium at a 10-fold dilution and statically incubated at 25°C or 37°C for 24 h to form biofilm. Cell viability was assessed using a Live/Dead staining kit and fluorescence microscopy. The biofilm was then separated (by shaking at 180 rpm for 1 min) into three cell populations: planktonic cells (PC), low-adherent biofilm cells (L-BF), and high-adherent biofilm cells (H-BF). We evaluated and compared these cell populations as follows: cell envelope charge measurement by a cytochrome c binding assay, assessment of bacterial cell surface properties by microbial adhesion to solvents (MATS) analysis, scanning electron microscopy (SEM) analysis, and cellular fatty acid composition analysis by the MIDI Sherlock Microbial Identification System.
Live/dead cell staining of the biofilm showed differences according to temperature, and mortality was lower at 25°C than at 37°C. The cell envelop charge of biofilm-forming cells (L-BF and H-BF) was higher than the charge of PC; however, the charge of L-BF and H-BF cells did not differ. Adhesion to chloroform was higher for L-BF than for H-BF. Adhesion to hexadecane differed according to temperature; at 25°C, it increased in the order of PC, L-BF, and H-BF, whereas at 37°C adhesion was decreased. At 25°C, cells had differences in both surface roughness and cellular fatty acid contents, including 12:0, anteiso-13:0, iso-14:0, 16:0, anteiso-17:0, 3OH-iso-14:0, and 2OH-14:0. Taken together, our data suggest that differences in cellular fatty acid contents of L. monocytogenes affect their cell surface properties and may contribute to physiological heterogeneity.

Microbiome; factors affecting microbial diversity and physiology

P2.17 Seasonality and geography have a greater influence than the use of chlorine-based cleaning agents on the microbiota of bulk tank raw milk

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Cleaning of the production environment is vital to ensure the safety and quality of dairy products. Although cleaning with chlorine-based agents is widely adopted, it has been associated with detrimental effects on milk quality and safety, which has garnered increasing interest in chlorine-free cleaning. However, the influence of these methods on the milk microbiota is not well documented. This study investigated the factors that influence the raw milk microbiota, with a focus on the differences when chlorine-based and chlorine-free cleaning of milking equipment are used. Bulk tank raw milk was sampled at three sampling months (Apr, Aug and Nov), from farms across Ireland selected to capture the use of different cleaning methods, i.e., exclusively chlorine-based (n = 51) and chlorine-free cleaning (n = 92), and farms that used chlorine-free agents for the bulk tank and chlorine-based cleaning agents for the rest of the equipment (n = 28). Shotgun metagenomic analysis revealed the significant influence of seasonal and geographic factors on the bulk tank milk microbiota, indicated by differences in diversity, taxonomic composition, and functional characteristics. Taxonomic and functional profiles of samples collected in November clustered separately from other months. In contrast, cleaning methods only accounted for 1% of the variation in the bulk tank milk bacterial community, and samples collected from farms using chlorine relative to chlorine-free cleaning did not differ significantly, suggesting that chlorine-free approaches used did not negatively impact microbiological quality. This study shows the value of shotgun metagenomics in advancing our knowledge of the raw milk microbiota.

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For the Developing Scientist Poster and Oral Award competition:
Enrolment: October 2018
Expected graduation: September 2022

Studying and enlightening food microbial ecosystem and microbiome

P3.1 Evaluation of the bacterial diversity of ready-to-eat African salad sold in Lagos, Nigeria

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In this study, the bacterial diversity of African salad, locally known as abacha, was assessed using polyphasic approach by combining culture-independent molecular techniques with culture-based genotypic typing methods. Microbiome profiling by 16S rRNA gene amplicon illumina sequencing revealed 52255, 36077 and 57060 reads of bacteria with 474, 538 and 364 OTUs from abacha samples ALC, ALE and ALW, respectively. In total, the most abundant Phyla in ready-to-eat (RTE) abacha were Proteobacteria, Firmicutes, Actinobacteria and a very few unclassified phyla, while at the species level Enterobacteriaceae group, Acinetobacter pittii group, Weissella confusa group, Acinetobacter baumannii, Enterobacter_uc (unclassified), Staphylococcus sciuri group, FMZ_s (not assigned a name yet), Streptococcus gallolyticus group, Bacillus, Staphylococcus saprophyticus group and other related species were identified. Cultured bacterial strains found include Staphylococcus simulans, Bacillus weidmannii, Lactobacillus hokkaidonensis and Phreatobacter oligotrophus. The combination of high-throughput illumina sequencing platform and culture-dependent genomic approach has enabled a broader description of the bacterial composition and diversity of abacha, as well as, identification of numerous low abundance bacteria that may constitute safety and quality issues, regarding freshly prepared RTE abacha. Conclusively, this study indicated that the bacterial composition of abacha is significantly more diverse than earlier reported and confirmed the occurrence of bacteria with pathogenic traits and unknown functions. There is, therefore, a great need for food processors and consumers to adopt hygienic practices to minimize risks of transmission of foodborne pathogens through abacha and other related RTE foods. Education of food handlers and the general public on food safety measures, effective Hazard Analysis Critical Control Point (HACCP) application and Good Manufacturing Practices (GMP) implementation is also imperative.

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P3.2

Comparative analysis of genomes and in vitro adhesion and invasion of human gut models for a collection of Arcobacter (Aliarcobacter) butzleri strains

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Arcobacter (Aliarcobacter) butzleri is a Gram negative emerging foodborne pathogen of the bacterial family Arcobacteraceae, which can cause enteritis in humans. The pathogenicity of these bacteria is still not well understood, despite the regular reports on human infection and its large occurrence in foods of animal origin.
The present study aimed at assessing in vitro the invasion/adhesion behavior of a collection of 32 A. butzleri strains, isolated from food, animals and human stool samples, employing different cell models. The behavior of the strains was defined by bacterial counts performed on mucus-producing (MP, Caco-2/HT29-MTX-E12) and not producing human cell lines (NMP, Caco-2 and Caco-2/HT29) to evaluate the colonization (bacteria adhering to and inside the cells) and invasion (bacteria present internally in the cells) ability of the A. butzleri strains tested. In addition, genomic DNA was sequenced with Illumina technology and annotated using the software Prokka to investigate possible correlations between observed phenotypic behavior and genetic features of the A. butzleri strains.
All strains colonized both models and higher colonization was observed on the mucus producing models. Moreover, 4 strains were not able to invade MP models, one of which was also not invasive on NMP. Phylogenetic analyzes showed the absence of a clear separation linked to the source of isolation of the strains, although three groups of isolates from pigs and one from humans are grouped in different dendrograms (among which MLST sequences, SNPs, core genome). The analysis of genomes confirmed the presence of different putative virulence genes. Part of these genes were linked to lipopolysaccharide metabolism (e.g. O-antigen, survival on host mucosa) and were located in a pangenome plasticity region. In addition, genes putatively associated to virulence mechanisms described in other Gram negative pathogens, as well as TonB complex (iron transport), hemolysis proteins (hecA, hecB, irgA) and gene expression regulation such as phoP/phoQ and walR genes, were identified. This study addresses a current lack of information on A. butzleri virulence mechanisms. Better understanding of the A. butzleri virulence is of relevance in elucidating their pathogenic role and will enable further transcriptomic studies.

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P3.3 Evolution assays of Salmonella Typhimurium in presence of ciprofloxacin lead to heat resistant variants

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Antimicrobial resistance (AMR) in bacteria still represents a serious hazard for public health. Due to several AMR drivers throughout the food chain (e.g., antibiotic misuse in food producing animals), zoonotic infections developed by AMR bacteria pose a threat to the consumer. Indeed, food isolates of Salmonella spp., the most frequent cause of foodborne outbreaks in the European Union in 2019, exhibited resistance to "critically important antimicrobials" in human medicine, such as ciprofloxacin (CIP). Nonetheless, it remains uncertain the extent to which AMR phenomena may impact on the effectiveness of food preservation methods, such as heat treatments (HT). Therefore, eventual genetic alleles in Salmonella spp. which confer AMR could lead to a less efficient food preservation treatment.

The objective of this study was to assess the presence of cross-resistance to heat of Salmonella Typhimurium LT2 (SeWT) resistant variants (RVs) obtained via evolution assays in presence of CIP.

For this purpose, two different 10-day evolution assays were carried out:

i) exposing a population of SeWT to sub-inhibitory doses (0.5×MIC, "Minimum Inhibitory Concentration") of CIP. Thus, the MIC of RVs against CIP increased up to 8-fold (0.031-0.0625 µg/mL), in comparison to SeWT (0.008 µg/mL). However, no differences in heat resistance were observed between SeWT and its RVs.

ii) exposing SeWT to a daily onward CIP gradient (1.85× higher for each successive cycle). Hence, the MIC of RVs increased up to 128-fold (0.0625-1.0 µg/mL). An isolated RV revealed a great increase in its heat resistance at pH 7.0: while a HT at 54 °C for 30 min inactivated 5.8 log⁻¹ cycles of SeWT population, only 2.5 log⁻¹ cycles of RV population were reduced.

These results demonstrate that AMR bacteria, such as CIP-resistant S. Typhimurium, might also show cross-resistance to food preservation methods, such as HT, compromising food safety. Whole genome sequence of RVs would reveal genetic modifications responsible for enhanced antibiotic and heat resistance. Furthermore, it is shown the potential of the onward gradient assay to isolate RVs with great increases in their MIC. More studies are required for the comprehensive understanding of the mechanisms of AMR bacteria against food preservation methods.

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P3.4 Metagenomic insights into the resistome of bulk tank milk filters: role of Moraxellaceae and Enterobacteriaceae as carriers of antimicrobial resistance genes

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In the present context of growing concern about antimicrobial resistance (AMR), understanding the distribution of AMR determinants in a complex food matrix such as milk has relevance in terms of protecting consumers and maintaining high food safety standards. Herein, the resistome of different dairy farms was investigated through a whole metagenome sequencing (WMS) approach, taking advantage of in-line milk filters as promising tools.
Bulk tank milk filters were collected from 10 dairy farms in May 2020; sampling was repeated in May 2021. DNA was extracted from each sample (N=20) and used for WMS to a sequencing depth of 50 M PE reads. Host-filtered reads were aligned to the MEGARes, CARD, ARG-ANNOT and Resfinder databases to perform a reads-based resistome characterization; taxonomic classification of host-filtered reads was assigned using Kraken2 and Bracken. Host-filtered reads were assembled de novo and ABRicate was used on generated scaffolds and metagenome assembled genomes (MAGs) to perform assembly-based resistome characterization. WAAFLE and MOB-suite were used to predict plasmid sequences and mobility and to identify horizontal gene transfer markers in MAGs.
The application of both the reads-based and the assembly-based approaches has allowed the identification of numerous AMR determinants, with aminoglycoside, β-lactam, tetracycline, multidrug and macrolide-lincosamide-streptogramin (MLS) being the most abundant classes. Most of the species harboring AMR genes were predicted to be Gram-negative bacteria, namely Enterobacter, Acinetobacter, Escherichia, and Pseudomonas spp. Thirty-seven plasmids carrying AMR-encoding genes were detected. De novo assembly resulted in 8 high-quality MAGs, including different members of the Moraxellaceae and Enterobacteriaceae families; seven out of eight MAGs harbored AMR determinants.
Our findings suggest that milk filters can successfully be used to investigate the resistome of bulk tank milk through the application of the WMS. In accordance with our results, raw milk can be considered a source of AMR bacteria and genes. This points out the importance of properly informing food business operators about the risk associated with poor hygiene practices in the dairy production environment and consumers of the potential microbial food safety risks derived from raw milk products consumption.

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P3.5

Metagenomics analysis of traditional starter cultures to manufacture nordic ropy fermented milk (Tettemelk)

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Nordic food culture has been historically associated with the consumption of traditional fermented milk products. Originally, they are manufactured through mesophilic Lactic Acid Bacteria (LAB) belonging to the genera Lactococcus and Leuconostoc in spontaneous fermentation and/or back-slopping processes. Tettemelk has been consumed for hundreds of years and it has traditionally been prepared by adding leaves of the Pinguicula plant. These products have also been traditionally honored with health-giving properties such as antioxidant, immunomodulatory, ACE-inhibitory, and anti-tumor activities. Overall, such cultures are composed of a mix of undefined strains of microorganisms that are only partially characterized. Therefore, in this study, we evaluated the microbiome of seven different starter cultures using metagenomic high-throughput sequencing (mHTS). Taxonomic profile using MethaPhlAn3 showed that both samples are dominated by bacteria belonging to the families Streptococcaceae (82% ± 9.9%) and Leuconostocaceae (18% ± 10.0%). At the species level, Lactococcus lactis (81.7 ± 10.2) and Leuconostoc mesenteroides (18% ± 10.0%) were the most prevalent across the samples. Although to a less extent manner, the species Lactococcus chungangensis (0.64%) and Lactococcus raffinolactis (0.58%) were also identified in one of the samples (TC). In total, 13 metagenome-assembled genomes (MAGs) were recovered in the complete dataset of seven starter cultures. Taxonomic classification with GTDB-Tk revealed that three different species could be identified: Lactococcus lactis (8 MAGs), Leuconostoc mesenteroides (3 MAGs), and Lactococcus raffinolactis (2 MAGs). The functional potential of the metagenomes with SUPER-FOCUS showed that the top-five pathways were related to the metabolism of carbohydrates, protein, DNA, amino acids and derivatives, and cell wall and capsule. Noteworthy, metagenome TE displayed a significant enrichment of elements associated with phages, prophages, and transposable elements when compared to the other six samples (8.2% vs 4.1% ± 2.1%). This study highlighted the microbial diversity/metabolic function observed in seven starter cultures involved in the production of Nordic ropy fermented milk and will serve as a basis to identify genomic traits of interest to the dairy industry.

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P3.6 Omics technologies for traceability of Greek PDO "Vostizza" currant

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Several independent agencies and quality authorities are looking for reliable tools for validating food products' geographical origin and authenticity. Due to this demand and the impact befallen on public opinion when a food fraud incident appears, researchers try to address this issue with innovative and analytical approaches. Food products which hold a PDO or PGI quality scheme because of their uniqueness and economic impact become prone to adulteration. Currant Vostizza (Vitis Vinifera L., var. Apyrena) is traditional and high valuable Greek PDO product. Vostizza currants are sun dried vine-products and according to nutrition scientists, they are an excellent source of antioxidants and polyphenol compounds. In this study, a combination of genomics and metabolomics approaches was performed for determination of Vostizza currant geographical origin. Therefore, non-targeted 1H NMR fingerprinting was used in combination with multivariate statistical analyses for the classification of Greek currants based on their geographical origins (Aeghion, Nemea, Kalamata, Zante and Amaliada). Additionally, the present study aimed also to explore DNA-based approaches (PCR-RAPD and PCR-DGGE) for authentication and traceability of Vostizza currants. Results from NMR analysis suggest that composition differences in carbohydrates, amino and organic acids of currants are sufficient to discriminate them in correlation to their geographical origin. In parallel, both PCR-RAPD and PCR-DGGE analysis proved to be very promising for discrimination of currants regarding their provenance, as well. In conclusion, currants metabolites which mostly contribute to classification performance of such discriminant analysis model present a suitable alternative technique for currants traceability. Moreover, by DNA-based analysis of currants, band profiles obtained can be unique for each provenance. Concluding, further analysis, could enhance the sensitivity and accuracy of omics technologies applied for determination of currants' geographical origin.

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P3.7 Meta-community of the Asiago-PDO cheese during the cheesemaking process and ripening

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Asiago cheese is a product with a certified designation of origin and is the eighth most consumed cheese in Italy. The interest towards the microbial consortia that govern fermented foods is increasing because it helps to understand the entire process of food production, including the cheesemaking process. This study aimed to investigate the Asiago-PDO Italian cheese microbiome, to define the structure of the cheesebiome, identifying all the microbial players showing resilience during the ripening process. Raw cow's milk, curd, and cheeses at different ripening times were collected from two certified Asiago-PDO dairies. The total bacterial count reached a maximum of ca. 9 log CFU/ml in cheeses after 2 months. The number of presumptive mesophilic lactococci in raw cows' milk was higher than that of presumptive mesophilic lactobacilli. The numbers of these microbial groups increased during ripening, showing temporal and numerical differences. The partial sequencing of the 16S rRNA of ca. 500 isolates was carried out. Thirty-five species were identified, most of them belonging to Lactococcus lactis subsp. lactis; Lactobacillus delbrueckii subsp. bulgaricus; and Streptococcus thermophilus. Potential probiotic strains like Lacticaseibacillus rhamnosus, Lactiplantibacillus plantarum, and Pediococcus pentosaceus were identified especially in cheeses after 2 and 3 months of ripening. Satellite members such as Bacillus siamensis, Enterococcus hirae, Bacillus cereus, and Bacillus licheniformis were also identified. Culture-independent methods (MiSeq-Illumina analysis) allowed the investigation of the bacterial evolution during production and ripening. The Bray-Curtis PCoA-plot of the □-diversity showed a clear separation of milk samples from the others, as well as the separation among samples from the two dairies. In raw milk, the phylum Firmicutes (up to 23% of the genus Streptococcus) and Proteobacteria (up to 65% of the genus Acinetobacter) dominated. The phylum Firmicutes was dominant in curd (up to 90% of the Streptococcus genus) and cheese samples (up to 71% of the Streptococcus genus and 51% Lactobacillus). We can conclude that although the microbial biodiversity of milk is much higher than its derivatives, this biodiversity is naturally guided by the starter during the cheese manufacturing process. Thus, this study identified strategic phases that characterized the manufacture and ripening of Asiago cheese.

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P3.8

A proteomic approach to analyze the response of Listeria monocytogenes to different environmental conditions

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Listeriosis is a foodborne disease caused by Listeria monocytogenes, with a significant morbidity and mortality rate in people with a weakened immune system, principally pregnant women, newborn, young, and elderly. Many outbreaks were associated with the consumption of raw milk and dairy products (principally white or soft cheeses), poultry and processed meat, seafood, and ready to eat foods (salads, raw vegetables, etc.). Severe clinical manifestations varied from self-limited gastroenteritis to septic abortion, encephalitis, bacteremia, and meningitis. Listeria monocytogenes can tolerate, grow and/or multiply over a wide range of environmental conditions that can be adopted to control food contamination (Chafsey et al., 2022), such as low pH, refrigeration temperatures, low water activity, and high salt concentrations. The current study evaluated the exposure effect of two L. monocytogenes 1/2a strains isolated from meat products at four different growth conditions: A (control): T 37°C, pH 7.0, NaCl 0.5%; B: T 37°C, pH 5.5, NaCl 7%; C: T 12°C, pH 7.0, NaCl 0.5%; D: T 12°C, pH 5.5, NaCl 7%. A proteomic approach was applied to evaluate which proteins were produced by the strains when exposed at different temperatures, pH, and salt concentrations. The whole proteome was analyzed resolving the protein extracts by 1D-electrophoresis. A shotgun proteomics analysis was performed to identify proteins of interest. The entire gel lanes were digested and analyzed by nLC-ESI-MS/MS technique. Several different targets were identified and characterized examining the corresponding gene list by ShinyGO tool (Ge et al., 2020). The analysis of the whole proteome profiles highlighted a significant inter-strain variation in the proteins produced. Most of the proteins identified were produced in growth conditions and were involved in basal cell metabolism. Differently, some proteins defined as unique for each condition are correlated with the pathway of pathogenicity and stress response. The proteomic data obtained in this study will be the base for further research to deeply investigate L. monocytogenes pathogenicity and stress adaptation, in order to prevent or mitigate future outbreaks.

Chafsey et al. (2022). Journal of Proteomics, 250, 104388.
Ge et al. (2020). Bioinformatics, 36(8), 2628-2629.

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P3.9 Prevalence of the LOS locus C type in Campylobacter jejuni strains belonging to the 21-clonal complex

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Campylobacter jejuni is an enteropathogen considered the most frequent foodborne illness. Although Campylobacter infection is generally a self-limited disease, in some cases, post-infection complications such as neuronal disorders Guillain–Barré syndrome (GBS) and Miller Fisher syndrome (MFS) may occur. Here, certain association seems to exist between the presence of genes associated with the sialization process during lipooligosaccharide (LOS) biosynthesis, and these postinfection complications. However, no relationship with genotype has been found. In this study, we analyzed the prevalence of the LOS locus class in Campylobacter jejuni samples and the potential correlation with a particular genotype.

Fifty-three strains of C. jejuni including different PFGE pulsetypes were sequenced using the Nextera XT DNA Library Preparation Kit and the MiSeq platform. Strains were taxonomically typed using mlst v2.17.6 and assigned to clonal complexes (CC) by the PubMLST database. Genes included in LOS biosynthesis locus were identified in the genome of each sample using NCTC 11168 C. jejuni strain as reference, and LOS structure was assigned to a LOS locus type (A-W) through tBLASTn analysis.

The most prevalent CC within the 53 C. jejuni strains was ST-21 (21), followed by ST-464 (6) and ST-353 (4). In the analysis of LOS locus, 33 strains had genes related to sialization (LOS A/B/C types), and the rest belonged to the groups D, E, F, H, I, K and L. Between these, ST-353 was associated to LOS D type, ST-443 to LOS E type and ST-354 to LOS I type. And more interestingly, all the strains included in the ST-21 CC presented a LOS A/B/C type, and even the LOS C type was exclusive to these ST-21 CC strains.

A correlation was found between the genotype of the C. jejuni strains and the structure of the LOS biosynthesis locus. Noteworthy is the relationship between the ST-21 genotype, the most frequent CC in C. jejuni, and the sialized LOS structure with the potential to induce postinfection complications, so the role of Campylobacter in human inflammatory diseases could be underestimated. These results highlight the potential usefulness of knowing the genetics of the strains in order to detect Campylobacter complications.

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P3.10 Looking into transcriptomic induction of resistance and/or virulence in L. monocytogenes cells forming a biofilm on an abiotic surface

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Listeria monocytogenes is an important pathogenic bacterium provoking listeriosis, a rare but quite life-threatening foodborne disease mainly for the vulnerable populations. According to the latest epidemiological data for Europe, 1876 confirmed cases of human listeriosis were recorded in 2020, resulting in 167 reported deaths, presenting a case fatality ratio of 13.0%. The persistence of L. monocytogenes in food processing areas (even for years) is believed to be linked, among others, to its ability to strongly attach to the surfaces of the equipment (e.g., cutting tables, and conveyor belts) and buildings (e.g., walls, ceilings, drains, and floors), creating robust biofilms on them that can later withstand the typically applied sanitization processes. Alarmingly, L. monocytogenes cells enclosed in biofilms may display an altered phenotype, presumably inducing increased antimicrobial resistance and/or virulence. Considering all these and aiming to shed some light on the cellular physiology of L. monocytogenes under biofilm-forming conditions, being considered the default growth mode for most microorganisms, the relative expression of ten key stress response and/or virulence associated genes (i.e., groEL, hly, iap, inlA, inlB, lisK, mdrD, mdrL, prfA, and sigB) was studied in this work, in three different strains, all isolated from foods and each belonging to an outbreak associated serovar (i.e., 1/2a, 1/2b and 1/2c). To do this, each strain was initially left to develop biofilm on a model polystyrene surface (petri dish) by incubating for 144 h (6 days) at 20 oC in tryptone soya broth (with medium renewal every 48 h). Following incubation, biofilms cells were recovered from surfaces, and their total RNAs were extracted and reverse transcribed to cDNAs. The same also happened for those RNAs extracted from the planktonic cells of each strain, also incubated under the same conditions. All cDNAs were then used as substrates in qPCR reactions to comparatively quantify the relative expression of each of the target genes between the two cell types (i.e., biofilm, planktonic), through the classical 2-ΔΔCT method. Results obtained increase our knowledge on the sessile physiology of L. monocytogenes and may thus assist our efforts to avoid its environmental persistence and food contamination, ultimately protecting public health.

Studying and enlightening food microbial ecosystem and microbiome

P3.11 16S rRNA gene sequencing a tool for process control

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A study was performed to demonstrate the use of 16S rRNA gene sequencing as a process control tool in the meat industry.
16S rRNA gene sequencing was performed to describe the microbial composition of the freshly produced meat products and environmental samples. Sliced meat products were collected at three different days, along with environmental samples from the production equipment used. The sliced meat products were stored, and the microbial composition were investigated at the expiry date and further, until the products were considered sensory unacceptable.
Bacterial counts were similar in the fresh products for all 3 samplings and increased 4-5 logs at the expiry date after which no further increase was observed.
The microbial composition of freshly produced meat products was distinct for each of the three different samplings. However, at the expiry date, the microbial composition for all samples were identical, and dominated by Leuconostoc carnosum and Lactobacillus sakei.
In fresh meat products, the relative abundance of L. carnosum and L. sakei was generally low.
On equipment, L. carnosum could be identified on almost all surfaces of the production equipment and dominated in one specific location, during all samplings.
This study demonstrated, that 16S rRNA gene sequencing has the potential for use as a process control tool in the food industry for:
- fast identification of bacteria causing spoilage.
- identification of spoilage bacteria in freshly produced products and production environment.
- provide faster and much more detailed information compared to traditional microbiological tests, resulting in knowledge on how uniform the microbial composition is, which gives the producer an opportunity to act and ensure optimal shelf life and quality.

Studying and enlightening food microbial ecosystem and microbiome

P3.12 Identifying competitive exclusion microorganisms against Listeria monocytogenes from animal wastes-based composts using next generation sequencing approaches

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Animal wastes-based composts are rich in microorganisms, and some of them may be competitive exclusion (CE) microorganisms against human pathogens, such as Listeria monocytogenes, a leading foodborne pathogen that contaminates a variety of food products. In this study, we used next-generation sequencing (NGS) approaches along with culturing methods to analyze changes of compost microflora as affected by L. monocytogenes intrusion and then identify potential CE microorganisms against this pathogen. Twelve dairy- and poultry-wastes based composts were artificially inoculated with L. monocytogenes. After 72 h incubation at room temperature, the bacterial DNAs were extracted from the composts and then analyzed by NGS. The top seven bacterial phyla were identified as Firmicutes (23%), Proteobacteria (23%), Actinobacteria (19%), Chloroflexi (13%), Bacteroidetes (12%), Gemmatimonadetes (2%), and Acidobacteria (2%). Although the interactions between L. monocytogenes and indigenous microflora were limited, some discriminatory species such as Bacillus, Geobacillus, and Brevibacterium between L. monocytogenes-inoculated and control composts were identified by Random Forest analysis. Furthermore, metatranscriptomic sequencing revealed changes in metabolic pathways and the increased abundance of bacteriocins category in the compost samples inoculated with L. monocytogenes. Using double- or triple-layer agar methods, CE strains (n=40) with anti-L. monocytogenes activities were isolated from these composts. In agreement with metagenomics analysis, more than 50% of the isolated CE strains were identified as Bacillus spp. Further analysis revealed that CE strains reduced the growth potentials of L. monocytogenes by >99 % in compost extracts. In conclusion, NGS is a powerful tool in exploring microbial community changes of complexed compost ecosystems and facilitating the discovery of potential competitive exclusion microorganisms.

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P3.13

In silico meta-analysis of the current peptidome datasets related to yogurt fermentation

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Yogurt is a milk-fermented product with a high nutritional content which is associated with various health benefits. Yogurt fermentation is driven by specific lactic acid bacteria (LAB). The main LAB involved in yogurt fermentation are Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus, but they can be co-cultivated with other microorganisms mostly probiotics such as Lactobacillus acidophilus, Lacticaseibacillus rhamnosus, Lacticaseibacillus paracasei and Bifidobacterium lactis. In recent years, research interest has moved to the study of bioactive peptides from food sources and their potential application in human health. The health benefits of yogurt are partly due to the release of bioactive peptides as a result of the proteolytic activities of the fermenting bacteria. Most of the peptides extracted from yogurt samples are fragments of □-casein, while others come from □S1-, □S2-, □-casein and a few from whey proteins (□-LG and □-LA). Our study aimed for the meta-analysis of peptides present in yogurt peptidomic datasets and reevaluate in silico their structural and physicochemical characteristics and biological functions using appropriate bioinformatic methods. For the elucidation of the physicochemical properties of the peptides, various parameters were calculated by the employment of software tools such as ProtParam. Among the calculated parameters, aliphatic index, and grand average of hydropathicity (GRAVY) were included. Moreover, the peptide structures were predicted utilizing PEP-FOLD and I-Tasser web servers to identify their structural characteristics. The bioactivities of the peptides were assessed with the following open access databases: FermFoodDB, APD, PepBank, BioPepDB, MBPDB, BioPep-UWM. The peptides could be characterized as antioxidant, antihypertensive, anti-inflammatory, immunomodulatory, antimicrobial and opioid. In several instances, some peptides had more than one function. We also attempted to characterize in silico the flavor of the peptides using tools like EROP-Moscow. Our analysis reveals a wealth of information concerning the yogurt peptidome which deserves further investigation in order to be properly exploited at the industrial scale.

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Studying and enlightening food microbial ecosystem and microbiome

P3.14 Probiotic chocolates; to maintain a healthy microbiome in your gut

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A human body contains about 100 trillion human cells in a body, and more than 1000 trillion bacterial cells are present in the human body which is 10 times more than human cells. The 30 thousand human genes present in a body responsible for the expression of different characteristics were 100 times more than bacterial genes present in our human body. These genes may involve in keeping us healthy. These microorganisms present in our body are called microbiome and microorganisms present in our gut are specifically called the gut microbiome. More than 90% of the count is present in the gut. These bacteria are not passively present in our bodies, but they are extremely important. Every part of our body is coved and protected by microorganisms. They digest our food, make vitamins, and also play important role in educating the immune system to keep bad microbes out. There are two types of microorganisms present in our body, one is helpful and another is harmful, the helpful microorganisms present in our body are called probiotic microorganisms. Scientific studies depicted that modulation of the specific microbial community is responsible for the specific disease condition. Hundreds of studies showed a consistent pattern of the microbiome was specific to the disease, some of the diseases associated with over 50 genera and some are 10-15 genus-level modulations were observed. Recent advancements in this field state that reverting to normal flora can overcome the disease by a therapeutic approach. In the present study, cultures were isolated and characterized for the probiotic candidate. Probiotics are live microorganisms when administrated in an adequate amount confer health benefits to the host. These cultures were identified and checked for their efficacy for immunomodulatory activity. The culture was lyophilized and mixed with the chocolate formulation. This probiotic chocolate formulation may improve the gut microbial diversity resulting in a healthy gut microbiome to live a longer and healthy life.

Studying and enlightening food microbial ecosystem and microbiome

P3.15 Identifying volatile spoilage indicators in minced beef packed under gaseous atmospheres

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The spoilage of meat is typically due to the production of microbial metabolites and the subsequent emergence of offensive off-odors. While modified atmosphere packaging (MAP) can be used for extending the shelf-life of meat and meat products stored under refrigerated conditions, the improvement of meat quality control calls for novel methods to monitor the spoilage of these products over storage time. Consequently, the application of spoilage-indicating volatile organic compounds (VOCs) as quality indices has gained great interest in the development of intelligent packaging technologies. However, the identification of relevant VOCs calls for extensive data collection under commercially relevant storage conditions and subsequent multivariate statistical analysis. The aim of this study was thus to identify potential volatile spoilage indicators in minced beef packed under gaseous atmospheres (air and high-O2 MAP) and stored at 4 °C. Microbiological and chemical analyses were performed at regular intervals throughout storage time: selected-ion flow-tube mass spectrometry (SIFT-MS) was used for real-time monitoring of VOC production in the package headspace. Different exploratory and selective statistical analyses were performed to characterize the beef volatilome and for identifying potential spoilage indicators. The results of the study indicate the impact of different atmospheres on both microbial growth and VOC patterns. Several VOCs were found increasing over storage time and microbial growth under the tested conditions, suggesting promising potential in spoilage monitoring. Overall, the results of the study contribute to the development of systematic and comprehensive identification criteria for volatile food spoilage indicators.

P3.16 Phenotypic and genotypic characterization of Escherichia coli MG1655 resistant variants isolated after prolonged exposure to complex essential oils

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Natural antimicrobials are emerging as an alternative to chemically synthesized food preservatives due to its better social acceptance and its high antimicrobial potential. Nevertheless, emergence of antimicrobial resistant bacteria by exposure to antibiotics has called into question whether the prolonged bacterial exposure to natural antimicrobials could lead to the emergence of resistant variants (RVs) with increased antimicrobial resistance. Our objective was to assess the emergence of RVs of Escherichia coli MG1655 by exposure to essential oils mixes, and to characterize RVs phenotypic and genotypically.

A population of E. Coli cells was cyclically exposed for 20 days to sub-inhibitory doses (0.5× minimal inhibitory concentration (MIC)) of two essential oil mixes (COLIFIT and AEN). After exposure, RVs were isolated and characterized. Comparison of MIC values of RVs and parental strain against COLIFIT and AEN were determined to evaluate their increase in direct resistance. RV isolated from evolution experiment with COLIFIT (EcCOLIFIT) showed a 25% increase in MIC value against COLIFIT; while RV from AEN experiment (EcAEN) showed a 28.6% increase against AEN. Likewise, MIC values against COLIFIT and AEN, thymol, cinnamaldehyde, amoxicillin and colistin were determined to assess cross-resistance. EcCOLIFIT showed cross-resistance against AEN (28,6% increase) and cinnamaldehyde (25% increase); and EcAEN showed cross-resistance against COLIFIT (25% increase) and cinnamaldehyde (25% increase). Interestingly, none of the RVs showed cross-resistance to thymol or to antibiotics. Whole genome sequencing (WGS) of RVs revealed their genetic changes in comparison to parental strain. In EcCOLIFIT, a deletion of more than 5,000 bp was identified, causing the loss of genes involved in environmental information processing and cell motility. In EcAEN, it was detected a single nucleotide variant in a gene involved in the response to lipid peroxidation of cell envelopes; and a deletion of ca. 5,000 bp in the same region to that detected in EcCOLIFIT.

In conclusion, RVs emerged by exposure to COLIFIT and AEN showed direct and cross-resistance against natural antimicrobials but not against antibiotics tested. In addition, the big deletion detected by WGS would indicate that both mixes have a similar mode of action.

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P3.17 Variation in organic acid resistance of multiple Listeria monocytogenes isolates

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Predictive Microbiology (PM) is an increasingly important element of food safety management and quantitative microbiological risk assessment. PM accuracy requires detailed insight of growth characteristics of pervasive foodborne pathogens like Listeria monocytogenes. This study took advantage of a large bank (n=177) of L. monocytogenes isolates at Moorepark Food Research Centre to assess the variability in resistance to commonly used food preservative or naturally occurring undissociated organic acids. These L. monocytogenes strains were originally isolated and collected from food, food production environments and clinical sources in the Republic of Ireland (ROI). The strains were screened to identify the most acid resistant variants by the microdilution method. Statistical data analysis and data visualising were performed with RStudio. Acetic, lactic and propionic acid molar concentrations ranging from 2 mM to 20 mM at pH of 5.3 was used to estimate growth limits. A large variation in resistance profiles was observed amongst strains and overall 15.3% of screened isolates exhibited significantly higher resistance to undissociated acid than the other isolates. The highest level of undissociated acid resistance was observed in strains sourced from dairy, vegetables and seafood environments. Results are important for refining predictive microbiology and microbiology risk management. In addition, genome sequence information is available for the strains and the genetic basis for acid resistance profiles will be explored.

P3.18 Evaluation of heat treatment in different antimicrobial resistance profiles of Salmonella

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Salmonella is one of the main pathogens which causes hundreds of thousands of foodborne illness cases worldwide annually. This fact associated with the increase in antimicrobial resistance makes this pathogen a significant public health concern. In addition, the emergence of Salmonella multidrug-resistant (MDR) strains brings new challenges to the food industry. The purpose of the current study was to assess the impact of antimicrobial resistance on the heat resistance of Salmonella. For that, 40 Salmonella strains with different antimicrobial resistance profiles [23 multi-drug resistant (MDR), 9 resistant (R) and 8 sensitive (S)] isolated from humans, cattle, poultry or food were evaluated. Microtubes containing 100 µl of trypticase soy broth (TSB) were inoculated individually with approximately 6 log CFU/ml of each Salmonella strain. The heat treatment was carried out in a heating block at 60 °C for 0, 2, 4, 5 and 6 min. After each time interval, the microtubes were cooled in running water until they reached 40 °C. Then, the Salmonella count was determined by drop plating on trypticase soy agar (TSA), with incubation at 37 °C for 24 h. After 2 min at 60°C, the reductions in the Salmonella counts ranged from 1.0 to 3.3 log CFU/ml. Six strains reached the limit of detection (1.0 log CFU/ml) after 5 min and 22 strains after 6 min of heat treatment. The five most thermal resistant strains had different antimicrobial resistance profiles (2 S, 1 R and 2 MDR), and were isolated from humans, cattle and food. In contrast, all the five lowest thermal resistant strains were MRD and isolated from chicken meat. Thefore, the results indicate that heat resistance is not directly related to antimicrobial resistance.

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P3.19 Isolation of resistant variants of Salmonella Typhimurium after sequential exposure to plasma activated water

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Plasma Activated Water (PAW) has been proposed as a novel chlorine-free disinfectant in the food industry. However, the mechanisms of bacterial resistance and inactivation by PAW are not completely understood. Many reactive oxygen and nitrogen species, as those present in PAW (such as H□O□, NO□-, NO□-), have been demonstrated to greatly contribute to bacterial inactivation by creating a high oxidation reduction potential. On the other hand, exposure to some biocides and disinfection agents represents a selective pressure that may favor the emergence of bacterial resistant variants (RVs). The study of genotypic and phenotypic characteristics of these RVs in comparison to parental strain might contribute to understand the mechanism of bacterial resistance against antimicrobial compounds. Therefore, the objectives of this study are the monitoring of emergence of Salmonella Typhimurium RVs by evolution assays with PAW; and their genotypic and phenotypic characterization.
The evolution assays consisted in the application of serial short-term (45 min) lethal treatments with PAW to S. Typhimurium population. Survivors were incubated overnight at 37 □C in liquid growth medium (TSBYE), until the stationary phase was reached. This procedure was repeated 30 times with four independent cultures or lines (L1-L4). After the 30th step, two colonies of each line were selected. All the colonies showed an increased resistance against PAW in comparison with the parental strain. Those RVs were further sequenced for genotypic characterization.
Genetic comparison of RVs with parental strain revealed up to five single nucleotide variants (SNV) in one of the mutated strains analyzed. The SNVs identified were very similar in the colonies from the same line and quite different between them (L1-L4). Some of these mutations affect to genes which contributes to the acid tolerance response, are involved in transcriptional regulation processes, ions and aminoacid transport or affect to global regulatory system that responds to oxygen.
Genotypic characterization of RVs provided relevant information on the mechanism of bacterial inactivation by PAW. This study demonstrates for the first time the appearance of RVs of S. Typhimurium against PAW. Emergence of RVs might compromise the efficacy of PAW, which should be considered in the design of disinfection protocols.

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P3.20

A meta-analysis of the wine proteome and peptidome deriving from must fermentation microorganisms and beyond

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Even though proteins and peptides constitute a minor percentage in wine, they play a significant role for its quality, organoleptic and functional characteristics.

The aim of the study was to collect the available dataset of these compounds reported for different wines and reassess in silico their physicochemical properties and functionality through state-of-the-art bioinformatics tools.

Mining for microbial proteins and peptides in wine published in original research articles resulted in a pool of 150 and 50 records, respectively. Components were screened through publicly available databases (NCBI, UniProt) and against the genomes of Vitis vinifera and Saccharomyces cerevisiae. Evidently most proteins/peptides derived from them. We expanded our search to include the genomes of additional non-Saccharomyces yeasts or bacteria. Interestingly, some proteins belonged to Lachancea thermotolerans, Botrytis cinerea, Clavispora lusitaniae, Candida pyralidae, Torulaspora delbrueckii, Wickerhamomyces anomalus and Pseudomonas syringae. Most of these microorganisms could be related to the plant ecosystem and some could reflect the health status of the grapes.

We then proceeded to the characterization of physicochemical features of proteins and peptides in our dataset. For that purpose, tools like the ProParam software were used to compute various parameters such as amino acid composition, theoretical pI and grand average of hydropathicity (GRAVY). Furthermore, structural models were built using prediction tools such as PEP-FOLD and I-Tasser. Annotation of the proteins revealed that most of them had some enzymatic function, but additional functions could also be identified (e.g. transcription/translation regulators, receptors, etc.). For an updated functional characterization of the peptides we used the following tools: FermFoodDB, EROP-Moscow, PepBank, BioPepDB, BioPep-UWM, AHTPDB. We were able to ascribe peptides into different categories, including antihypertensive, antioxidant, antimicrobial and immunomodulatory. Some of the aforementioned databases also allowed us to correlate the peptides with sensory attributes (sour, umami or bitter).

The present study reveals important information from the metanalysis of proteins/peptides isolated from wines that can provide targets for validation with wet experiments and organoleptic tests.

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P3.21 Challenges of Campylobacter jejuni biofilm control

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Campylobacter jejuni is a biofilm-forming, multi-resistant pathogenic bacterium that is widely distributed throughout the food production chain and causes gastrointestinal disease in humans. Interrelated properties important for biofilm formation, such as intercellular signalling and the ability to attach to different abiotic surfaces, allow C. jejuni protection and survival outside its natural host. It is necessary to improve the control of C. jejuni biofilm as this will reduce the risk that C. jejuni poses to public health and the food industry. Therefore, we need new methods to study C. jejuni biofilms and find alternative antimicrobial agents active against biofilms traits.
The aim of this study was to: i) select and optimise a method to study intercellular signalling of C. jejuni; ii) develop the method to study C. jejuni biofilms using confocal laser scanning microscopy (CSLM); iii) test different Lavandula sp. formulations against C. jejuni biofilms; iv) understand the mechanisms of action of the Lavandula sp. formulations.
Our results showed that the biosensor assay using V. harveyi MM30 is the most suitable for studying the intercellular signalling of C. jejuni and has 100-fold better sensitivity compared to HPLC-FLD. Using CSLM, we were able to study C. jejuni biofilms at the air-liquid interface and separate live and dead biofilm cells. Various Lavandula sp. formulations, i.e. essential oils and ethanol extracts from dried flowers and waste material after essential oil distillation, were found to be successful in biofilm removal and prevention. Lavandula sp. formulations reduced significantly C. jejuni intercellular signalling and adhesion. Transcriptome analysis of the planktonic C. jejuni culture after treatment with Lavandula sp. essential oil confirmed the results obtained at the physiological level, where a down-regulation of biological process important for biofilm formation, i.e. locomotion, or flagellum-dependent cell motility, was observed.
All results together give an insight into the mechanisms important to combat C. jejuni biofilms. By using alternative strategies and novel targets our innovative approach has the potential to develop new effective agents with biofilm-preventing and biofilm-degrading activity.
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P3.22 Epidemiological study of Campylobacter spp. isolates from human clinical specimens in the city of Burgos (Spain)

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In previous studies strains of Campylobacter spp. were isolated from different steps of the poultry food chain (farm, slaughterhouse and retail) and simultaneously, with isolates causing Campylobacteriosis from the city hospital. From the latest, 34 isolates shared the same pulsotype with other isolates from the chain steps mentioned: 10 from farm, 5 from slaughterhouse and 20 from retail. Additionally, two strains (H1, H2) were added from clinical samples without relations with food chain steps. Thirty four out 36 isolates belong to C. jejuni and 2 to C. coli. All isolates were whole genome sequenced for further detailed study using the workflow Wombat.

Twenty-three strains were isolated from males and 13 for women. Age distribution was: <1 year (2 isolates), 1-10 years (21), 10-20 years (3), 21-59 (3) and > 60 years (5), from 2 patients no age data was available. Genome length of all isolates was between 1.6-1.8 Mbp and GC content between 30.2-30.5 % except C. coli strains with 31.4 %. MLST reveals the presence of 12 well identified clonal complex (CC). However, 7 strains couldn't be assigned to one CC. Among the CC, ST 21 CC was the majority with 7 ST: ST 50 (4 isolates); ST 21, ST 148 (H1 and H2) and ST 883 (2); ST 3769, ST 760 and ST 19 (1) followed by ST 464 CC 464 (3 isolates) and ST 354 CC 354 (2). The other C. jejuni STs belonged to different ST/CC with only one isolate. The two C. coli isolates belonged to the same CC 828, but different STs; 827 and 854.

Only 5 patients had to be admitted to the hospital. Four of them caused by C. jejuni STs/CC: 148/21; 50/21; 356/353; 677/677 and one by C. coli 854/828. Three of the STs corresponding to CC 21 and ST 356/353 showed similar antibiotic resistance gene profile, harboring the resistance genes tet(O), blaOXA-193 and the point mutation gyrAT86I (resistance to quinolones). ST 677/677 harbored only resistance to blaOXA-447. C. coli ST 854/828 showed genomic antimicrobial resistance to gyrAT86I, tet(O) and a point mutation in 23S A2057G that confers resistance to macrolides.

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P3.23

Growth patterns of luminous Photobacterium on chilled chicken meat and genotypic characterization of individual isolates

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Chicken meat spoilage is caused by a dominant fraction of the initial microbial association that consists mainly of Pseudomonas, Brochothrix thermosphacta, Enterobacteriaceae and lactic acid bacteria (LAB). The recent application of next generation sequencing technologies on spoiled chicken meat has revealed the presence of genus Photobacterium at significantly high abundances. This study aims to a) assess the presence and growth patterns of luminous Photobacterium on chicken breast fillets during refrigerated (4°C) storage and b) estimate their genotypic biodiversity. Chicken breast fillets (n=6 samples/3 batches) were periodically analyzed for the estimation of total mesophilic bacteria and presumptive Photobacterium spp. Ninety luminous Photobacterium strains were isolated from Marine Broth (MB) agar medium at the beginning (time of first detection), middle and end of storage. Genetic diversity of isolates was assessed through RAPD and rep-PCR DNA fingerprinting techniques, while identification at the genus/species level of representative isolates was performed by sequencing of 16S rRNA, gyrB and fur genes. Luminous bacteria were scarcely encountered at the beginning of storage in one out of three batches at ca. 2.0 log CFU/g. However, they were found at significantly higher populations, ranging from 5.3 to 7.0 log CFU/g, at later stages of storage in samples from two batches. In the third batch, they were only occasionally found throughout storage at populations of up to 3.9 log CFU/g. Pattern similarity based on RAPD-PCR and rep-PCR fingerprint profiles allowed the discrimination of bacterial isolates in 18 clusters when a coefficient of similarity of 85% was used. Moreover, the sequence of the three genes assigned the isolates to Photobacterium genus. In conclusion, the relatively high enumerated numbers of luminous Photobacterium along with the genotypic differentiation suggest this genus as potential chicken meat spoiler with high genetic diversity.
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P3.24 Oxidative stress adaptation of Bifidobacterium spp.

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Bifidobacteria are the dominant inhabitants of the human gastrointestinal tract. Due to the health benefits of this probiotic, its inclusion in the production of functional foods, especially fermented dairy products, is popular. Oxygen is easily incorporated during yoghurt production and results in toxic and damaging ROS that significantly affect the viability of incorporated bifidobacteria cells. The mechanisms underlying the oxidative stress response of three Bifidobacterium strains were induced to produce variants with increased aerotolerance. A stress adaptation treatment was developed based on the activity of three strains of bifidobacteria under oxidative stress conditions. To determine the effect of sub-lethal doses of O2 and H2O2 on the viability of Bifidobacterium spp., the growth of three strains was determined using turbidimetric methods paired with standard plate counting under conditions of sub-lethal doses of O2 (3, 5, 10, 20 %) or H2O2 (0.01, 0.1, 1 mM). After sub-lethal treatment of O2 and H2O2, the critical enzymes involved in detoxifying ROS in bifidobacteria, NADH oxidase and AhpC, were purified and characterised. Proteomic techniques involved 2D-PAGE and allowed us to identify the proteins whose production was changed after the stress adaptation treatment. The viability of adapted Bifidobacterium spp. was assessed using a flow cytometry-based approach. By exposing cells to sub-lethal doses of O2 and H2O2, the genes involved in the stress response of Bifidobacterium spp. were induced, allowing for an upregulated expression of stress response proteins without compromising cell viability, resulting in variants able to survive previously lethal doses of oxidative stress. The viability of stress-adapted Bifidobacterium spp. during the fermentation and storage of yoghurt, and yoghurt with inulin or lactulose was investigated using a metabolomic and proteomic approach. To evaluate the ability of oxidative stress adapted Bifidobacterium spp. to remain viable when exposed to oxidative stress during yoghurt fermentation and storage was analysed by standard plate counting during a shelf-life study. The metabolomic approach allowed us to determine whether other critical metabolic processes of bifidobacteria were affected, resulting in the production of functional, beneficial bioactive compounds.

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P3.25 Protocols for isolation of RNA from Campylobacter jejuni biofilm

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Campylobacter jejuni is the leading cause of foodborne bacterial gastroenteritis worldwide. Biofilms serve as a pool for antibiotic resistance and new contaminants and play an important role in the survival and persistence of C. jejuni in food production, by providing a physical barrier. For this, extracellular polymeric substances are the most important with the key role also in the structural support, functional integrity and intercellular communication of these communities. On the other hand, this matrix is the main reason why mechanism at the RNA level is not yet been explored, as it can interfere with nucleic acid extraction and purification.

Therefore, we evaluated three different protocols to isolate RNA from C. jejuni biofilms by combinations of RNA isolation protocols with purification using silica membranes, named P1, P2 and P3. After each, we determined RNA quantity and quality based on the absorption ratios: A260/A280 as indicators of contamination with protein; A260/A230 as indicators of contamination with polysaccharides, phenols, and/or chaotropic salts.

P1 was selected first because RNA was successfully isolated from planktonic cells. In P1, we used TRIzol, a single-phase solution of phenol and guanidinium isothiocyanate that simultaneously solubilizes biological material, denatures proteins, and provide highly effective inhibition of RNase activity during lysis. The isolated RNA was present in low quantity and purity. P2 is a modified version of P1 using a combination of chemical lysis and mechanical cell disruption. Mechanical disruption of biofilm cells did not improve the concentration and ratio of isolated RNA. Finally, in P3, we used a lysis buffer containing □-mercaptoethanol and a cationic surfactant, CTAB. Using P3, we successfully isolated a high concentration of pure RNA (A260/A280 1.9-2.0; A260/A230 > 2.0).

Our results showed that not all methods are suitable for isolation of RNA from C. jejuni biofilms due to the sample composition, especially the presence of the extracellular matrix. We chose extraction with lysis buffer containing □-mercaptoethanol and a cationic surfactant to obtain sufficient quantity and quality for RNA.

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P3.26 The impact of calcium on the transcriptional profile of Lactococcus lactis strains

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The fermentation of protein-rich dairy substrates with lactic acid bacteria may result in bitter-tasting products. Yet, it is not clear whether bitter peptide formation is related to milk-endogenous enzymes or the applied starter cultures. As no bitter peptide formation is observed in products with regular protein contents, the increased calcium content which is introduced with the casein micelles was considered as crucial. The proteolytic system of L. lactis consists of the cell envelope peptidase PrtP, transport systems for the uptake of di-, tri-, and oligopeptides, as well as intracellular peptidases. The global transcriptional regulator CodY is putatively regulating the expression of genes encoding this proteolytic system.
Genomic and plasmid DNA of two Lactococcus lactis subsp. lactis and cremoris strains was isolated and sequenced by Illumina next-generation sequencing. The genomes were compared to each other and to a set of reference genomes. The presence of genes encoding transport systems and enzymes of the proteolytic system was confirmed. Cells of one prtP-positive strain per subspecies were grown in media containing the same casein content with either regular or double calcium content. Biomass was harvested for RNA preparation in the late exponential phase. Transcriptomic analysis via RNA-Seq was performed in biological triplicates.
The subspecies lactis strains were closely related to each other and the reference genomes, while the subspecies cremoris strains were not. Contrary to expectation, for both prtP-positive strains individual genes encoding transport systems and enzymes of the proteolytic system as well as codY were not significantly differentially regulated at different calcium contents. Gene set enrichment analysis did reveal an upregulation of the genes of the proteolytic system in the L. lactis ssp. cremoris strain, while the respective gene set in the L. lactis ssp. lactis strain was not found to be significantly enriched. Enrichment was observed especially for the peptide transporter genes.
Thus, even closely related strains react to changing environmental conditions in an individual manner and the impact on the enzymatic level might outweigh that on the transcriptomic level. These findings stress the importance of an in-depth evaluation of individual strains for the selection of suitable starter cultures.

Studying and enlightening food microbial ecosystem and microbiome

P3.27 Microbial functional and genetic diversity characterisation of soft drink effluent

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Microbial ecosystems in soft-drink effluent consist of a wide range of microorganisms due to an abundance of organic matter. In South Africa, very little effluent treatment takes place before discharge and the microbial composition is seldom explored. This study aims to map the microbial consortia functionality as well as determine the core microbiome in soft drink effluent in an attempt to establish a consistent profile. Composite effluent samples were collected hourly over 10 days. Community Level Physiological Profiling (CLPP) based on sole-carbon substrate utilisation was measured every 24 hours using EcoPlates. Genetic diversity was determined by using 16S and ITS targeted metagenomic sequencing at 2 x 300bp on an Illumina Mi-Seq. The metabolic fingerprint of microbial communities revealed that the most utilised carbon compound group was carbohydrates (34,7%), followed by carboxylic acids (24,3%) and amino acids (20%). However, the most utilised carbon substrate was found to be □-Hydroxybutyric Acid at 4,4% mainly because it is a very important metabolite produced by bacteria in times of starvation and during the action of stress factors to increase chances of survival. NGS results showed 40 phyla, 341 families and 685 genera. These Amplicon Sequence Variants (ASVs) were Clustered in QIIME 2 at 99% similarity, with 783 belonging to the bacterial taxa and 212 belonging to the fungal taxa. Proteobacteria was found to be the most abundant bacterial phyla at 45,42% and Ascomycota (76,6%) for the fungal taxa. Shannon's diversity indices were calculated for both the functional and genetic fingerprint, these illustrated that the core microbiome of the effluent is rich and diverse. Both the carbon utilisation and ASVs characterised also correlate positively to the nutrients in soft drink effluent which are present in the form of sugars, artificial sweeteners and preservatives. This study presents an understanding of the core microbiome of soft drink effluent on a functional and genetic level, a valuable step towards future applications for industry-specific effluent.

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P3.28 Microbial community diversity and functionality in red meat abattoir effluent

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Abattoir or slaughterhouse processes require large quantities of water and in turn produce large quantities of wastewater with high organic concentration. In South Africa this effluent is discharged directly into the municipal sewer systems, often without pre-treatment, causing strain on already overburdened sanitation systems. Physicochemical qualities of abattoir effluent and the perceived environmental effects have been studied, but limited information exists focused on microbial diversity of effluent from this industry. The aim was to construct a diversity profile of the microbial component in red meat abattoir effluent on genomic and metabolic level. Daily sampling was performed for 10 consecutive days by collecting composite samples from the pipe through which effluent is discharged into the sewer system. Community Level Physiological Profiles (CLPP) were constructed using EcoPlate sole-carbon substrate utilisation analysis and genetic diversity determined through 16S and ITS targeted metagenomic sequencing on the Illumina MiSeq. Simpson Index (D) of 0.962 (±0.0012) displayed a very diverse community while evenness index (E) of 0.97 depicted a large abundance of species present. Functional diversity showed significant similarity amongst the samples (p=0.0003). The relative abundance of metabolic activities was related to polymer, carbohydrate and amino acid metabolism. Glycogen and L-asparagine were the most utilised substrates, and no utilisation of 2-hydroxy benzoic acid was evident. Bacterial diversity showed consistent dominance of 3 phyla, namely Bacteroidetes, Fibrobacteres, Planctomycetes. Bacteroidetes represent major members of microbiota found in the gastrointestinal tract of animals. Fibrobacteres includes many major rumen bacteria and lastly Planctomycetes consists of widely distributed aquatic and terrestrial bacteria important for global carbon and nitrogen cycles, with many species capable of anaerobic ammonium oxidation. Genus richness did not present any discernible pattern or consistency in distribution. Phascolarctobacterium, Ruminococcaceae and Succinivibrio appeared to be the most abundant genera. Dominant fungal phyla were either Ascomycota or Neocallimastigomycota. In contrast to the common distribution of bacteria amongst the different sampling days, the fungal community was dominated by a particular genus in each sample. These included Caecomyces, Neocallimastix, Trichosporon, Anaeromyces, Kazachstania, and Diulina. This was the first step in understanding the complexity and behaviour of the red meat abattoir wastewater microbiome.

Studying and enlightening food microbial ecosystem and microbiome

P3.29 Succession of microbial communities during chilled storage of whole and gutted sea bass

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Various factors such as the type of product, the composition of the initial microbiota, the applied storage conditions, microbial interactions etc., can determine the succession of microbial communities and the selection of Specific Spoilage Organisms (SSOs) in seafood. The aim of this work was to study the effect of primary processing in the microbial communities' succession, of whole and gutted sea bass during chilled storage. For this purpose, 16S metabarcoding analysis was carried out for both whole and gutted sea bass throughout a storage period of about 15 days (end of shelf-life). Results indicated that the presence of Thermus was noteworthy at the initial stage of storage in both products, followed by Propionibacterium and Rahnella in gutted fish and Kluyvera and Lelliotia in whole fish. In later stages of storage, Pseudomonas was by far the most abundant genus in the middle and the end of shelf-life of whole and gutted sea bass, while Shewanella was found at similar abundances in both products. However, the presence of Carnobacterium solely in whole fish, as well as the presence of Psychrobacter in gutted fish, demonstrated a slight effect of primary processing in the microbiota profile at the final stages of storage. To conclude, primary processing affected the microbial communities' composition, however both products were dominated by the same SSOs at the end of shelf-life.

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P3.30 Bacterial communities on raw salmon fillets packed in a modified atmosphere assessed by cultural method and by Next-Generation Sequencing

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Salmon is one of the most widely consumed fish worldwide. It is nutritious, rich in omega-3 fatty acids, essential vitamins, proteins, minerals, and form part of a healthy diet. However, salmon is highly perishable as a fish despite the health and nutritional benefits. Storage temperature, handling, and packaging conditions affect microbial growth and thus the shelf-life of fish. This study was undertaken to explore the bacterial communities of raw salmon fillets packed in a modified atmosphere of carbon dioxide and nitrogen, stored at refrigeration (5°C) and slightly abused (8°C) temperatures through conventional cultural methods along with next-generation sequencing (NGS) analysis. Totally of 15 samples were tested.
The main microbiota of raw salmon fillets samples 3 days after commercial packaging consisted of Lactobacillus (29.9% ± 3.1%), Photobacterium (22.8% ± 8.8%), Streptococcus (21.6% ± 6.9%), and Lactococcus (8.1% ± 2.1%). During storage Photobacterium becomes dominant, and after 6 days they constitute 94.5% ± at 5°C and 91.0 ± 0.4% at 8°C. Carnobacterium was found in small amount (3.9% ± 1.6% at 5°C and 3.2% ± 0.3% at 8°C). Lactococcus made up 3.3% ± 0.5% at 8°C.
After 10 days of storage, Photobacterium constitutes over 95% of the identified genera, regardless of the storage temperature. Most sequences were assigned to Photobacterium phosphoreum.
Conventional plate counting with growth media commonly used in spoilage studies did not always correspond to the microbial community profiles derived from NGS analysis. Lactic acid bacteria (LAB) count reached less than 3 log CFU /g after 3 and 6 days of storage and after 10 days at 5°C. While after 10 days at 8°C LAB reached 4.8 log CFU/g. The number of Enterobacteriaceae was 6.1 log CFU/g after 10 days at 8°C, while by NGS, their presence was not demonstrated. The number of presumptive Photobacterium on marine agar was 5.4 log CFU/g after 3 days and over 7.0 log CFU/g after 6 and 10 days of storage of raw fillet samples (MAP) regardless of the temperature.
Using a polyphasic approach for seafood products could provide better insights into residential bacteria dynamics and their impact on food safety and quality.

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P3.31 Microbial changes of sea bass fillets stored under air and MAP at various temperatures using 16S metabarcoding

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The microbial communities' succession during fish storage depends on factors such as the applied storage conditions (e.g., temperature, atmosphere) and microbial interaction. The present work aimed to monitor the microbiota succession of sea bass fillets, during storage at various temperatures (4, 8 and 12oC) in both air and MAP conditions. The results obtained through 16S metabarcoding analysis indicated that the initial microbiota consisted of three main dominant genera, Pseudomonas, Acinetobacter, and Psychrobacter, as well as of several others at a lesser extent. However, Pseudomonas was exclusively the most abundant genus in all temperatures and atmospheres in the middle and the end of shelf-life, except for the end of shelf-life of MAP-stored fillet at 12oC, where Serratia was by far the most dominant genus. Interestingly, at the end of shelf-life, beyond Pseudomonas, several different bacterial genera co-existed at remarkable abundances, the presence of which was mainly dependent on the different storage atmospheres and not the different temperatures (Acinetobacter and Shewanella for air-stored fillets, Brochothrix for MAP-stored fillets). However, there was a noticeable different bacterial profile between the MAP-stored sea bass fillets of 4oC and those of 8oC and 12oC, where Carnobacterium (4oC) was present in the top-three most abundant genera, instead of Serratia (8 and 12oC). To conclude, the application of different storage conditions affects the microbial profile of stored sea bass fillets.

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P4.1 Antimicrobial effect of i-PAW

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The aim of this study was to evaluate the effect of three different Iced Plasma Activated Water (i-PAW) on the microbiota of cuttlefish (Sepia officinalis) along its storage.
Three types of PAW were produced by modifying the generation process (direct, recirculation and bubbling) and then characterized -concentration of nitrates, nitrites, hydrogen peroxide, hydroxyl radical (OH*), nitrogen monoxide (NO*), nitrogen dioxide (NO2*) and peroxynitrite radical (ONOO-)- before and after freezing. Those PAW were frozen in flakes (i-PAW) and used to store the cuttlefish at 2°C for 12 days. During the storage, the microbial load of the cuttlefish (mesophilic and psychrophilic bacteria) was evaluated.
Before freezing, the highest concentrations of OH*, NO*, NO2* and ONOO- were detected in the bubbling-PAW, followed by the recirculation-PAW and the direct-PAW. These chemical species seem to be generated by secondary reactions due to the instability of nitrites at acidic pH (pH < 3.5). H2O2 was not detected in any PAW studied, consequently, ONOO- radicals would be generated due to the reaction between either OH* and NO2* (pH < 3.5) or NO* and O2- (pH < 6.5). Just after thawing (≈6°C) PAW (pH > 3.5) displayed a significant reduction of OH*, NO* and NO2* concentration, but ONOO- increased. It is believed that secondary reactions are affected by that pH (> 3.5), thus balancing the reaction towards ONOO- production.
The microbial loads of cuttlefish fillets stored with i-PAW made of recirculation-PAW and bubbling-PAW were significantly lower than those of the control (stored in ice prepared from distilled water) along all the storage time, leading to an increase in the microbiological shelf life of the cuttlefish of, at least, two days.
The results of this study prove that the application of i-PAW is a very promising alternative for extending the shelf life of cuttlefish and that bubbling would be the most interesting PAW generation process for this purpose.

P4.2 Rapid sourdough yeast identification using panfungal PCR combined with High Resolution Melting analysis

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The microbial composition of the sourdough starter affects the sourdough bread properties. Therefore, it is crucial to find a tool for rapid, time-saving, and economical identification of the sourdough microbiota. We focused on the rapid identification of sourdough yeasts. We designed a panfungal real time-PCR targeting the ITS2 region (ITS-amplicon) and a fragment of D1/D2 region of 26S rRNA gene (U-amplicon) and used high resolution melting analysis (HRM) for subsequent species identification. The sensitivity and specificity of our method were tested on the reference yeast cultures. We obtained divergent melting peaks (Tm). The further analysis of melt curves suggests the possibility to discriminate yeasts on the genus- and some on species-specific level in the mixed sample. The applicability of this method in routine practice was evaluated on nine sourdough samples. Revealed melt curves of U-amplicons were predominantly characteristic of the sourdough. The evaluation of the Tm and the shape of the melt curve was used to assess the sourdough yeasts. Additionally, using HRM-PCR method the contamination of the ergot fungus DNA was revealed. Our data showed HRM-PCR is a simple, rapid, and inexpensive tool useful in identifying sourdough yeasts.

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P4.3

Discrimination of milk from different animal species using FTIR features

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There is a trend for spectroscopic instruments to be miniaturized for integrating them across the food supply chain enabling continuous monitoring of quality, safety, and fraudulent practices. Feature selection limits the needed spectral span, enabling the instrumental simplification both in size and cost.
Herein, features were selected from Fourier Transform Infrared (FTIR) spectra to discriminate raw milk from different animal species.
Raw milk (i.e., from goat, sheep, and cow) was provided by 50 producers for a period of six months. The samples were transported in the laboratory for FTIR spectra acquisition. In total 73 samples were analyzed in triplicates (n=219). FTIR sensor range is 4000 to 400cm-1, but just the 3100 to 900cm-1 range was used, excluding the noisy signal at the edges of the spectrum and the water peak. Afterwards, stratified sampling (to combat with imbalance among the classes) was applied, so as 75-25% of the datasets to be used for development and external validation of the models. Feature selection was performed by exploring the distributions of mean values and standard deviations of goat, sheep, and cow milk. Those wavenumbers exhibiting a significant amount of deviation (> std) across the 3 classes of milk were considered as important while the rest were excluded. The models were (SVM classifiers) developed and externally validated using all features, for a base efficiency of the models, and then with the selected ones. Intuitively, non-overlapping wavelengths were considered. The most informative features found to be coherent in the range of 1750 to 1194cm-1, enabling the development of problem specific sensor development.
The external validation in the case of all features was 90.74%, while for the selected features the accuracy was 88.89%. Thus, while the accuracy scores are similar, the wavenumbers used in the latter case are the 25% of the total.
FTIR coupled with SVM showed potential for milk discrimination in the region 1750-1194cm-1 with similar results when all features were used. Further analysis is important to be applied using milks from other animal species (e.g., buffalo, donkey) and adulteration scenarios.
This work has been supported by the project "DiTECT" (No. 861915).

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P4.4 One-day methods for the identification of Salmonella enterica and Campylobacter spp. in poultry meat

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Salmonella and Campylobacter spp. are the two main foodborne pathogens contaminating poultry meat. They are leading cause of foodborne diseases in Europe, and provoke severe syndromes to infected people. To facilitate the quick identification of contaminated batches, we compared two protocols based on a short enrichment step followed by molecular analysis with either isothermal Loop Amplification Mediated PCR (LAMP) or Real-Time PCR. Two batches of chicken minced meat were contaminated with Salmonella enterica or Campylobacter jejuni and Campylobacter coli at three different concentrations (10^1, 10^3 and 10^5 c.f.u./g). The ability of LAMP and Real-Time PCR to detect the pathogens was tested at four enrichment times (0, 2, 4 and 6 hours post inoculation). Real-Time PCR was performed according to ISO 10272-2:2017 and Salmonella Iq-Check (Biorad, CA, USA), while LAMP was carried out using primers previously described in the literature and lavaLAMP kit (Lucigen, WI, USA) for one hour at 69°C and 67.5°C for Salmonella and Campylobacter spp., respectively. LAMP results were visualized with the QIAxcel (Qiagen, Hilden, Germany) system for capillary electrophoresis. Real-time PCR detected Salmonella at all contamination levels since T0, while Campylobacter was detected only from the 10^3 c.f.u./g contamination level since T0. Conversely, LAMP detected Salmonella and Campylobacter spp. only after 6 hours enrichment of the 10^1 c.f.u./g and 10^5 c.f.u./g initial contamination level, respectively. Our results show that when results are urgently needed direct PCR, possibly after a short enrichment, can be performed to obtain a preliminary diagnosis in one day. LAMP, which is generally considered to have higher sensitivity and to resist inhibition by components of complex food matrices, had worse performance than Real Time PCR at the conditions tested. However, for Salmonella enterica, LAMP, which is easily applied not requiring a thermal cycler, could be considered for field applications on-plant or at site of inspection.

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P4.5 Salmonella Typhimurium & Salmonella Enteritidis: a real-time PCR assay for detection from various food matrices

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Introduction
Detection of precise subtypes of pathogens is primordial to ensure public health safety. The whole Salmonella genus is considered as adulterous agent, however only a few serotypes are considered epidemiologically relevant. Among them Salmonella Typhimurium and Salmonella Enteritidis are known to be the most pathogenic and prevalent serotypes worldwide.
We developed a real-time PCR assay based on two single markers allowing the simultaneous detection of S. Typhimurium and S. Enteritidis from complex food matrices.
Method
Sensitivity, specificity and limit of detection were determined. Biological performances of the GENE-UP® SEST assay were evaluated on multiple matrices including poultry, eggs and pork in comparison to the NF EN ISO 6579-1 Salmonella spp. reference method (according to ISO 16140-2 procedure).
Results
Sensitivity was established at 100% for S. Enteritidis, and at 98.2% for S. Typhimurium. Specificity was established at 95%. Limits of detection of the S. Enteritidis and S. Typhimurium PCR was determined and are similar to the one of the Salmonella PCR assay.
Evaluation of the biological performances for the detection of S. Enteritidis and S. Typhimurium on poultry and pork matrices showed a good correlation between this alternative method and the ISO reference method.
Conclusion
The GENE-UP® SEST assay shows good performances allowing its use as a detection method directly on complex food matrices, or as a confirmation method of the GENE-UP® Salmonella 2 detection assay from enrichment and/or on isolated colonies.This work has been supported by the project “DiTECT” (No. 861915).

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P4.6 A novel biological method for detection of antibiotic residues in foods based on electrical impedance

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Antibiotics are regularly used in livestock to maintain an optimal animal health and productivity. Nevertheless, their abusive and/or incorrect use prompt the development of antimicrobial resistance and may cause toxicity and allergies to consumers when entering the human food chain. Consequently, livestock and derived food products are routinely subjected to exhaustive controls by authorities. Preferred methods for analysis of antimicrobial residues are binary screening tests (positive/negative) that use a sporulated microorganism sensitive to a wide range of compounds as a biological indicator, while chromatographic analyses are merely used to ratify the presence and concentration of the chemical. Most commercial screening tests involve the detection of pH or redox potential changes caused by the metabolic activity and growth of the microorganism when the food sample is free of antibiotics; however, composition of the food matrix might interfere with the reading method. This work aimed to evaluate a new screening method based on measuring impedance changes in the detection medium as a reporter of microbial activity. To this end, the impedance change induced by several sporulated strains of Geobacillus spp. and Bacillus spp. in different growth media was tested, where G. stearothermophilus ATCC 10149 showed the fastest and the largest shift. Kinetics of impedance change upon G. stearothermophilus ATCC 10149 growth allowed to settle a cut-off impedance value to test antimicrobial susceptibility. Our screening method was able to detect the presence of frequently used antibiotics, such as doxycycline, oxytetracycline, amoxicillin and sulfamethoxypyridazine with a detection limit of 20 ppb, 50 ppb, 10 ppb and 20 ppb, respectively. Hence, impedance-based tests are promising easy-handling, fast and reliable methods for screening the presence of antimicrobials in foods.

P4.7 Multispectral Imaging (MSI) coupled with machine learning algorithms for the microbiological quality assessment and discrimination of seaweed Alaria esculenta

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Alaria esculenta is a widely distributed edible seaweed in Europe. The expansion of seaweed aquaculture sector along with the rapid deterioration of these products enhance the importance of implementing rapid, real-time techniques for their quality assessment.
Seaweed samples (n=380) of different harvest year and geographical origin (Irish and Scottish) were stored under various temperature conditions for specific time intervals. Microbiological analysis was performed throughout storage, assessing the Total Viable Counts (TVC), while in parallel, MSI analysis was conducted. Machine learning models (Partial Least Square Regression (PLS-R)) were generated and validated to assess the correlation between MSI and microbiological data. The datasets were split into training and test set, while the slope of the regression line, root mean squared error (RMSE) and coefficient of determination (R2) were used as metrics for the evaluation of models' performance. Additionally, Linear Discriminant Analysis (LDA) was developed for the discrimination of samples based on geographical origin and harvest year.
Microbial counts ranged from 2.5 to 9.0 log CFU/g. The results of the PLS-R model for the A. esculenta from Scotland indicated a good prediction performance on the external test dataset, in spite of the rather high RMSE value (1.83). In A. esculenta from Ireland the results for the linear regression are a slope of 0.49, R-Square value of the fit 0.51 and the RMSE of the predictions 0.95, indicating a relatively good prediction performance. Moreover, 98% of samples were grouped correctly based on their origin, while the discrimination based on the different harvest year was also successful as 96% of the samples were grouped into the correct category.
Multispectral Imaging could be combined with conventional analytical techniques for the quality and authenticity assessment of seaweeds enabling industry to make fast, real-time and reliable decisions.
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P4.8 Classification of Listeria species using Near-Infrared Hyperspectral imaging

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Near infrared (NIR) hyperspectral imaging and multivariate analysis was evaluated for its potential use in classification of Listeria species. The analytical tool combines both spatial and spectral information for non-destructive food analysis. Although this technique was reported to be capable of differentiating between different bacterial genera, limited work has been done on distinguishing between species of the same genus. The aim of this study was to investigate the classification of three different Listeria species in the wavelength range of 950-2500 nm. Three Listeria species namely L. monocytogenes (ATCC 23074), L. innocua (ATCC 33090) and L. ivanovii (ATCC 19119) were grown for single colonies on Brain heart infusion agar for 22 hours at 37°C. After incubation, a pushbroom HySpex short wave imaging system was used to collect images of agar plates. Principal component analysis (PCA) was used to remove unwanted pixels of the petri dish, agar, and background pixel noise. Images were preprocessed with standard normal variate correction and the Savitzky-Golay smoothing technique (3rd order polynomial with 25 points). After all irrelevant pixels were removed, PCA was recalculated with five principal components. The PCA score plot showed slight separation between the three groups with L. monocytogenes and L. ivanovii grouping close together. There was overlapping of some bacterial pixels. The overlapping was attributed to the fact that all bacteria are Gram-positive and fall in the same sensu stricto clade. Based on the loadings, differences in bacteria were attributed to protein and carbohydrate composition in the bacterial cell wall. Partial least squares discriminant analysis was employed for classification. Classification accuracies for L. monocytogenes, L. innocua and L. ivanovii were 91%, 89% and 86% respectively. The F1 scores were used to assess the quality of the model. The model showed good performance for L. monocytogenes (0.90) and L. innocua (0.89) however, L. ivanovii had an F1 score of 0.28, which showed poor predictability. The results demonstrated that hyperspectral imaging has notable potential to classify bacteria within the Listeria genus. Nonetheless, more classification models and bacterial strains need to be investigated in further research.

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P4.9 Lacticaseibacillus rhamnosus as potential preservative agent in functional grape juices

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Today, there is mounting pressure on food manufacturers to either completely avoid the use of chemical preservatives or to adopt "natural" alternatives. Functional cultures and microbial derivatives seem to play a significant role in the prevention of food spoilage. In this vein, selected probiotic strains can be used for inhibition of spoilage and pathogenic species. However, dried cultures are preferable to wet cultures due to multiple technological advantages. Since the drying and food production processes, as well as storage are usually related to a significant reduction of cell viability, cell immobilization often acts as a protecting shield, securing high cell loads. Hence, the aim of the present study was to investigate possible resistance to microbial spoilage of grape juice containing freeze-dried free or immobilized Lacticaseibacillus rhamnosus OLXAL-1 cells on apple pieces after deliberate spiking with Saccharomyces cerevisiae or Aspergillus niger, usually responsible for juice spoilage. Initial levels of both free and immobilized L. rhamnosus OLXAL□1 cells in grape juice were ~ 7.5 logcfu/g. At room temperature, counts of free L. rhamnosus OLXAL□1 cells decreased significantly compared to the initial levels, while levels of immobilized L. rhamnosus OLXAL□1 cells in apple pieces remained significantly higher (> 7 logcfu/g) after 4 days of storage. Storage at refrigeration temperature resulted in L. rhamnosus cell loads > 7 logcfu/g for both free and immobilized cells up to 10 days and no spoilage was noticed. Deliberate contamination of juices fortified with freeze-dried immobilized cells with S. cerevisiae or A. niger cells resulted in significant growth inhibition both at room and refrigeration temperatures compared to the juice supplemented with free cells and the unfortified products. In conclusion, probiotic cultures may serve as biopreservative agents to prolong the self-life of juices and prevent spoilage. However, more research is still required to verify their effectiveness in industrial production.

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P4.10

An Information Technology-based platform for microbiological quality

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TCurrently, consumers demand constant reassurance of quality and safety of the food products they purchase. Therefore, food industries, retailers and authorities seek effective and relatively low-cost solutions for real-time quality and safety monitoring systems. In this context, various analytical approaches are exploring the utility of data science and machine learning strategies to streamline food validation tasks.

In recent years, online resources and data repositories have played a major role in advancing food-related research, by providing researchers with means for the dissemination of research, regardless of geographic dispersal. The Symbiosis Online Research Framework - SorfML (www.sorfml.com) was developed to provide the food research community with an interactive web application for experimental data management, analysis and visualisation/knowledge exchange. The application is currently used as a central data repository for various EU (e.g., PhasmaFOOD) and national (NovelEYE, QAPP) projects.

Our recently funded EU project DiTECT aims to develop a modular, online big data-enabled platform that will integrate the existing and future analytical/machine-learning functionalities of SorfML, to supplement existing food validation infrastructure at all applicable stages of production and distribution, summating data collected with non-destructive chemical and biological sensors, in addition to more conventional lab-based methods.

We have developed a series of online-ready regression models based on multiple sensor technologies, viz. Multi-spectral imaging (MSI) and fluorescence (MSIF) sensors, Fourier Transform Infrared (FTIR), Freshdetect, a portable fluorescence detection device, and a biomimetic sensor e-nose implementation. The developed models have achieved a high predictive accuracy for bacterial total viable counts. Additionally, the models' readiness via the SorfML platform ensures their application to predict freshness profiles for new food batches as soon as they are scanned and recorded using the DiTECT online platform.

This work has been supported by the Horizon2020-funded project "DiTECT" (GA No. 861915).

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P4.11 Screening of Lactococcus lactis starters with Absorbance Reduction Activity Method (ARAM)

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Lactococcus lactis is one of the most used species in the dairy industry. This species has the ability to reduce to very negative values the redox potential (Eh) of food matrices, making it one of the most reducing Lactic Acid Bacteria (LAB) [1]. This reducing activity influences oxidation-reduction reactions involved in the organoleptic quality of the product and prevents the undesired microorganisms developments [2–4]. The main mechanism involved are exofacial thiol groups, NoxE NADH Oxidase and the Electron Transport Chain mechanism [5-7].
For Eh measurement, the commonly used method consists of a redox probe placed in a liquid medium or food matrix [8]. With this probe, Cachon et al. (2002) proposed an innovative procedure allowing to compare reducing activity of LAB, according to their maximum reduction rate, based on their reduction kinetic vs. time. Despite its accuracy, this method can be complex, time-consuming and expensive for the screening of many strains.
Michelon et al. (2013), proposed an agar milk screening using colored oxidation-reduction indicators to quickly categorize, with a reducing power score, a large number of LAB strains. This method was used successfully for screening a large bank of LAB and mutants' strains. However, while this screening method showed significant interspecies differences, it was difficult to identify intraspecies ones [9].
In order to differentiate several strains belonging to the same species, we developed a new method combining Cachon et al. (2002) and Michelon et al. (2013) procedures with high throughput screening. This rapid method allows to compare until 30 L. lactis strains simultaneously and can be applied on frozen and freeze-dried mixed starters.

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P4.12 Database building to study the microbiology of plant-based milk

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Microbiology of plant-based milk is much less researched than that of bovine milk. A preliminary search on the microbiology literature by Google Scholar showed that significantly more studies (>23K) have been published on "Bovine milk" than on "Plant-based milk" (<500), the latter ones mostly focusing on fermentation and production processes; except for example Misiou et al. 2021, who analysed bacterial growth kinetics in plant-based alternatives. The link between the biochemical components of, and the bacterial kinetics on, plant-based milk is also way under-represented in the literature, though this question is at least as important as in the case of bovine milk.

Plant-based milk is becoming an important commodity for the health-conscious segment of consumers, in parallel with the emergence of personalized nutrition. A key factor in this development will be the utilization of the data that will be generated in the next decades. This poster demonstrates the preparation of an appropriate database ontology that will be suitable to combine nutritional and food microbiology data of plant-based milk ready to be analysed by predictive microbiology methods.

The question is not whether such databases should be set up but how to populate them in the most efficient way. It is vital to digitize observations and to store them in a systematic, programmable structure. The interpretation of relevant publications itself is rather challenging, and the most effort goes to converting published data into a compatible format.

We expect that nutritional data of plant-based milk, such as fat, carbohydrate, etc content will be shown to have quantifiable effects on the inactivation and re-growth of both pathogenic and spoilage organisms; most importantly on their maximum specific death / growth rate.

No doubt that a properly set-up database is vital to draw conclusions and even predictions, helping to make decisions. Such database can also be used to find gaps in the available data and fill them with new laboratory observations. Once the database reaches a critical mass, predictive modelling and computational means will be decisive for a scientifically proper exploration of the microbiology of plant-based milk. Our poster hopes to contribute to this aim.

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P4.13 Microbial quality, safety and diversity of brown algae Alaria esculenta deriving from two geographical areas

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The increasing interest for cultivation of marine algae for human consumption and the limited number of studies about their microbiological quality and safety enhance the need for relevant studies. The aim of this work was the assessment of the microbial diversity of A. esculenta throughout storage as well as the evaluation of their spoilage potential.

Fresh samples of A. esculenta were harvested from multi-trophic aquaculture stations from Scotland (2020 harvest) and Ireland (2020 and 2021 harvest) and stored at 5°C for certain time period. Microbiological analyses were performed for the enumeration of Total Viable Counts (TVC), Pseudomonas spp., Enterobacteriaceae, Bacillus spp. and fungi. The presence of food pathogens Salmonella, Listeria monocytogenes and Vibrio spp. was also investigated. Additionally, bacterial colonies from the general growth medium (Marine Agar) were isolated from various time points throughout storage and subjected to partial 16S rDNA sequencing, so as to characterize bacteria during spoilage.

The initial TVC of A. esculenta from Scotland was 3.2 log CFU/g, while microorganisms reached the level of 7.0 log CFU/g on day 4, while the specific spoilage microorganisms Bacillus spp. and Pseudomonas spp. were found at levels similar to TVC. The initial TVC of A. esculenta from Ireland for 2020 was also similar to this from Scotland, while products of 2021 were of enhanced microbiological quality and microbial counts did not exceed the level of 7.0 log CFU/g even after 7 days of storage. DNA sequencing results revealed the presence of Psychrobacter, Cobetia and Pseudomonas species in algae cultivated in Scotland, whereas Pseudoalteromonas and Psychrobacter species were found predominant during the spoilage of algae cultivated in Ireland in 2020. Psychrobacter and Bacillus species were identified during the spoilage of algae harvested in 2021.

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P4.14 Developing functional food ingredients using immobilized presumptive probiotic Pediococcus acidilactici cells

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In recent years, the development of novel functional foods, containing probiotic microorganisms has attracted the interest of both academics and professionals. Probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer health benefits on the host". In order to deliver the health benefits, functional foods shall contain adequate amounts of probiotic microorganisms at the time of consumption, a condition that still remains a challenge for the food industry. Cell immobilization may occur naturally by adherence to surfaces and offers technological advantages, such as maintaining cell viability during food processing and storage. Although the probiotic sector has been dominated by the Lactobacillus and Bifidobacterium species, there are some interesting inputs in the market and the literature by Pediococcus acidilactici strains. The aim of the present study was the production of functional and stable food ingredients containing immobilized cells of a wild-type Pediococcus acidilactici strain isolated from human feces that has been studied to evaluate its effect in reducing cholesterol levels. Hence, cell immobilization was tested on cereals (oat flakes or ground flaxseed), nuts (almond or peanut) and fruit pieces (apple or blonde raisin). Cell viability of wet, freeze-dried or thermally-dried immobilized cells stored at room temperature (18-20°C) and at 4°C was monitored and compared to free cells. Storage at both room temperature and at 4°C led to a significant decrease of cell viability in thermally-dried, as well as in-freeze-dried immobilized cells on fruits. However, cell populations of thermally- and freeze-dried immobilized cells on cereals and nuts remained > 9 and 8 logcfu/g, respectively, during storage for up to one year at 4°C, whereas the corresponding counts of thermally- and freeze-dried free cells were 8.08±0.05 and 8.33±0.05 logcfu/ml. At room temperature, cell levels ranged > 8 and 7 logcfu/g in thermally- and freeze-dried immobilized cells on cereals and nuts stored for 12 and 6 months, respectively, in contrast to thermally- and freeze-dried free cells viable counts, which were 5.12±0.12 and 4.06±0.18 logcfu/ml after 1 year of storage, verifying, thus, the positive effect of immobilization technology in maintenance of cell viability.

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P4.15 Partial Least Square Regression models development for the fish sea bass (Dicentrarchus labrax) quality prediction, using microbiological and multispectral data analysis

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The European sea bass (Dicentrarchus labrax) is a Mediterranean fish of high commercial interest in the European Union and the main country of production, Greece. The evaluation of fish quality and the prediction of fish shelf life are a constant target of producers and the consumers. Multispectral imaging in combination with data analysis methods are widely studied to explore new ways of determining food spoilage, with the aim of saving time. For this purpose, the microbiological alteration of farmed whole ungutted seabass and its fillets was evaluated by conventional microbiological methods, along with multispectral imaging analysis (MSI). Fish samples were stored under different packaging (aerobic and modified atmospheres-MAP) and temperature (isothermal 0, 4, 8 and 12 oC and variable in the range 2-15 oC) conditions. At regular intervals during storage, duplicate samples of 25 g of fish dorsal ham were microbiologically analysed to determine total viable counts (TVC), in parallel with MSI measurements taken from the skin side of the products using Videometer. The results transformed to log CFU/g and the multispectral data. The least squares partial regression (PLSR) was used to determine the correlation between spectral imaging data and microbial measurements, with the former being the input and the latter the output variables. The models were calibrated and validated with the data collected from the conditions under study. The values of the rout mean square error of the path for the calibration/ validation/prediction of the models were 0.92, 0.88, 0.67/ 1.48, 1.29, 0.83/1.58, 1.63, 1.21, for whole sea bass, sea bass fillets stored in air and under MAP, respectively. Based on the above results it is considered that MSI is a promising rapid and non-invasive technique for the quantitative monitoring of microbiological deterioration of fish and the subsequent assessment of their quality.

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P4.16 DiTECT – Tracking Biological Hazards & Contaminants Across Food Chain within a EU-CHINA project (no.861915)

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In the DiTECT four pilots have been designed i.e. PILOTS; P1- maize (corn and infant foods) P2- poultry; P3 – Cattle and P4 Fish (as food), where selected microbiological hazards were monitored across food chain, using rapid, non-invasive sensors for their detection and monitoring.

To achieve this a database is created, that concerns the entire food chain covering not only the primary production but also the remaining stages of the food chain.

The contents of this database are contamination maps (prevalence, biogeography and metagenomics) of biological and chemical hazards as well as environmental contaminants on selected foods (animal origin and infant foods) and routes at retail level.

A wide range of data was collected as follows (i) Data derived from instruments based on vibrational spectroscopy e.g., FTIR/NIR/ Fluorescence/ RAMAN as well as from UV/VIS (ii) Data from convectional microbiological analyses (iii) Product characteristics/processing, distribution and storage conditions (e.g., pH, aw, atmosphere etc.).

The successful implementation of the above – mentioned database build within DiTECT project is expected to significantly reduce microbiological hazards, through means of early detection before they make their way to the final product. Moreover, the collaboration between the EU-China food businesses and research partners will result in enhancing consumers' confidence in the safety of food traded between the two regions, throughout the farm-to-fork continuum.

DiTECT is a HORIZON 2020 EU/CHINA Project (Contract N. 861915)

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P4.17 Evaluation of the microbiological status and the self reported behaviour of the food handlers in Quintile 1 – Quintile 3 (Q1-Q3) schools of the Lejweleputswa area, South Africa

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Introduction
Microbiological food safety is one of the key factors in the prevention of food-borne diseases. The knowledge, attitude and practices of food handlers is important in any food establishment to ensure food safety, hygiene and the prevention of food borne illness from occurring. Majority of the food-borne illness results from the lack of knowledge of the food handler related to food microbiology, how to avoid cross contamination, proper hand wash and cleaning techniques including safe handling temperature of food. In South Africa food served within the National School Nutrition Program (NSNP) is prepared by Voluntary Food Handler (VFH) who have no formal training or educational background on proper sanitation, food safety and hygiene practices that may need to be employed in a food handling facility except from the indigenous knowledge that they have gained from their mothers through different generations.
Methodology
Surface swabs and an inspection checklist was used to assess the hygiene status of the food preparation area. The checklist was formulated from the regulations governing the general hygiene requirements for food premises, the transport of food and related matters (R638). However, not all aspects of these regulations were applicable because the NSNP setting is not similar to a formal trading facility and the fact that most of the food preparation areas were once classrooms turned into kitchens. In addition to the above a focus group was conducted to assess the knowledge, attitude, practices and self reported behavior of the VFH in the food preparation area, during preparation, handling and serving.
Results & Discussion
The results obtained from the focus group interview and microbiological samples led to qualitative and quantitative data. Indicating a strong correlation between the the knowledge,practice and hygiene status of the food preparation areas

Conclusion
Lack of infrastructure, resources, knowledge of food handler's and pre-requisites programs within the National School Nutrition programs are the major setbacks. However, despite these challenges and never being trained in food safety previously where possible the food handlers devise control measures that aid in reducing the cross contamination and ensuring that those who benefit are not compromised.

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P4.18 The use of qPCR for the rapid assessment of seafood freshness

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Microbial spoilage is the main cause of quality deterioration of fresh fish. As spoilage microorganisms grow, produce metabolites which deteriorates fish freshness. Freshness is assessed by microbiological, chemical and sensory analysis. It is important for the stakeholders, such as industry, consumers and authorities, the rapid freshness assessment of such perishable products. The aim of this work was the use of qPCR for rapid freshness assessment of fish. European sea bass fillets were provided by a leading aquaculture company of Greece and stored aerobically at various temperatures (0, 4 and 8oC). At various time intervals sensory attributes (odour and appearance), were evaluated, while samples were taken for classical microbiological analysis (TVC). At the same time molecular analysis using qPCR was conducted in a Q Thermocycler. More specifically DNA was extracted from fish samples and the V3-V4 region of 16S rRNA gene was amplified using gene-specific primers. Afterwards, the Ct values from qPCR were fitted versus log cfu/g and sensory score. The results showed a very good correlation of both Ct-log TVC and Ct-sensory score, allowing a rapid (less than 4 hours) estimation of freshness status.

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P4.19 Using organic LED in intelligent food packaging for quality monitoring of meat products (OLED_Lumin_FoodPack)

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The technological and IT explosion has a significant impact on all active domains in the production and distribution of consumer goods. The aim of the project is the development of intelligent packaging that provides continuous and on-line information to manufacturers and consumers regarding food quality and package integrity through the entire supply chain. To achieve this, organic photonics based on the recent technological breakthroughs in the domains of printed electronics, carbon nanotechnology, silicon photonics, and biotechnology (sensors), which offer the prospect of developing a new generation of optical sensors, or redesigning conventional sensors, will be applied. The main goal is to monitor the changes in concentration of volatile metabolites associated with food quality loss, through molecular probes of optical output. The latter will be achieved with the predominant use of fluorescent- or color changing probes and electroluminescent devices (OLEDs), for color-coding reading. The individual objectives are: 1) Selection of target analytes and calibration of sensing elements, 2) Incorporation of sensors in packaging material, 3) Development of the transducer and 4) In situ application of developed sensors. The effectiveness in developing a multi-sensor that can give more information about complex spoilage changes than sensors or indicators based on targeting a single quality indicator will be evaluated. The novelty of this work relies on the predominant use of OLEDs for colour/fluorescent-coded reading, instead of absorbance spectrum changes for read-out as is the case with almost all proposed devices so far. New intelligent packaging applications providing continuous information on the food condition or packaging integrity is not only beneficial for the customer, but also enables the detection of calamities and possible abuse through the entire supply chain. This undoubtedly results in a safer and more efficient supply chain, reducing food loss and waste and timely preventing unnecessary transport and logistics. Collectively, the food industry can be benefitted via improved logistics, warehouse management systems, and transportation management systems to distant places around the world. Acknowledgments: This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship, and Innovation, under the call RESEARCH-CREATE-INNOVATE (project code: Τ2ΕΔΚ-04175).

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P4.20 Helicobacter pylori detection in organic fresh vegetables by real time PCR

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Helicobacter pylori is considered the primary etiological agent of peptic ulceration and chronic gastritis and has been related with gastric cancer. It has been suggested that H. pylori could reach humans through contaminated raw vegetables. Over the last decades, the consumption of organic fresh fruits and vegetables has increased considerably. The knowledge of H. pylori contamination levels in these foods is, thus, essential in order to better asses potential human health risks. Therefore, the aim of this study was to determine the presence of H. pylori in organic fresh vegetables which are raw-consumed.

For this purpose, a total of 60 organic vegetables samples (30 lettuces and 30 spinach) were purchased from 10 different ecological markets and greengroceries in Valencia (Spain) between November of 2020 and February of 2022. The samples (25g) were individually homogenized with 225mL of Wilkins-Chalgren Broth supplemented with Horse Serum (10%) and Dent Selective Supplement. One ml aliquots were analyzed by real-time PCR using specific VacA gene primers, before and after 48h of selective enrichment under microaerobic conditions at 37°C.

Twenty-two (36%) out of the 60 samples analyzed, 12 lettuces (40%) and 10 spinach samples (33.3%), were positive for H. pylori, with amplicons showing the expected melting curve. H. pylori was detected in 15 out of the 60 samples before the enrichment step, while seven samples yielded positive results only after 48 h of enrichment. The massive growth of other competitive organisms might explain the loss of detection in some samples after enrichment. According to our results, H. pylori is highly prevalent in organic fresh vegetables and, as they are generally consumed minimally processed, it could be a source of transmission for H. pylori to humans.

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P4.21 Helicobacter suis detection in pork food products

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Helicobacter suis is the most prevalent non-Helicobacter pylori (NHPH) species detected in stomach of humans. This organism is considered a zoonotic pathogen, causing of gastric diseases, such as peptic ulcer, gastritis and mucosa-associated lymphoid tissue (MALT) lymphoma. Pigs are the natural host of H. suis, and this bacterium has been reported in different farm pigs around the world. It has been suggested that consumption or manipulation of contaminated pork meat might be the route of transmission of H. suis bacteria to humans, although the risk of exposure is not clear. Thus, the aim to this study was to determine the presence of H. suis among pork products for human consumption in Valencia (Spain).

Twenty-five pork carcasses were collected from different local butchers in Valencia (Spain). Twenty-five grams of each sample were homogenized with 40mL of Brucella Broth, supplemented with 10ml of Fetal Bovine Serum, and enriched at 37°C for 48h under microaerophilic conditions. Ten milliliters aliquots of the broths were taken, before and after enrichment. The broths were concentrated and analyzed by specific multiplex PCR, using HH-F5/HH-R4 for the non-coding region, and carR2F/carR2R for the carR gene of H. suis. All amplicons of PCR positive samples were sequenced to confirm the H. suis presence.

Both, the 348-bp (HH primers) and the 220-bp (carR2 primers) specific bands of H. suis were detected by PCR in 8 out of the 25 samples studied (32%). Detection rates were higher before enrichment (7/8) and only one of the positive samples remained positive after the enrichment period. H. suis has fastidious growth requirements and the massive growth of other organisms might explain the low detection levels after enrichment. According to our results, H. suis is relatively common in pork products in our geographical area and the direct multiplex PCR could be a specific and sensitive method to detect this pathogen from pork meat.

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P4.22 Evaluation of disinfection methods: A case study on Greek Kalamon table olives

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Food products are required to address certain quality requirements. The main aspect of these quality criteria is to ensure consumers safety. In terms of food safety microbiological safety is considered to be crucial. Microbes such as pathogenic bacteria and fungi can contaminate food throughout the production chain. Therefore, a variety of disinfection approaches has been developed, which target to minimize the presence of pathogens in the final food product. These methods can either be physical, chemical or biological and their application on different pathogens and food matrices is constantly being explored. Among physical methods commonly used are exposure to ultraviolet radiation and ultrasounds, while chemical methods include treatment with ethanol solutions. Interestingly, their use on table olives has scarcely been examined. Table olives are a product of great dietary and economic importance throughout the Mediterranean countries. Therefore, it would be of great significance to study disinfection methods on a popular and high nutritional value Greek food product. The aim of the present study was to evaluate the disinfection potential of UV, ultrasound or ethanol treatments against foodborne pathogens artificially contaminated on Greek Kalamon table olives. Table olives were spot inoculated with a suspension containing either Candida albicans, Aspergillus brasiliensis or Listeria innocua, a common surrogate for the pathogen Listeria monocytogenes, and left to dry. Afterwards UV, ultrasound, or ethanol treatment were applied and the microbial load was calculated using traditional culture dependent techniques. UV and ethanol resulted in reduction of the microbes' concentration although ethanol was the most effective. Ultrasounds slightly reduced A. brasiliensis, however had no effect on C. albicans. In conclusion, the results of this study highlight the usefulness of implementation of these techniques for the disinfection of food products in food industry. However, further research should be conducted to find the optimum parameters for each treatment among different food types, as well as their impact on the physicochemical and sensory attributes of food products.

Food Safety in 2050 – Is it too early?

P5.1 Acrylamide mitigation by probiotics: Formation, properties, detection methods and reduction approaches

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Acrylamide is a chemical compound that forms in starchy food with the availability of asparagine as an amino acid when food is heated at a temperature above 120°C. Potato chips, bread, biscuits, coffee, and similar products contain higher levels of acrylamide in comparison with other foods. Acrylamide is recognized as a potentially cancer-causing compound by the International Agency for Research on Cancer. Acrylamide might cause endometrial cancer and kidney cancer.
The European Food Safety Authority and the World Health Organization published different guidelines to educate industries about mitigation strategies and kept screening acrylamide in food over the years. Many approaches have been conducted to reduced acrylamide in food, which targeted reducing asparagine, reduced sugar, playing a role in the formation reaction, or having a role in changing some of the surrounding factors such as temperature or pH. Most of the methods have an impact on food quality and sensory characteristics. Biological reduction methods of the other hand showed positive results in reducing acrylamide and in cases improved food quality. Probiotics appeared to be an ambitious alternative. Literature has shown gaps in studying acrylamide reduction using probiotics and further effort is needed to understand the fullest potential of those types of bacteria and their mechanism of removal. Therefore, the aim of this paper is to provide a comprehensive revision of the reduction methods of acrylamide in food products focusing on the biological approaches using probiotics.

P5.2 Microbiological Quality and Safety Assessment of Ostrich and Chicken Meat Patties Treated with Gamma Radiation and Kale Leaf Powder

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The current study was designed to determine the effects of gamma irradiation and kale leaf powder (KLP) on ostrich and chicken meat on microbial quality and safety at different storage intervals. Gamma irradiation is processing technique that is used to produce safe and quality meat products by eliminating the pathogenic microbes. However, some non-thermal processing technologies are being used to produce safe and quality meat in which gamma-irradiation are used. Gamma irradiation (cesium 137 or cobalt 60) are enhanced the meat product shelf life by inhibiting the growth of bacteria. These rays are eliminated the pathogenic bacteria from meat products and aid in improving human health. The different parameters including TVBN, TBARS, POV, antioxidant measurement (DPPH, ABTS, FRAP and TPC) and microbial analysis (TBC and coliform) of ostrich and chicken meat patties were performed to observe the changes on different treatments at storage intervals. Gamma irradiation (3 kGy) with or without kale leaf powder (1 % and 2 %) was applied. The outcomes showed that kale leaf powder, irradiation and storage intervals were changed significantly in the overall samples. The variations in TBVN, TBARS and POV values were observed significantly in ostrich and chicken meat during different treatments and storage periods. Outcome of our study shows that kale leaf powder and gamma irradiation reduced the TBC and coliform load in ostrich and chicken meat. However, it was concluded that the significance of irradiation (3 kGy) and kale leaf powder is suitable and helpful for microbial quality, safety, and stability of ostrich and chicken meat patties.

Food Safety in 2050 – Is it too early?

P5.3 Identifying viable Listeria monocytogenes cells after exposure to sodium hypochlorite using PMA-qPCR and Fluorescence Microscopy

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Exposure of Listeria monocytogenes to sublethal stresses related with food processing environments may induce different physiological states, i.e. sublethal injury, persistence, viable but non culturable (VBNC) state, with varying resuscitation capacity. Bacterial heterogeneity, injury and VBNC state-limitations may lead to undetected hazards, causing foodborne outbreaks or be the reason for insufficient evidence to enable source attribution.
The objectives of the present study were to investigate potential VBNC induction during exposure to sodium hypochlorite, to outline the proportion of metabolically active, sublethally injured, VBNC and dead cells using fluorescence microscopy coupled with CFDA/PI staining, to identify size colony variations (SCVs) associated with persistence and to compare the results regarding cell's viability from PMA-qPCR and fluorescence microscopy.
Sodium hypochlorite (SH) 200 and 400 ppm at 20oC for 3 h were used to investigate viability of L. monocytogenes, Scott A. Phenotype heterogeneity was evaluated through visual inspection after treatment with SH following 2 days of incubation at 37oC. To differentiate sub-lethally injured cells from the total population, Tryptic Soy Agar with 0.6% Yeast Extract (TSAYE) supplemented or not with 5% NaCl was comparatively used. To define sublethally injured (CFDA+/PI+) and VBNC (CFDA+/PI-) cells, fluorescence microscopy coupled with CFDA (viable) and PI (dead) staining was used. Viability was also assessed with Propidium Monoazide-qPCR.
Treatment with SH 200ppm for 3 h at 20oC resulted in 3 log reduction of the total population. At the same conditions, sublethal injury was detected after 2 and 3 h of exposure, using plate counting. Fluorescence microscopy did not suggest sublethal injury (double stained sub-populations). Exposure to 200ppm SH at 20oC, resulted in two sub-populations on TSAYE, one with the phenotype of untreated cells and one resembling SCVs. A significant percentage of viable cells was detected after exposure to SH 200ppm, using PMA-qPCR. After incubation in 400ppm SH at 20oC for 3 hours, the whole population was found non-culturable. However, cells appeared as CFDA+, indicating viability, collectively suggesting the induction of VBNC state.
Our results may assist in the reliable detection of the VBNC state during food quality control, preventing false-negative results that may lead to public health threats. This work has been supported by a PID2019-105691RB-I00 Research Project from Ministerio de Ciencia e Innovación, Spain.

P5.4 Deeper insight into stochastic microbial inactivation

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Traditional deterministic inactivation models do not consider the heterogeneity in the resistance of individual cells to a lethal stress. Lately, single cell individuality has attracted the scientific interest and several approaches have highlighted its importance leading to stochastic inactivation models. In a previous work of ours a statistical modeling approach was applied based on probability distributions for the description of individual cell time to death and the evaluation of population inactivation dynamics using simulation techniques. The resulting inactivation curves (population evolution over time) are characterized by an increasing variability with time in the number of survivors at a specific time point and the time required for a given decrease of the population. Do the number of survivors or the time required for a certain decrease follow a certain distribution? How the distribution of individual cell time of death is affecting the variability on both axis? The objective of the present study is to provide a deeper insight into the above questions in order to enhance the understanding of microbial inactivation curves and the applicability of stochastic inactivation models.

Food Safety in 2050 – Is it too early?

P5.5 Clean label alternatives to the use of nitrite in cooked ham – results of a pilot study

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Environmental and health concerns over the use and consumption of artificial additives like nitrite have prompted consumer wishes and desire for "cleaner" products containing natural ingredients whose names they know and understand what they are used for. In this context, the clean label movement is trending with the goal of offering natural products while having in sight their safety. However, this is quite challenging for the food industry. Consumers demand cleaner food labels, but they will also not allow concessions in terms of quality, shelf life and safety. The aim of this work was to assess if natural sources of nitrate in combination with nitrate-reducing starter cultures would be good substitutes of chemical nitrite in cooked ham, ensuring its microbiological safety without compromising its organoleptic characteristics. Four cooked hams, combining rich nitrate vegetable sources with two different nitrate-reducing commercial starter cultures, were manufactured at pilot plant scale by Primor. A cooked ham produced using chemical nitrite (150 ppm) was used as control. Microbiological, and physical-chemical characteristics testing were performed every week after the products were sliced. In parallel a challenge test was carried out using a cocktail of different strains of Listeria monocytogenes. After inoculation, products were stored at the recommended storage temperature (4 °C) and at temperature abuse conditions (10 °C).

Preliminary results show that the test hams and the control have a similar microbiological profile, except for Enterobacteriaceae counts (only observed for test hams). Regarding texture and colour analysis, similar results were obtained for both control and test samples throughout the 28 days of storage. Water activity and pH values remained constant and similar between control and test samples during the test period.

When inoculated in the products, L. monocytogenes reached similar values in both control and the test hams. A 2 log difference was observed between the two storage temperatures, being that at 10 °C bacterial counts were higher.

These preliminary results demonstrate the potential of using natural sources of nitrates combined with nitrated-reducing starters as a clean label alternative to the use of nitrite in cooked ham, maintaining microbiological safety.

Food Safety in 2050 – Is it too early?

P5.6

Molecular characterization of Shiga toxin-producing Escherichia albertii strains exhibiting high vero-cell cytotoxicity

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Escherichia albertii, an emerging human pathogen, has been historically misidentified as Escherichia coli or other Enterobacteria due to their similar biochemical properties. Molecular characterization is thus needed for improved detection and to further understand the virulence traits of this pathogen.

This study examined genomes and pathogenicity potential of Shiga toxin-producing E. albertii (STEA). A total of 10 environmental STEA strains were isolated from wild birds in a leafy greens-growing region in California. The genomes were sequenced using PacBio RSII and compared with the genomes of 11 clinical STEA strains available in GenBank as January 2020. Production of Stx was quantified by ELISA. The Shiga toxin (Stx) activity was examined using a fluorescent Vero cell-based assay, and the cytotoxicity differences were determined using One-Way ANOVA followed by Dunnet's test. Both Shiga toxin-producing E. Coli (STEC) and stx-negative E. Coli strains were used as controls.

All E. albertii strains carried eae, encoding intimin, and tir, encoding the receptor protein for translocated intimin, which both were located on a LEE Island ranging from 37,597 bp to 52,799 bp. All environmental strains possessed genes encoding type II cytolethal distending toxin, a subtype prevalent in clinical strains. All environmental strains carried stx2f; whereas only two clinical strains were stx2f-positive. A large variation in Stx production was observed. Among the 10 environmental STEA strains, six strains exhibited higher cytotoxicity than that of stx2f-carrying STEC strains (Dunnet's test, $P<0.05$). Among these six strains, two strains exhibited even higher cytotoxicity than that of enteroheorrhagic E. Coli (two O157:H7 outbreak strains carrying stx2a+stx1a or stx2a and one O145:H28 outbreak strain carrying stx2a) (Dunnet's test, $P=0.05$). Higher cytotoxicity appeared to correlate with a higher Stx2f level. Although the MLST-based phylogenetic analyses failed to discriminate environmental strains from clinical ones, a linage containing the two clinical strains and the two environmental strains that exhibited the highest cytotoxicity was detected. All strains within this lineage possessed a gamma intimin. The data suggest that hypervirulent STEA strains are present in wild birds, which may serve as a pathogen reservoir in a leafy greens-preharvest environment.

Food Safety in 2050 – Is it too early?

P5.7

Effect of Chlorella vulgaris supplementation of pigs during the fattening period on the microbiological profile of Longissimus lumborum

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The growth in human population and the increase in living standards is pushing up the demand for high-quality animal products. When comparing to terrestrial crops, microalgae constitutes a more sustainable feed resource with high nutritive and functional values. Microalgae supplementation of pigs during growth and finishing period has been reported to improve fatty acid profile of meat. However, to fully take advantage from microalgae, knowledge of effects on microbiological profile of meat is of paramount importance. The present study aimed to evaluate the effect of dietary inclusion of 1% Chlorella vulgaris in the diet of pigs from 14-15 weeks of age until slaughter (25-26 weeks of age) on the microbial profileof Longissimus lumborum meat. The trial was carried out in a commercial pig farm and after slaughter a sample of Longissimus lumborum was collected from a total of 120 pigs (50% males and 50% females) fed the control diet (without C. vulgaris inclusion; 60 animals) and the experimental diet (1% C. vulgaris inclusion; 60 animals). Microbiological analysis was carried out on pork meat according to ISO standards: enumeration of total viable counts, Enterobacteriaceae, Escherichia coli, Coagulase-positive staphylococci (CPS), Yersinia enterocolitica, Campylobacter spp., and detection of Listeria monocytogenes and Salmonella spp. Total viable counts, Enterobacteriaceae, E. Coli, CPS and Y. enterocolitica were present in numbers less or equal to 10^4 CFU/g. Salmonella spp. and L. monocytogenes were detected in four and thirteen samples, respectively. No significant differences ($p > 0.05$) were observed between the feeding conditions applied to the pigs.
Although microalgae have been shown to exert antimicrobial activity against several microorganisms present in meat, the supplementation level tested in the present study kept unaffected the microbiological composition of the pork meat.
This work was supported by project ALGAVALOR - Microalgas: Integrated Production and Valuation of Biomass and Miscellaneous Applications (POCI-01-0247-FEDER-035234; LISBOA-01-0247-FEDER-035234; ALG-01-0247-FEDER-035234) funded by Lisboa 2020 ; ALGARVE 2020 ; COMPETE 2020 ; Portugal 2020 ; FEDER - Fundo Europeu de Desenvolvimento Regional ; AICEP - Agência para o Investimento e Comércio Externo de Portugal, E.P.E.

Food Safety in 2050 – Is it too early?

P5.8 Detection of HAV & NoV in food products in Greece

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HAV and NoV are two of the most prevalent food-borne viruses and are responsible for multiple acute gastroenteritis outbreaks and can cause severe disease. HAV can cause acute liver failure and is deadly in many cases, while NoV may result in death, especially in elderly people and immunocompromised individuals. So, their presence in fresh and preserved produce must be surveyed and monitored with the use of universal protocols, in order to protect public health. In recent years, there has been a serious development of innovative tools and methods for the detection of food-borne viruses and many of these methods have been implemented in the testing of produce. The prevalence of these two viruses in fresh and preserved produce as well as the standardized methods used for their detection are the main subject of this study. The whole process from the sampling of the food sample to the detection of the viral RNA with the method of RT-qPCR analysis will be described, which is done according to ISO standards, as well as a statistical analysis of the results of the last few years of the presence of HAV and NoV GI & GII in produce. Although the molecular methods used have high sensitivity the percentage of positive samples for both viruses were found to be quite low. Although, the percentage of the contaminated samples was low the threat to public health and the risk of outbreaks still remains so there should not be any negligence in the control of fresh and preserved produce. In addition, not all countries use the same control methods or have the same standards for produce testing for HAV and NoV and, in addition, all testing methods demand a highly trained analyst. This leads to the need for universal and globally accepted and executed methods for food-borne virus testing of fresh and preserved produce. Finally, the method used in this study shows high potential for implementation in the food industry for the rapid detection of food-borne pathogens in general.

P5.9 Food Risk Analysis: Towards a Better Understanding of "Hazard" and "Risk"

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Understanding the difference between "hazard" and "risk" is important for risk communication. Clear definitions of these terms can be found in the Codex Alimentarius, but also in the General Food Law Regulation of the European Union (EU). Hazard refers to any physical, chemical, or biological agent or condition with the potential to cause an adverse health effect. Risk means a function of the probability of occurrence of an adverse effect from exposure to a hazard. The use of these terms as synonyms or their interchange is a problem that has not yet been overcome, despite awareness-raising by food organisations.

Using a newly developed software tool, we were able to detect this issue in food regulations written in all official EU languages except English. To validate our findings, we asked 29 native-speaking experts working in the field of food safety for their opinion. All responses were coded and processed through cluster analysis. The results indicate that the two terms are interchanged at different levels in almost all languages. The substitution of "hazard" for "risk" was most frequently observed in Romance languages, but also in Dutch, Danish, Slovene, Czech and Lithuanian. The use of the word "hazard" instead of "risk" was more frequent in Slavic languages such as Slovene, Croatian, and Polish, but also in Lithuanian, Estonian and Hungarian. These observations were also confirmed by the native speaker experts. However, there were a few cases where the experts considered the use of the two words as synonyms acceptable for reasons of linguistic freedom. Furthermore, the results showed that corrections are only made randomly when comparing an old version with a new version of the same regulation, with some regulations having some corrections while others have almost none.

To conclude, food legislation translated from English into other official EU languages reveals constant interchanging of "hazard" and "risk". For stakeholders to better understand these terms and use them correctly in their work and oral communication, we recommend correcting the food regulations and ensuring the correctness of future documents

Food Safety in 2050 – Is it too early?

P5.10 Novel and emerging foodborne bacteria identified in commercial South African Ready-To-Eat (RTE) leafy greens during a study for the prevalence of Listeria monocytogenes and Salmonella species

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With increasing focus on healthy living, consumption of leafy greens is on the rise. It is consumed as ready-to-eat, washed, and bagged due to current fast paced lifestyles. Fresh produce is known to have a large microbial load owing to farming, environmental, and processing conditions. However, plastic bags and pre-washed labels instilL a false sense of security. As the concentration of chlorine during washing affects the organoleptic properties of leafy greens, it is generally kept low. This may lead to sub-lethal chlorine exposure consequently enabling survival and resistance development. Current industry standards cannot be sufficient, as leafy greens are progressively linked to disease outbreaks by pathogens such as Listeria monocytogenes, Salmonella and Escherichia coli in developed countries. Developing countries like South Africa face a compounded risk due to farming practices and water scarcity leading to contaminated irrigation water. Furthermore, intensified production outputs prevent successful tracing and eradication of contamination sources. This study screened 60 leafy green samples from three retailers in the Western Cape for Listeria species (ISO 11290-1:2017), Salmonella species (ISO 6579-1:2017), E. Coli (3M petrifilms) and total viable counts (TVC) (3M petrifilms). The sample set consisted of 30 bagged, and 30 unbagged or partially bagged samples. Presumptive colonies were confirmed using the VITEK® 2 compact automated platform. Confirmed isolates were tested for antimicrobial resistance (CLSI M100). Of the 60, six (10%) tested positive for the presence of Salmonella spp. Forty-six samples (77%) tested positive for Listeria spp. encompassing 50% bagged samples, and 50% whole unbagged and partially bagged samples. Of these, three (6,5%) tested positive for L. monocytogenes. Additionally, a novel Listeria spp., L. rocourtaie, and bacteria with clinical significance, Globicatella sanguinis and Kocuria kristinae were isolated. This study is the first of its kind in South Africa, resulting in a higher prevalence than that of similar studies conducted in other countries for Listeria spp. It emphasises the need for better surveillance and drastic changes in the South African fresh leafy green sector.

Food Safety in 2050 – Is it too early?

P5.11 Potential of bacteriocinogenic cultures on the biopreservation of soils

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The agricultural practices influence soil microbiota and contribute to the presence of foodborne pathogens on soils. Therefore, soil is a potential source of vegetable contamination, which can lead to foodborne diseases, especially due to the consumption of ready-to-eat products. With the purpose of reducing these risks, the use of lactic acid bacteria (LAB) as biocontrol agents emerges as a potential alternative to the use of other practices with negative environmental impact.

The main purpose of this work was to evaluate the survival of two bacteriocin-producers LAB in two different organic soil amendments.

Pediococcus acidilactici HA-6111-2 and Enterococcus faecium 10A10 were separately added to both soil amendments (identified as sample 1 and sample 3) in levels of 10^9 Colony Forming Units (UFC)/g. Enumeration of LAB was performed in MRS immediately after inoculation (day zero) and along 37 days of storage at room temperature. Uninoculated soil amendments were used as controls.

No significant differences were observed in the numbers of P. acidilactici and E. faecium in sample 1 at day zero and day 37 ($p>0.05$). However, significant reductions of about 2 and 4 log cycles occurred in sample 3 for P. acidilactici and E. faecium, respectively. No LAB were found in control samples.

Despite being preliminary, these results point to the survival of bacteriocinogenic LAB in two different soil amendments. Although their composition seems to have an effect on this survival, the high numbers found after one month indicate the potential of these bacteria to control foodborne pathogens in contaminated soils.

This work was developed in the scope of the project HSoil4Food - Healthy soils for healthy foods (NORTE-01-0145-FEDER-000066) co-financed by the European Regional Development Fund (FEDER) through the Northern Regional Operational Program.

Food Safety in 2050 – Is it too early?

P5.12

Assessment of the Probiotic and Technological Properties of Bacillus spp. isolated from Burkinabe Soumbala

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Soumbala is an alkaline traditional fermented food condiment very prized in Burkina Faso. It harbors a various microbiota dominated by fermentative Bacillus spp. as functional microorganism with less confirmed health promoting properties. The present study aimed to evaluate six (06) Bacillus strains previously isolated and identified from soumbala and selected as presumptively safe bacteria for probiotic and technological characteristics. Thus, their strains were assessed for in vitro probiotic criteria (tolerance to low pH (2 and 4), gastric juice, 3% (m/v) bile salt, intestinal juice and 0.3% (v/v) phenol, cell surface hydrophobicity and auto-aggregation capacity, antimicrobial activity against 19 food-borne pathogens, antibiotic susceptibility, biofilm production and technological properties, including protease, amylase, lipase and tannase activities, poly-y-glutamic acid production and thermo-tolerance.
All the tested Bacillus strains presented variably relevant probiotic properties (good tolerance to acid condition (pH 2 and pH 4), gastric juice, bile salt, and intestinal juice and phenol), with marked difference in hydrophobicity and auto-aggregation capacity ranged from 73.62 - 94.71 % and 49.35 - 92.30 %, respectively. They exhibited a broad spectrum of activity against food-borne pathogens depending on indicator pathogen, with the highest activity exhibited by strain F20 (29.52 mm) against B. cereus 39 ($p < 0.001$). They were found to be sensitive to the majority of antibiotic used as medicine and presented good production of biofilm. They also exerted variable enzymatic activities for protease (43.00 - 60.67 mm), amylase (22.59 - 49.55 mm), lipase (20.02 - 24.57 mm), and tannase (0 - 10.67 mm). All the tested Bacillus strains tolerated temperature up to 50 °C, while only strains F26 and F44 showed the best PGA production. Overall, the tested cultures exhibiting potential probiotic and technological characteristics, particularly B. subtilis F20, B. subtilis F21, B. subtilis F26, and B. subtilis F44 could serve as relevant probiotic-starter cultures of commercial interest in the production of high-quality soumbala.

Food Safety in 2050 – Is it too early?

P5.13 Studying the production of metabolic factors that may influence the growth of Listeria monocytogenes during co-culture of different strains of the pathogen

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The simultaneous presence of more than one strains of Listeria monocytogenes in/on the same food product may affect their growth capacity. The present study evaluated the production of metabolites that may influence the growth of the pathogen during co-culture of different strains.
L. monocytogenes strains C5(4b) and 6179(1/2a) were selected as resistant to different antibiotics (enabling selective enumeration) and inoculated (2.0-3.0 logCFU/mL) in Tryptic Soy Broth with 0.6% Yeast Extract (TSB-YE) in single and two-strain cultures (1:1 strain ratio). Bacterial growth was assessed during storage at 7□C, under aerobic conditions(AC). After reaching stationary phase, single and dual cultures were centrifuged and filtered, and the spent medium(SM) was either characterized by Fourier transform infrared (FT-IR) spectrometry or re-inoculated, after the addition of concentrated TSB-YE (for nutrient replenishment), with single and two-strain cultures for the evaluation of growth under the influence of metabolites produced from the same singly and co-cultured strains in all the different combinations of strains and SM origin (7□C/AC)(n=2x3).
By the end of storage singly-cultured C5 and 6179 had reached 9.2±0.2 logCFU/mL, while in dual cultures, 6179 was affected by the presence of C5, reaching 6.7±1.1 logCFU/mL. FT-IR spectra of SM produced by singly-cultured 6179 and the co-culture were almost identical. Characteristic peaks in FT-IR spectra of SM of singly-cultured C5 at 1219, 1457, 1647 and 1741cm-1 represent functional groups, which were not present in the SM of co-culture. These molecules may be located inside or mounted on bacterial cell surface and removed from the supernatant during cell filtration. Both singly and co-cultured 6179 managed to grow similarly at every SM. Contrarily, both singly and co-cultured C5 managed to outgrow 6179 in SM, which contained its metabolites, while in SM produced by singly-cultured 6179 did not grow, suggesting that SM of 6179 contained metabolites, which may influence its growth and in parallel C5 may produce molecules in the presence of which, it counteract the inhibitory effect of 6179.
The findings shed more light on the mechanism behind the inter-strain interactions indicating that both contact of cells and produced metabolites may influence the behavior of the different co-existing strains.
05/2016

Food Safety in 2050 – Is it too early?

P5.14 Molecular characterization of lactic acid bacteria isolated from water buffalo's milk functioning as reservoirs of mobile antibiotic resistance genes

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Lactobacilli are common microorganisms in food and are also highly represented within the intestinal microbiota of humans and of most animals.They can also survive food processing and persist in finished products, constitute a large portion of the natural microflora in many fermented carabao's milk products.
In carabao farming, antibiotics are routinely used to treat carabao and also incorporated veterinary medicine and animal husbandry (Tenover and Hughes, 1996). Lactic acid bacteria (LAB) have a long history of safe use as food-processing aids, and as probiotics, they are associated with health benefits (Pham et al., 2008). However, when present in the food chain and in the intestinal tract of animals and humans, these bacteria may function as reservoirs of mobile antibiotic resistance genes that can be transferred to pathogenic bacteria, such as Listeria monocytogenes (Salyers et al., 2004). This could complicate the treatment of a patient with an antibiotic resistant bacterial into feed meals as prophylactic agents. This has lead to emergence of antibiotic-resistant bacteria due to the excessive and inappropriate use of antibiotics in infection or disease.
L. monocytogenes is widely distributed in the environment and can be isolated from soil, water, sewage, both domestic and wild animals (Budzinsha et al., 2012). L. monocytogenes is also associated with dairy products, soft cheese and non-pasteurized milk are mentioned as carrier of the fatal listeriosis (Farber and Peterkin, 1991). L. monocytogenes is the main cause of listeriosis and can cause severe disease in neonates, elderly people, pregnant woman, and immune-compromised persons (Freitag et al., 2009). L. monocytogenes is able to migrate to the intestinal, blood-brain, and fetoplacental barriers (Jacquet et al., 2004). L. monocytogenes causes gastroenteritis, meningitis, septicemia, meningoencephalitis, abortion, or perinatal infection. L. monocytogenes is reported to be a highly invasive intracellular pathogen. It is able infect macrophages via phagocytosis, or epithelial cells, which cause changes to the cytoskeletal and plasma membrane (Edelson and Unanue, 2000).
Thus, present study was conducted to determine the antibiotic resistance, occurrence of plasmids, molecular characterization of Lactobacillus species isolated from carabao's milk and fermented carabao's milk product.

P5.15 Influence of the slaughter process on inactivation of Campylobacter jejuni and compliance to a Performance Objective (PO) in poultry meat in France

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Campylobacter jejuni is the leading bacterial cause of human gastrointestinal disease worldwide. Broiler meat is considered as the most important source of human Campylobacteriosis.
The objective of this study was to assess the influence of poultry slaughter process on the subsequent log reduction of C. jejuni during cold storage in order to assess the compliance with a Performance Objective (PO) in France.
Three strains of C. jejuni inoculated at 4, 6 or 8 log10 CFU/g on chicken cuts were consecutively submitted to heat (54°C for 3 min) and cold (3°C for 2 h) stresses, inspired from the two main slaughtering steps. Fillets were then stored at 6°C during 17 days under modified atmosphere (70% O2 / 30% CO2). Inactivation curves of C. jejuni were obtained from plate counting on selective Casa plates and fitted by the Weibull model.
High variability associated with the Weibull shape parameter led us to use log reduction rate raw rather than model adjusted data. For all strains, C. jejuni inactivation during storage was shown to depend upon inoculum, being the lowest at 8 log CFU/g (-0.61 ± 0.22 log10 CFU/g), than at 6 and 4 log10 CFU/g (-1.64 ± 1.14 and -2.23 ± 0.61 log10 CFU/g) respectively. One strain exhibited enhanced resistance during storage after application of stress, suggesting an impact of the cell history on further bacterial resistance. The contamination level of C. jejuni predicted at the end of a 6-day storage, showed compliance of chicken meat ready-to-be cooked with an hypothetical PO of 2.55 log10 CFU/g as suggested by the ICMSF (International Commission on Microbiological Specifications for Foods) group.
This study opens the path to assess the compliance to a PO of C. jejuni in poultry meat and more generally provides inputs to refine microbiological risk assessment.

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P5.16

Development of updated food safety messages to reduce the burden of foodborne illness in Europe

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The most commonly used and widespread recommendations to consumers about how to procure, prepare and store food safely are the "Five Keys to Safer Food" by the World Health Organization (WHO). Since these food safety messages were established about 20 years ago, a wealth of scientific knowledge about pathogens and how they transfer, multiply and survive during food preparation, has been generated, together with a much broader understanding of consumers' behaviour and food consumption. Risk communication models have also evolved, taking into account a broader understanding of the scope of communication and how information should be designed to obtain risk reduction through behavioural change.

In the European Research and Innovation project, SafeConsume (safeconsume.eu), a process to develop new evidence-based food safety messages, with expected high impact, was conducted. Common unsafe consumer practices were identified, based on the most problematic food pathogens in Europe as well as consumer behaviour. Qualitative information about kitchens, consumers and their practices was collected through kitchen fieldwork (behaviour observation and interviews in six European countries), interviews with students and their teachers (five European countries), and collection of "food safety myths" across Europe. Quantitative data were obtained through a web-based survey in 10 European countries. The effects of observed and self-reported practices on food pathogens were identified based on existing literature and by experimental work. For a subset of practices that were common among consumers, and where a change would result in a high potential reduction of exposure to the top five pathogens representing a health burden in Europe, the project steering board (consisting of nine people with different competencies and backgrounds) developed an initial ranking, in terms of consumer motivation, capability, and opportunity for behavioural change. The ranking was finalised through an expert survey among the consortium participants that had participated in the research and innovation activities. The aforementioned research will be used to develop food safety messages with a high impact potential in a workshop with project members and policy actors, and presented at FoodMicro2022 in August.

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P5.17 Survival of the indigenous microflora including Escherichia coli and Pseudomonas aeruginosa as well as an added culture of Salmonella Typhimurium in fat from the black soldier fly stored at 5 or 21°C

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The use of insects for feed and food products is attracting an increased attention. One of the most promising candidates in that regard is the black soldier fly (Hermetia illucens; Diptera: Stratiomyidae). Previous studies have demonstrated the presence of a diverse microflora in the larvae of the black soldier fly including at some instances E. Coli. However, to our knowledge there are no data on the composition and survival of the microflora in fat produced from this species. We analyzed a number of batches from a manufacturer by classical enumeration using PCA agar (aerobic mesophilic flora) and Rapid E. Coli agar. The microbial counts showed a wide variation ranging from 3.9 to 7.2 log CFU/g for the aerobic, mesophilic count and from <1 to 5.0 log CFU/g for E. Coli. The bacterial levels did in overall not increase nor decline during storage for three months at 5 or 21oC. In addition to E. Coli the microbial flora included a number of taxons identified by MALDI-TOF and/or 16S sequencing including Acinetobacter baumanii, Bacillus cereus, Citrobacter freundii, Enterococcus casseliflavus, Klebsiella pneumonia, Moraxella osloensis, Pseudomonas aeruginosa, Pseudomonas nitroreducens, Stenotrophomonas maltophilia and other species.
We inoculated two batches of the products with Salmonella Typhimurium 4/74 in initial levels of 7.3-7.6 log CFU/g. These levels were only slightly reduced, to 5.9-7.2 log CFU/g after storage at 5 or 21oC for 1.5 months. The results show that the fat from the soldier fly present an excellent medium for survival of E. Coli, an indicator of the hygienic level, as well as for survival of pathogenic bacteria such as Pseudomonas aeruginosa and Salmonella. This study demonstrates the need for hygienic precautions in order to prevent undesirable microbial contamination of this product during insect rearing and subsequent processing.

P5.18 Risk-benefit assessment of shifting from traditional diets to plant-based meat alternative diets

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The shift to plant-based diets such as those recommended by the EAT-Lancet Commission is underlined by the perceived greater health benefits and sustainability. Although highly nutritious and associated with positive health effects, legumes can pose a risk to consumers from a toxicological point of view. In order to provide advice regarding the shift from traditional diets to meat alternatives, we used the risk-benefit assessment approach. Recent data indicate the contamination of one of the most used meat alternatives, soybeans, with genotoxic and carcinogenic mycotoxins such as aflatoxins. In this paper we present the toxicological risks of soybean consumption. Using scientific assessment tools provided by the European Food Safety Authority we assessed the chronic dietary exposure of Italian consumers to aflatoxins. Alternative scenarios were developed in order to assess the risk that Italian consumers expose themselves to by consuming soy-based food. The risk of liver cancer and the health burden due to exposure to aflatoxin were estimated for exposure to contaminated soy-based food. This risk is even higher when modelling data with increased consumption frequency that reflect the current growing trend of legume consumption. Currently, no maximum limits are established for aflatoxins in soy. Our findings indicate the need for authorities to investigate legumes' contamination levels with mycotoxins and establish limits as it is the case for cereals and other foods.

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P5.19 Survey on food safety and hygiene practices in Brazilian households

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Epidemiological data show that most outbreaks of foodborne illnesses occur in homes, but information on hygiene practices, handling, and storage of food in Brazilian households is scarce. This study aimed to obtain information on hygiene practices during the handling and storage of foods in Brazilian homes, in addition to obtaining temperature data from domestic refrigerators. An online questionnaire consisting of 29 questions was applied, with anonymous participation, through Google Forms. The temperature was collected during three days from 216 refrigerators of participants residing in the State of Sao Paulo. A total of 5,000 individuals, from all Brazilian states responded the questionnaire, with a majority participation of females, aged between 25 and 55 years and with an income of up to four minimum wages. The results showed that regarding the cleaning of fruits, only 28.5% of participants sanitize as recommended with running water and a chlorinated solution. For other vegetables the rate is 37.7%. The handling and consumption of animal products was also evaluated, in which 46.3% of the participants reported to have the habit of washing meat in the kitchen sink, 24.1% usually consume undercooked meat and 17.4% consume raw or undercooked eggs. Analyzing the purchase of food in supermarkets, most participants (81%) do not use thermal bags to transport refrigerated or frozen foods to their homes. 11.2% of participants reported to store leftover foods after 2 h of preparation, which represents a food safety risk. It was also evidenced that it was common among the participants to thaw food at room temperatures (39.5%) or inside a container with water (16.9%). Regarding the temperature of refrigerators, 91% of the records collected are between the recommended 0 to 10°C temperature range. In the context of hygiene practices, handling and storage of food, there are certain actions performed inappropriately by a significant portion of the respondents. Thus, better food safety communication strategies should be implemented as a way to prevent foodborne diseases in Brazil.

P5.20 The effect of temperature and carbon dioxide climatic stress factors on Escherichia coli growth kinetics

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Globally, the processing of dairy products results in vast volumes of dairy processing effluents. Whey is a critical effluent in the dairy production chain, characterised by high pollutant potential due to high levels of solids and chemical and oxygen demand. Furthermore, due to its high content in protein, whey has been recognised as an excellent substrate for the growth of various bacterial contaminants such as Escherichia coli, Streptococcus, Lactobacillus. Consequently, the continuous disposal of the whey on farms or fields may lead to health and ecosystem crises. Concomitantly, the global level of atmospheric carbon dioxide is expected to increase in the coming century resulting in temperature changes in many areas and a reduction of the pH in aqueous solutions. Due to the survival of stress-adapted bacteria, climate changes may raise the risk of adverse effects on food safety and public health. In this study, the influence of temperature (range: 27 – 42oC) and dissolved CO2 (range: 30 – 1200 ppm) on the specific growth rate of E. Coli BL21 (DE3) pD454 MBPeGFP was analysed, thus assessing its response to induced environmental stress factors. A kinetic assay has been performed utilising a microplate reader with a spectrofluorometer. To estimate the specific growth rates, growth curves were generated using enhanced green fluorescent protein as a reporter to calculate the generation time in Miller's Lysogeny Broth. A response surface modelling approach was developed to correlate the environmental conditions of temperature and CO2 with the growth of E. Coli BL21 (DE3) pD454 MBPeGFP. At 42oC and 30 ppm dissolved CO2, the maximum specific growth rate was estimated at 1.35 1/h. At the same temperature, the increase in dissolved CO2 from 30 to 380 ppm resulted in a 0.81 1/h decrease in the specific growth rate. However, this increase in dissolved CO2 raised the specific growth rate by 0.72 1/h at a lower temperature of 27oC. The results obtained in this study can be considered a warning indicator regarding microbial proliferation under climate stress conditions, highlighting the need for corrective measures to control microbial contamination in the food industry.

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P5.21 Molecular basis for the resilience of Listeria monocytogenes LL195 in a deli meat food matrix

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Listeriosis was one of the two most severe diseases with the highest case fatality rate. Infection mainly results from the consumption of contaminated food, and in order to cause disease, L. monocytogenes need to survive and, in many cases, grow in the food matrix. Numerous large outbreaks of listeriosis have documented the severity of food safety gone wrong. Deli meats feature prominently among the food matrices associated with listeriosis. A better understanding of the molecular mechanisms behind L. monocytogenes resilience toward the stress conditions in deli meats is crucial, not only for developing effective methods to reduce pathogen fitness but also to assess food safety risks better. Therefore, the main objective of this study was to understand the genomic basis underlying the interactions of L. monocytogenes with "Lyoner", a common deli meat in Germany.

A mariner transposon-based Tn insertion mutant library was cloned in L. monocytogenes LL195. The saturated library contains a collection of 2,700 individual mutants, corresponding to a Tn insertion every 1000 bp. This library was screened on Lyoner, and mutants with an effect on the fitness of L. monocytogenes were identified. Candidate genes were defined by a nested PCR protocol to determine the exact location of the Tn in the genome.

Among others, clones with mutations in pbpC, sau3AIM, and a putative transcriptional regulator showed a significant growth defect on deli meat at refrigerated temperatures compared to BHI. pbpC encodes a transpeptidase responsible for peptidoglycan biosynthesis. sau3AIM encodes a methylase that protects DNA from cleavage by the corresponding Sau3AI endonuclease. Interestingly, Δsau3AIM strains grew well in BHI at optimum temperatures. Components of the temperature dependent restriction-modification system might in the future be used as a preventive strategy during cold storage of deli meats. The downstream regulon of the transcriptional regulators of unknown function that were identified in this study may also yield potential targets for preventive strategies. Clean deletion mutants of these genes will be cloned for further investigation, and the regulon of the transcriptional regulator will be determined.

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P5.22 Traditional Foods, Food Safety Practices and Food Culture in the Middle East

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Food preferences between societies have evolved, offering differences from traditional food to modern food. Throughout history the Middle East countries have been known for its authentic and delicious traditional food. Traditional food in the Middle East countries around the world may act as the foundation for people who lives within this area. People's identity, culture and tradition can be seen during the presence of food. Sustaining heritage is important considering the acknowledgement that the next generation should have toward their past; hence continuing the practice of consuming traditional food as it acts as a significant reminder of the culture and identity. Middle Eastern traditional food is known for its exotic, rich and aromatic flavours that may be present in both daily and special occasions. However, consumer perception toward traditional food within the Middle East have changed due to globalisation along with business and marketing. This presentation discusses various aspect of traditional food in the Middle Eastern countries, their practises, culinary preparations and nutritional/health aspects. In addition, consumer perception and relevance of traditional food consumption in these countries along with future prospects and awareness efforts to sustain the presence of traditional food are also briefly outlined.

P5.23 Quantitative Microbiological Risk Assessment: What about a real-time product specific decision making tool?

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Quantitative Microbial Risk Assessment (QMRA) is the fundamental framework for characterizing the nature and likelihood of harm resulting from human exposure to foodborne hazards. A plethora of QMRA studies has been performed covering most of the major food-hazard combinations while various objectives are addressed for several specific types of products or geographical regions. Food industry, following risk assessors, has shown increased interest in risk-based food safety decision tools. Risk assessment models customized for specific products or even for facilities and vertical integrated brands can support food business operators' decision making. The ongoing development of sensors and on-line monitoring devices for QMRA important environmental parameters can result in a "real-time" decision making tool. Based on the above, the concept of a real-time, product specific QMRA model as a decision making tool for vertical integrated producers is outlined. In traditional QMRA, after the development of the conceptual model, the microbial propagation throughout the continuum is considered. Data obtained from existing literature or databases, fitted to appropriate probability distributions, are used to populate the QMRA. In RT-QMRA assigned distributions are optionally substituted by point estimates for a particular case under study. Distribution and storage phase are the first candidates where "real-case" temperature data, during transportation (truck) or storage (catering facility or restaurant), for a specific lot or product can be used in the model. Real-time temperature monitoring can be achived with thermocouples or termperature data loggers. Wireless technology can facilitate on-line integration of such data. The real-time tracking of the hazard levels enables the progressive (re) evaluation of the consumer risk for the studied product or lot. Even the consumer profile (eating preference and/or age) may be taken into account. The final risk estimate retains its stochasticity and becomes less variable as single value inputs are integrated. Finally, since the consumer risk is updated based on the elapsed stages, the need or the extent for corrective measures to achieve the accepted level of risk (compared to traditional QMRA) can be decided. It is expected that the proposed approach will support food business operators' decision making.
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P5.24 The control and management of foodborne virus hazards, from 2022 and beyond

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Quantitative microbial risk assessment (QMRA) allows risk managers to consider the impact and likelihood of adverse consumer health outcomes caused by exposure to microbial hazards. This gives the ability to compare the outcomes for different control scenarios and select between them. Moreover requires modelling the effect of these control measures on the hazard in question: its growth, persistence, survival, and removal. In turn, these models need data on these questions for the specific combination of hazard and food.
Virus hazards require special consideration when it comes to managing foodborne microbial risk. Compared with bacterial hazards, viruses tend to be more persistent, more difficult to remove, and more infectious in low doses. Control measures intended to prevent bacterial risk, such as low temperatures, can be counter-productive in managing virus risk. There is also a problem with data available for modelling the effect of virus control measures, largely due to past difficulties in detecting and quantifying infectious viruses in food.
This talk will address the main methods for managing and controlling virus risk. It will consider the most common food matrices associated with virus risk and the most appropriate measures for each. Both thermal and non-thermal inactivation methods will be considered, as will prevention of contamination. Advances in detection methods have allowed a greater understanding of the scope and control of virus risk, and the data shown will reflect this.
The conclusion drawn is the greater need for prevention over inactivation and the hygiene and control strategies that can be implemented. This includes rules and systems such as HACCP and GMP. Food safety measures in 2050 and beyond will need to consider virus risk and the sources of contamination. A combination of hygienic controls and predictive modelling of inactivation methods will be needed to manage virus risk. This in turn will need better routine detection methods to model and implement, which is ultimately where the future of virus QMRA lies.

P5.25 Quantitative Microbiological Risk Assessment (QMRA) of fresh poultry spoilage

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Over the last decade, food spoilage has become a major issue with approximately 1.3 billion tons of food per year lost or wasted globally due to spoilage. Specifically, fresh poultry are classified as highly perishable food products and are usually spoiled within a short storage period even when stored at sufficient refrigeration conditions. The aim of this study was to develop a probabilistic QMRA model to assess and effectively control the risk of spoilage of fresh poultry products.
The QMRA spoilage model was based on the growth of pseudomonads which are the specific spoilage organisms (SSO) of aerobically stored fresh poultry and combined data on their concentration at the time of packaging and data on the retail and domestic temperature conditions in Greece with a mathematical model for the effect of temperature on pseudomonads growth during storage. In addition, with simultaneous microbiological and sensory analysis, a "spoilage-response" relationship was developed with a beta-poisson model describing the probability that a consumer rejects the food at the time of package opening as a function of SSO level in the food at that time. The latter relationship represents the heterogeneity on the perception of spoilage and/or the individual differences in senses among consumers.
The use of the developed model as a framework to effectively assess and manage the risk of spoilage is demonstrated. The proposed risk-based approach can support the FBOs in selecting an effective expiration date, leading to the maximum exploitation of the "true" product's shelf life, while minimizing the risk of spoilage.
This research was part of the project DiTect, "Digital Technologies as an enabler for a continuous transformation of food safety system" under funding from the European Union's Horizon 2020 research and innovation program under grant agreement No861915.

Controlling and predicting microorganisms in food ecosystems

P6.1 Dynamic modeling of the shelf life of fresh fruits and vegetables in real-time – a multivariate approach

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High amounts of the foods produced are wasted throughout the supply chain, especially of fruits and vegetables. An accurate knowledge on the exact product status during delivery is supposed to improve logistic processes, use the sales period more efficiently and thus reduce food waste. The combination of product-related temperature monitoring, data exchange throughout the supply chain and real-time shelf life modeling based on temperature data is an innovative approach to achieve this goal. In order to establish a multivariate prediction model and data workflow, raspberries and Lamb's lettuce were chosen as food models. A total of 160 packaging of Lamb's lettuce and 154 packaging of raspberries were investigated in storage trials under isothermal conditions (2, 4, 10, 15°C). During storage, the microbial, sensory and physicochemical quality of the products were investigated at eight subsequent investigation points. Microbial contamination of spoilage as well as pathogenic bacteria (total viable counts, Enterobacteriaceae, Pseudomonas spp., Lactic Acid Bacteria, Yeasts, Moulds, Listeria spp., Bacillus cereus, Escherichia coli) were assessed using classical enumeration techniques. The analysis of sensory quality was conducted by a trained sensory panel and comprised purchase decision, defect assessment. The physicochemical analysis covered weight loss, pH-value, colour, texture, brix-value and titratable acids. The total viable count at the beginning of storage was 4.23 log10 cfu/g (± 0.46) for raspberries and 6.08 log10 cfu/g (± 0.16) for Lamb's lettuce. The screening for pathogenic bacteria showed no noticeable problems. Spoilage bacteria showed an atypical growth pattern and no specific spoilage organism could be identified. Thus, a combination of different predictors was chosen to develop the shelf life models. The implementation of dynamic shelf life models into practice improves transparency, helps to enhance supply chain efficiency and thus reduces food waste in the sector of fresh fruits and vegetables.

P6.2 The effect of water activity, storage temperature and packaging on the behavior of Staphylococcus aureus in dry-cured ham

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Staphylococcus aureus is the predominant specie involved in staphylococcal food poisoning outbreaks. This bacterium is able to grow over much adverse conditions than others pathogens, such as low water activity (aw) (>0.83). S. aureus growth and enterotoxin production is a concern for dry-cured ham (DCH), particularly when stored at room temperature, as confirmed by the application of growth/no growth predictive models to the physico-chemical characteristics of DCH commercialized in a convenient format (i.e. sliced and packaged). In this framework, the main objective of the study was to determine the capability of S. aureus to growth in DCH as a function of storage temperature and aw for different packaging conditions. DCH slices with different aw (0.87-0.94) were inoculated with a cocktail of S. aureus strains (CECT4466, CECT976 and CTC1008), packaged under 3 formats: aerobically, modified atmosphere (MAP 80:20 N□:CO□) and vacuum, and stored at different temperatures (2, 8, 15, 20 and 25 °C) for up to 3 months. S. aureus were periodically enumerated on chromogenic agar. Growth or inactivation models were fitted to the S. aureus counts depending on the observed behavior. Behavior of S. aureus was dependent on the three factors studied, i.e the storage temperature, product aw and packaging. No growth was observed in vacuum and MAP at all aw stored at the lower temperatures (< 15°C). Nevertheless, in samples with high aw stored at 20 and 25 °C, inactivation of S. aureus was observed after 30 days of storage. On the other hand, growth was observed in aerobic conditions for DCH with the highest aw content, increasing to 1.2 and 3 $\log_{10}$ in 2 days at 20 and 25 °C, respectively. At 25 °C, the pathogen continued growing until day 5 achieving a total increment of ca. 4.5 $\log_{10}$.
New data to validate predictive models will be needed for future assessment and management of S. aureus in DCH.
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P6.3 Evaluation of the protective effect on marinated pork of a mix of three lactic acid bacteria against Listeria monocytogenes and their impact on the product´s microbiome

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Lactic Acid Bacteria (LAB) are being broadly studied for their antimicrobial properties and they seem to offer a feasible alternative towards the demands of more natural foods. This research aims to evaluate the employment of a cocktail of three LAB strains, Lactococcus lactis, Lacticaseibacillus paracasei and Lactiplantibacillus plantarum (inoculated at 10⁷ cfu/g), in marinated pork to control the growth of Listeria monocytogenes (inoculated at 10^3 cfu/g). During the study, the product was stored under vacuum or modified atmosphere packaging at 7°C for 8 days followed by 12°C for 4 days. The impact towards some physico-chemical characteristics (pH and aw), the product´s microbiome (through amplicon 16S rRNA gene sequencing), and the consumer acceptability were evaluated.

The growth potential of L. monocytogenes in marinated pork under vacuum was of 0.9, 0.7, and 0.4 log CFU/g after 4, 8 and 14 days, and was reduced with the use of LAB in 0.7, 0.9 and 0.3 log cfu/g, respectively. Under MAP, L. monocytogenes showed a growth potential of 0.9, 0.4 and 0.4 log CFU/g after 4, 8 and 14 days, which was reduced with the use of LAB in 0.7, 0.6 and 0.4 log CFU /g, respectively. While LAB gradually increased in counts throughout the storage period, counts of psychrophilic bacteria when co-inoculated with LAB showed a reduction at the end of the storage compared to control samples. The treatment with LAB had no marked effect on aw, but a steady decrease in pH was observed for all samples. The use of LAB had a significant influence over the taxonomic profile (adonis, p=0.001) and explained a 53% of the variation observed, while there was no influence of the packaging method employed. Regarding alpha diversity indices, control samples had significant higher values for richness, Simpson and Shannon indices than LAB-inoculated samples, and alpha-diversity indices decreased along storage time, although significant differences were only observed for control samples. Overall, the LAB inoculated rapidly became the predominant taxa among the product´s microbiota. No significant differences between samples were appreciated in the sensorial analyses, which makes feasible the incorporation of the LAB as protective cultures in marinated pork.

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P6.4 Investigating the impact of an ultraviolet light-emitting diode (UV-LED) technology on the microbiota of chicken meat during refrigerated storage

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Ultraviolet (UV) light has been shown to be capable of reducing the bacterial burden on meat surfaces. Nowadays, new UV devices like Light-Emitting Diodes (LEDs) offer an economical and highly efficient approach for potential application in the food industry. However, the impact of UV light on the background bacteria during storage needs further attention before this technology can be used for the disinfection of raw chicken meat. This study aims to investigate the effectiveness of a LED device to reduce background levels of bacteria on chicken meat and extend microbial shelf life. Chicken fillets were purchased at a local supermarket and aseptically cut into square pieces of ~ 10 g. The chicken pieces were treated with UV light at 280 nm for 6 min based on previous studies using an LED device and untreated samples were used as controls. These samples were then packed aerobically in 'plastic trays', simulating retail conditions, stored for 7 days at 4˚C, and sampled at day 0, 1, 3, and 7. Microbiological analysis was carried out with suspensions of stomached samples which were serially diluted in maximum recovery diluent. Total viable counts (TVC) and total Enterobacteriaceae counts (TEC) were determined using Petrifilms which were incubated for 48 h at 30˚C and 24 h at 37˚C, respectively. Furthermore, stomached chicken samples were filtered and centrifuged at 7000 rpm for 5 min. DNA extraction was carried out using the DNeasy PowerFood Microbial Kit and DNA extracts were sequenced on the Illumina MiSeq platform using a 16S rRNA amplicon sequencing approach before bioinformatics analysis. Thus, UV light extended the shelf life of chicken by 3 days. TVC levels of 4.09 ± 0.51 log CFU/g and TEC levels of 1.30 ± 0.67 log CFU/g were observed in UV-treated samples compared to controls with TVC levels of 5.13 ± 0.20 log CFU/g and TEC levels of 2.53 ± 0.31 log CFU/g. The results of the 16S rRNA amplicon sequencing will provide insight on the bacterial background of raw chicken meat and a deeper understanding how UV affects the chicken microbiota and thereby, extends shelf life.

Controlling and predicting microorganisms in food ecosystems

P6.5 Impact of food-relevant conditions on efficacy of prenylated isoflavonoids as natural preservatives against Listeria monocytogenes

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The growing demand for natural preservation methods has motivated us to explore novel antimicrobials from plant sources. Prenylated isoflavonoids of the Leguminosae/Fabaceae family have shown remarkable antimicrobial activity against foodborne pathogens and spoilage bacteria, highlighting the possibility of using these compounds as natural food preservatives. The substitution of the isoflavonoid skeleton with a prenyl group (5-carbon isoprenoid group) is essential to their antimicrobial activity, although the position and type of prenylation are critical aspects. Excellent candidates from this subclass of compounds are glabridin (monoprenylated isoflavan) and 6,8-diprenylgenistein (diprenylated isoflavone). Although these novel antimicrobials have been previously studied, their activity has mainly been tested in standard laboratory settings, and the impact of food-relevant conditions on their antimicrobial activity has not been investigated.

The antimicrobial activity of glabridin and 6,8-diprenylgenistein was tested against L. monocytogenes, a robust and highly adaptable foodborne pathogen. Their activity was tested at various conditions relevant for food application, such as different temperatures (from 10°C to 37°C), pH (5 and 7.2), and presence or absence of oxygen. The antimicrobial activity was measured in vitro in a nutrient-rich plant-based medium using a micro broth dilution assay. Glabridin and 6,8-diprenylgenistein showed good inhibitory and bactericidal activities. Their minimum inhibitory concentrations were between 0.8 µg/mL and 12.5 µg/mL, comparable to commonly used antimicrobials in food and clinical settings. Our results showed that their bactericidal activity decreased when these compounds were tested at 10°C compared to 37°C. A four-fold higher concentration was needed for glabridin to achieve the same antimicrobial efficacy, whereas 6,8-diprenylgenistein did not show bactericidal activity at the tested concentrations. However, growth inhibition was still observed with low concentrations of compounds (6.3 – 12.5 µg/mL) at 10°C. In general, lower pH increased the activity of the compounds (from 2 to 8-fold) and restored the antimicrobial activity of 6,8-diprenylgenistein at 10°C. Lastly, similar activities were observed for glabridin and 6,8-diprenylgenistein in aerobic and anaerobic conditions.

Overall, these findings highlight the potential of the tested prenylated isoflavonoids to control (an)aerobic growth of L. monocytogenes at refrigeration temperature and/or at mildly acidic conditions relevant for food.

Controlling and predicting microorganisms in food ecosystems

P6.6

Assessing Listeria monocytogenes growth in artisanal fresh goat milk cheeses over the distribution chain through the development and application of predictive models

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Introduction: Listeria monocytogenes has been considered a hazard of concern in fresh goat cheeses produced in the Andalusian region (Spain), which is evidenced by a listeriosis outbreak resulting in 1 death and 13 cases (May-2021), and recent food safety alerts concerning this pathogen in these products (September-2021 and March-2022).
Purpose: This work was aimed at developing a L. monocytogenes growth model in artisanal fresh goat cheeses at different storage temperatures and on its application to assess the exposure of consumers to the pathogen during the product shelf-life.
Methods: The effect of storage temperature (4-25°C) for up to 18 d on L. monocytogenes was evaluated in a lab-scale goat fresh cheese initially inoculated with a three-strain cocktail of the pathogen (ca. 2-3 log cfu/g). To describe the relationship between L. monocytogenes concentration with growth rate (μmax, log cfu/d), storage temperature and time, the Baranyi and Ratkowsky models were fitted to the collected growth data following a one-step approach. Furthermore, a probabilistic exposure assessment model, following a Modular Process Risk Model methodology, was designed using temperature-time profiles of a real distribution chain encompassing steps from the storage at the factory to household storage. Both the growth and exposure assessment models were built using R considering different time-temperature scenarios for model simulations.
Results: The μmax of L. monocytogenes showed a positive correlation with temperature, with values ranging from 0.296±0.023 to 2.122±0.466 log cfu/d from 4-25°C. The estimated secondary model parameters were b=0.057±0.006 and Tmin= -4.7±1.1°C and the global model showed a good fit to data (RMSE=0.849). The exposure assessment model simulations showed that L. monocytogenes could reach concentrations as high as 5.84 log cfu/g in fresh goat cheeses during the early distribution chain.
Conclusions: The results confirm that the distribution chain conditions of fresh goat cheeses enable L. monocytogenes growth over their shelf-life. Hence, focused control measures, as reinforcing manufacturing, cleaning and disinfection practices and strict storage temperature controls, should be implemented to reduce the L. monocytogenes consumer's exposure. In next steps, the exposure assessment will be linked to a dose-response model for risk estimation of listeriosis.

Controlling and predicting microorganisms in food ecosystems

P6.7

Assessing the microbiological safety of beef aging process: Impact of temperature and relative humidity conditions

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Dry-aged beef meat is becoming more and more popular among the consumers. Despite the increasing number of works dealing with the technological aspects of dry-aging of meat, the microbiological safety has been scarcely studied. The aim of the present study was to evaluate the safety of four different aging processes regarding the temperature and relative humidity combinations. The impact on the decrease of aw and the pH change on the meat surface was monitored. Subsequently, the growth of L. monocytogenes as the relevant microbial hazard was simulated under dynamic conditions of the key factors associated with dry aging of beef. The two loins of ten commercial Holstein culling cows were selected, cut in 10 pieces (approximate 20cm length) and submitted to a dry aging in an aging room set at: (i) 0°C and 60%RH; (ii) 0°C and 78%RH; (iii) 3°C and 60%RH; (iv) 3°C and 78%RH. The actual temperature was recorded with a datalogger every 30 sec. Loins were sampled periodically, from day 0 to 56. The pH was measured on the dried surface, then, the aged piece was deboned, and a 5 mm-thick slice of the surface was cut to determine the aw. The recorded environmental temperature, and meat aw and pH were used as input values for the microbial predictive model (Mejlholm et al. (2010)) about the growth of Listeria monocytogenes. The temperature during aging was kept under control in the different processes (standard deviation <0.3). The aw of the meat surface decreased differently depending on the process. At 0 °C the drying was slower (particularly at 78 %RH) than at 3°C (similar at 60 and 78 %RH). The pH slightly increased with no apparent differences among the four processes. According to the predictive model the aging conditions recorded in processes at 0 °C did not support the growth of L. monocytogenes. Contrary, a slight increase of the pathogen was predicted (ca. 1 log) during the first 3 weeks. This growth would be inhibited (<0.1 log increase) at ≤ 2 °C, which would be the critical temperature limit to assure a safe beef dry-aging process.

Controlling and predicting microorganisms in food ecosystems

P6.8 Tunable Diode Laser Absorption Spectroscopy for Non-destructive Detection of Microbial growth in Aseptic Food Products

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Tunable Diode Laser Absorption Spectroscopy (TDLAS) can be used to monitor carbon dioxide changes due to microbial growth in a container headspace which has the advantage of being rapid and non-destructive. This study aimed to provide detailed scientific evidence for the application of TDLAS technology as a method to determine product sterility (both spoilage and microorganisms of concern) in real food products. TDLAS growth detection of Bacillus fengqiuensis, Candida albicans, Lactococcus lactis, Microbacterium luteolum, Paenibacillus chitinolyticus, Staphylococcus pasteuri and Listeria monocytogenes was studied in various Ready To Feed (RTF) dairy matrices. TDLAS was capable of detecting growth of L. lactis within 20 h and S. pasteuri in 55 h when foods were contaminated with as low as ~100CFU/ml. However, the spore former B. fengqiuensis was not detected after 72 h in three matrices when inoculated at low levels. The lowest cell density detected at 4.47 CFU/ml was for the yeast (C. albicans) after 28.99 ±1.82 h and the highest at 8.53 CFU/mL was for the Actinomycete (M. luteolum) at 37.02 ±1.84 h in non-hydrolysed RTF matrices. Overall, the TDLAS equipment was shown to be reliable with some specific limitations in identifying microbiological contamination by typical spoilage microbes in commercially sterile RTF products.

P6.9 Antifungal effect of Staphylococcus xylosus against ochratoxin A- producing Aspergillus westerdijkiae in a dry-cured ham model system

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The environmental conditions during the ripening of dry-cured ham favour the colonization of their surface by ocratoxigenic moulds such us Aspergillus westerdijkiae. The use of autochthonous antifungal microorganisms usually found in this meat product is a promising strategy to control the hazard of ochratoxin A (OTA). Previous work has reported the efficacy of Staphylococcus xylosus Sx8 to decrease the growth of toxigenic moulds and the production of mycotoxins. However, it is necessary to understand the antifungal mechanisms of S. xylosus Sx8 in order to optimise its correct application during the ripening of dry-cured ham. The aim of this work was to elucidate the mechanism of action of S. xylosus Sx8 against A. westerdijkiae in a dry-cured ham model system. For this, the nutritional utilisation pattern, niche overlap index (NOI), interactions by dual-culture assays, antifungal effect of volatile compounds, OTA detoxification and proteomic analysis were determined. The number of carbon sources metabolised by A. westerdijkiae were higher than those utilised by S. xylosus. The NOI values showed that both microorganisms can coexist. The volatile compounds did not show antifungal effect, and S. xylosus neither degraded nor adsorbed this mycotoxin. However, in the interaction assay, Sx8 was able to lower the growth of ocratoxigenic mould and the production of OTA. In addition, proteomic analysis showed an increase in proteins involved in catabolic processes and a decrease in those implicated in the metabolic of nitrogen compounds. In conclusion, the antifungal action of S. xylosus Sx8 is likely related to competition for space and nutrients with A. westerdijkiae triggering alterations in the fungal metabolism. Therefore, this strain could be considered to control OTA production in dry-cured ham.
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Controlling and predicting microorganisms in food ecosystems

P6.10

Project SACADA: SARS-CoV-2 transmission in meat processing plants

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In France and worldwide, during the COVID-19 pandemic, workers in the food production sector were considered as part of the critical infrastructure workforce. In spring 2020, clusters in the food production sector are reported in different countries. Meat processing plants have been repeatedly identified as hot spots for SARS-CoV-2 transmission in many countries, including France. Health authorities have identified barriers to effective prevention and control of COVID-19 in these plants, with socio-economic challenges that may contribute to workers in this industry continuing to work while ill.

The objective of the SACADA project (2021-2022) is to better understand the circulation of SARS-CoV-2 in meat processing plants in order to provide preventive or mitigating measures for workers and consumers.

In this project, we have collected the data necessary to understand the circulation of the virus in this type of workplace. We will use these data to build a simulation model of the spread of SARS-CoV-2. We follow four work packages. Firstly, the factors of transmission and persistence of SARS-CoV-2 in meat processing plants will be studied, based both on literature and experimental data. In a second task, and in order to understand the emergence of clusters, a description of the working conditions and environmental factors in meat processing plants was carried out, following epidemiological investigations but also through questionnaires, interviews and visits.

All of these elements support the third step, which is the construction of a mathematical model to simulate the spread of the virus in a meat processing plant. The objective is to evaluate the impact of certain prevention or mitigation measures on the probability of transmission of the virus to employees and the contamination of products and the environment.

Finally, in a last task, we will propose elements for engaging employees in an effective safety management policy, gaining cooperation and support, and maintaining a positive safety culture.

Controlling and predicting microorganisms in food ecosystems

P6.11 Differences in thermal inactivation of spore-formers in plant-based milk alternatives vs. bovine milk and culture medium: The example of B. licheniformis

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The consumer demand for plant-based milk alternatives has been increasing in the last years. The potential food spoilage induced by spore-forming bacteria from plant-based ingredients is an emerging research topic. The objective of this study was to assess whether there are differences in thermal inactivation of spores between milk alternatives, bovine milk and culture medium.
Bacillus licheniformis was chosen as a model micro-organism due to its common involvement in spoilage incidents in milk and vegetables. To investigate the impact of food matrix on the inactivation profile of spores, experiments were carried out in culture medium, bovine milk and several plant-based milk alternatives with different composition. Spores of B. licheniformis CTCPA 3107001 were inoculated in matrices that were subjected to different time-temperature conditions in capillary tubes. The temperatures ranged from 100 to 120°C.
The initial inoculum level was approximately 9 log CFU/ml and the target reduction was at least 5 log. Survival of heat-treated spores was checked with plate enumeration and all analyses were performed in triplicates.
Inactivation was more variable and delayed in food matrices comparing to culture medium, suggesting a potential protective effect of the food matrix. For instance, at 100°C, the D-values determined in plant-based milks were systematically higher than in culture medium. In addition, differences were observed between the different plant-based formulations in terms of time required to reach the target reduction and inactivation plateau.
Overall, this study provides insights on the inactivation kinetics of B. licheniformis spores in plant-based milks and confirms the food matrix impact during thermal processing. The latter indicates the need for specific models to accurately predict the inactivation of spoilage organisms in plant-based milks to achieve high product quality.

Controlling and predicting microorganisms in food ecosystems

P6.12 Effect of organic herbs on growth kinetics of Salmonella Enteritidis in chicken broth

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Salmonella is a major cause of foodborne illness in humans worldwide. Its frequent presence in poultry intestines may increase the risk of contamination of poultry products within the industry handling raw meat, chicken products and ready to eat products. The current study aimed to estimate the effect of organic herbs on the growth kinetics of Salmonella Enteritidis in chicken broth. Approximately, one hundred Salmonella Enteritidis cells were inoculated in chicken meat broth (prepared by cooked chicken portions for the better simulation of the food matrix) with organic herbs. Different concentrations and mixtures of the following organic herbs: oregano, thymus, summer savory, crithmum were tested. A laboratory medium, meat extract (ME) broth, was used as control. The growth of Salmonella was monitored using a microplate reader for 24 hours at 37°C, and the growth parameters were estimated by Baranyi and Gompertz model. According to the results, the organic herbs affected the growth of the pathogen. The observed differences were related to the herb species, the added quantity and the growth medium used. More specifically, the lag phase of Salmonella was increased in most cases, when pathogen was grown on chicken meat broth with herbs. Notable differences in lag phase were also observed in ME broth. Additionally, the growth rate was affected by the different growth conditions. The observed differences between the effect of the same herb on the growth of Salmonella in different growth media, highlight the importance of choosing the most appropriate medium to simulate the food matrices. Such studies are fundamental for proposing the necessary amendments to control the foodborne pathogens throughout the food chain.

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P6.13 The enantioselective metabolism of lactate in Propionibacterium freudenreichii

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Propionibacterium freudenreichii plays a fundamental role in the manufacturing of Swiss-type cheese. By utilizing DL-lactate as the main carbon source, it is responsible for the development of the typical sweet and nutty taste of this cheese variety, but also for eye formation. In order to reduce late fermentation issues that may arise during cheese maturation, we assume that selective utilization of only one lactate enantiomer would be a desirable property for dairy P. freudenreichii strains. Here, we propose an Adaptive Laboratory Evolution strategy for the development of new enantioselective mutants, using D-lactate spent medium from Lactobacillus delbrueckii subsp. lactis in a chemostat. Moreover, we phenotypically and genotypically characterize existing L-lactate-negative strains and aim at elucidating the metabolic pathways responsible for L-lactate utilization by comparative genomics and knock-out / rescue experiments.

Controlling and predicting microorganisms in food ecosystems

P6.14 The effect of soy sauce starter cultures towards the survival of Staphylococcus aureus in the development of low-salt soy sauce fermentation

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Soy sauce is made by fermenting soybeans in a high salt condition (18-22%) in a process called moromi fermentation. Extreme salinity is required to control undesirable microorganisms and develop organoleptic properties. High salt diets can lead to cardiovascular diseases, thus developing low-salt soy sauce would promote a healthier diet. However, salt reduction would cause the appearance of pathogenic microorganisms. Several fermented food studies have shown that starter cultures can control undesirable microbe growth caused by NaCl reduction. Although Tetragenococcus halophilus and ZygoSaccharomyces rouxii are used as soy sauce starter cultures for flavor development, no studies on their safety have been done. The effect of soy sauce starter inoculation against Staphylococcus aureus during low-salt soy sauce fermentation was studied. A preliminary study was done in tryptic soy broth (TSB) with 12% glucose and 6% NaCl. S. aureus were inoculated to a final concentration of~5 log CFU/mL. S. aureus then was treated with (i) no starter cultures (SA), (ii) Tetragenococcus sp. only (SAT), (iii) Z. rouxii only (SAZ), (iv) Tetragenococcus sp. and Z. rouxii (SATZ), (v) Tetragenococcus sp., followed by Z. rouxii inoculation after 1 week of incubation (SATZZ). All samples were incubated for 4 weeks at 25°C. The results were then validated in real moromi. Viable cell counts of S. aureus was monitored by culture using Baird Parker Agar (BPA). Inoculating Z. rouxii inhibited the growth of S. aureus after 4 weeks of incubation. SATZ had the greatest reduction in S. aureus (2.57 log CFU/ml) compared to SA, while Tetragenococcus sp. had no effect on S. aureus growth (0.1 log CFU/ml). SATZZ had significantly fewer colonies (1.288 log CFU/ml) than SATZ. Both SATZ and SATZZ were then tested in a real moromi, prepared by mixing koji and brine solution (6%NaCl), with S. aureus (~5 log CFU/mL). S. aureus thrived regardless of treatment and reached a final population of 6.3-6.5 log CFU/mL, indicating no effect of initial pH reduction on growth ($p>0.05$). In conclusion, adding only the soy sauce starter culture seems insufficient to prevent S. aureus growth. This study's findings may help assess microbial hazards in low-salt soy sauce production.

Controlling and predicting microorganisms in food ecosystems

P6.15

Combined effect of mild heat and Origanum essential oil treatment on culturability and viability of Escherichia coli ATCC 25922 in vitro and in inoculated fruit juice

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E. Coli is a pathogen frequently involved in fruit juices outbreaks, despite the intrinsic fruit juices low pH. Novel food preservation technologies are emerging to develop safe food products with extended shelf-life and preserved nutritional and organoleptic characteristics, according to consumers' changing demand. In the present study, the individual and synergistic effect of Origanum vulgare essential oil (OEO) with mild heat treatment on E. Coli ATCC 25922 growth was evaluated in vitro and in a commercial fruit juice artificially inoculated. The treatments efficacy was assessed by traditional culture-based and Flow Cytometric methods for applications designed at monitoring food safety, to achieve understanding into viability and cells physiology (e.g. Viable But Not Culturable State). Bacterial survival ratio in vitro and in a fruit juice challenge test was estimated by plating culture on MacConkey agar, and by applying FCM methods with SYBRGreen I/Propidium Iodide double-staining. Results obtained in vitro showed E.coli ATCC25922 inactivation by increasing the treatment temperature (55°C, 60°C, 65°C). Interestingly, we highlighted an overestimation of the dead population using the culture-based method and the preponderance of injured cells with a potential resuscitation detected only by the FCM analysis. The challenge test on fruit juice at its own pH (3.8) and buffered at pH 7, displayed a bactericidal action of OEO and a higher efficiency of the mild heat at 65°C for 5 min combined with OEO aimed at food preservation. Overall, the combination of mild heat and OEO increased the treatment antimicrobial efficiency, thus representing a promising alternative to improve safety of fruit juices. Furthermore, FCM proved to be able of ascertaining antimicrobial efficiency and becomes essential as an early warning monitoring system for preventing the risk of food-borne disease spreading.

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P6.16

Monitoring the quality of chicken broth with organic herbs stored at refrigeration temperature

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The effect of herbs and their substances to enhance the quality characteristics and safety of foods is well known. In this study, we aimed to produce a chicken broth with organic herbs and monitor the microbiological, organoleptic and physicochemical quality during storage at refrigeration temperature. For this purpose, chicken portions were cooked with organic herbs to prepare the chicken broth. Different amounts and mixtures of organic oregano, thymus, summer savory, crithmum were evaluation. After organoleptic assessment of different samples during storage at 4°C, the samples with the higher values were considered for further experiments. The chicken broth was prepared and stored at 4°C for 2 months. Chicken broth without organic herbs was used as control. Microbiological analysis, pH measurement and organoleptic evaluation were performed in different time intervals. The concentration of NaCl and total nitrogen and protein content in fresh chicken broth were also determined. In addition, Fourier transform infrared spectroscopy (FT-IR) in combination with partial least squares (PLS-R) was used to estimate the storage time. According to the organoleptic characterization, the organic herbs were found to prolong the storage time of broth. The microbiological quality of the chicken broth was acceptable during storage at 4°C, while the pH value ranged from 6.63 to 6.79. The rest physicochemical values were estimated to 10g/L NaCl, 1560mg/L total nitrogen and 9.75mg/L protein. A good correlation of storage time with the FT-IR spectra was achieved through the PLS-R models, where the square of the correlation coefficient (R^2) was estimated at 0.975 and 0.958 (training and external validation, respectively). In conclusion, a new chicken broth with organic herbs was produced with good microbiological, physicochemical and organoleptic characteristics. In addition, FT-IR combined with partial least squares was found to be a promising rapid method to estimate the storage time of the new product.

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P6.17 Germination of B. weihenstephanensis spores under industrial processing conditions of Refrigerated Processed Foods of Extended Durability

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Consumers´ demand for Refrigerated Processed Foods of Extended Durability (REPFED) is increasingly growing because their ease of preparation and long shelf-life facilitate current busy lifestyle. However, food safety and stability of REPFED still present some challenges mainly posed by psychrotrophic bacterial spores, including toxin-producing Bacillus weihenstephanensis, which can survive thermal pasteurization, germinate and multiply during refrigerated storage. Improving control of these microorganisms implies the design of effective preservation methods that prevent germination and/or outgrowth while maintaining food quality, which inevitably requires a deep knowledge on spore behaviour in industrial settings.
In this work, we studied the germination capacity of three B. weihenstephanensis strains (WSBC 10204, WSBC 10202 and SC) in a meat-based model after applying a mild or intense pasteurization and storage at an ideal or abusive temperature in aerobic or anaerobic conditions. Furthermore, the impact of sporulation temperature on spore germination was evaluated. Although germination capacity varied among strains, all spores obtained at the minimum sporulation temperature (Tmin) germinated slower than those obtained at the optimal and maximum temperatures, and such differences were higher when stored at 4°C than at 10°C. The application of a mild heat treatment (70°C/2 min) barely affected germination in all the strains sporulated at any temperature. In contrast, a more intense pasteurization (90°C/10 min) largely improved germination of spores of the most heat-resistant strain (WSBC 10204) at 4°C, especially those obtained at Tmin, in both aerobic and anaerobic conditions. As such, spores of WSBC 10204 could reach up to 90% of germination, independently of their sporulation temperature, after conventional pasteurization and 48 h of aerobic or anaerobic storage at 4°C, highlighting the relevance of B. weihenstephanensis for maintaining food safety and stability of REPFED.

P6.18 Effect of sodium nitrite on outgrowth and toxinogenesis of psychrotrophic Clostridium botulinum Group II type B following an extended cooling of cooked ham

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Botulism is a severe disease that still occurs sporadically in Europe and foodborne origin constitutes one of the most common forms. Foodborne botulism poisoning may occur in case of the ingestion of C. botulinum neurotoxins. Meat products could be contaminated after carcass contamination linked to inappropriate slaughtering or handling practices. Sodium nitrite (NaNO2) is commonly used in meat processing for its antimicrobial effect against Clostridium spp. In a previous study, Lebrun et al (2020) demonstrated that incorporation rates of NaNO2 ≥ 30 mg/kg prevented the outgrowth and toxinogenesis of psychrotrophic C. botulinum Group II type B in a cooked ham model subjected to thermal treatment classically used in the meat processing industry. A new experimental trial was recently performed to examine the impact of an extended cooling time on this pathogen using the same cooked ham model prepared with different NaNO2 (0, 30 and 60 mg/kg) incorporation rates. This is an important point to consider since prolonged cooling can be encountered during food processing failure or in undesirable hot spots (slowest cooling) within cooling chambers. Cured ground pork batters were inoculated with a cocktail of 3 strains of C. botulinum Group II type B at 3.5 log10 CFU/g, portioned and samples of 50 g were vacuum packed then cooked and cooled according to two different regimens i.e. an usual (10.0 hours) versus a prolonged (28.5 hours) cooling period. Cooked ham model samples were then stored under reasonably foreseeable conditions of use and storage i.e. for 14 days at 4 °C and 35 days at 8°C with a cold chain break for 1 h at 20 °C on day 21. Results indicated that cooling regimens influenced the behavior of C. botulinum Group II type B during storage of cooked ham samples. Only an ingoing amount of NaNO2 of 60 mg/kg inhibited toxinogenesis of C. botulinum Group II type B in cooked ham samples subjected to the extended cooling regimen. In comparison, 30 mg NaNO2 per kg were sufficient to prevent toxinogenesis in cooked ham cooled for 10.0 hours.

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P6.19 Fate of Salmonella Newport on leafy greens when challenged with bacteriophage cocktails

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Introduction
Salmonella spp. is one of the major causes of foodborne diseases and recently implicated in an outbreak with leafy green greens in the USA. Bacteriophages have emerged as a promising agent to reduce pathogens in foods and could potentially be used to reduce Salmonella on leafy greens.
Objective
The objective of this work was to determine the effectiveness of two bacteriophage cocktails in reducing Salmonella inoculated onto spinach or romaine lettuce.
Methods
Phage cocktails consisting of either three (HER19, 06, and SE13) or five (Felix 01, HER19, 03, 06, and SE13) phages were sprayed to deliver 6 log PFU/cm2 onto a 4cm2 marked area of spinach or romaine lettuce plants in a greenhouse one day before or one day after a challenge with Salmonella Newport (5 log/cm2). Salmonella and phage populations were measured on days 0 and 1 by excising the treated area and processed by adding 20 ml water (phage) or TSB (Salmonella) followed by vigorous vortexing for 1 minute. Salmonella samples were diluted and enumerated on onto XLD. Phage samples were passed through a 0.22µm filter for analysis by plaque assay. Three trials were completed with duplicate samples (n=6).
Results
The largest Salmonella population reductions occurred when the challenge of Salmonella preceded the phage application regardless of phage or leafy green type. For example, the population reduction on spinach was significantly higher ($P<0.05$) when Salmonella application occurred prior to a five phage cocktail (1.6 Log CFU/cm2) than after (0.3 Log CFU/cm2). Likewise, population reductions on romaine were significantly higher ($P<0.05$) when Salmonella application occurred prior to a 3-phage cocktail (1.4 Log CFU/cm2) than after (0.6 Log CFU/cm2). Reductions ranged from 0.2 Log CFU/cm2 (spinach, 3 or 5-phage application prior to Salmonella) to 1.6 Log CFU/cm2 (spinach, Salmonella application prior to 5 phage).
Conclusion
The data suggest that bacteriophage may be useful for reducing Salmonella contamination on the surfaces of spinach and romaine lettuce.

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P6.20 Screening for lactic acid bacteria strains with anti-Listeria activity: Challenge test in a meat model and raw sausages

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The presence of lactic acid bacteria (LAB) is important for the fermentation process in raw sausage. LAB reduce the pH of the product during the ripening process and consequently suppress and retard the growth of pathogens and spoilage organisms. In addition to general acidifying properties, some LAB produce antimicrobial compounds that include bacteriocins, which are promising for biocontrol concepts to increase food safety and reduce artificial additives. Listeria monocytogenes is a high-risk organism that can be found in various food products, including meat and fish products. In a previous study, 39 LAB strains belonging to Lactobacillus sakei (n=30), Lb. plantarum (n=6), Lb. curvatus (n=2), and Leuconostoc carnosum (n=1) were isolated from a broad variety of fermented meat and fish products and exhibited strong activity against relevant Listeria monocytogenes serovars 1/2a (ATCC15313), 1/2b (SLCC2755), and 4b (ATCC19115). In this study, the anti-Listeria activity of these 39 strains was determined in a challenge test using a meat model in petri dishes with minced meat, nitrited curing salt, and other spices. The model was incubated for four days at temperatures simulating the 14-day ripening process of raw sausages (4.5 h at 22°C, 21 h at 24°C, 14 h at 22°C, 14 h at 20°C, 42 h at 18°C). A total of seven strains showed clear inhibition of L. monocytogenes up to 3 log CFU/g over a simulated 4-day ripening period compared with control samples, solely inoculated with L. monocytogenes. Furthermore, Lc. carnosum DH25 and Lb. sakei DH42, which showed the strongest anti-Listeria activity, were applied in a challenge test using two different raw sausages (Salami and Mettwurst), resulting in growth inhibition of L. monocytogenes up to 0.5 log CFU/g over the 14-day ripening period. In order to evaluate inhibition capacity at low temperatures, Lc. carnosum DH25 was finally applied in a challenge test using the meat model at 8°C, resulting in successful inhibition of L. monocytogenes (-1 log CFU/g) over 14-day storage. The findings of this study can contribute to a product and application-near selection of anti-Listeria LAB strains for application in fermented meat products, that includes anti-Listeria activity at refrigerator temperatures.

P6.21 Suitable surrogate microorganisms for Salmonella spp. as safety indicators in fruit juice production

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Salmonella enterica is one of the major bacterial foodborne pathogens. Characterizing its inactivation kinetics under processing conditions allows for the development of more powerful elimination methods to increase food safety. For safety reasons, pathogens can usually not be used to evaluate these treatments in an industrial area. The solution may be the application of surrogate microorganisms as their nonpathogenic counterparts that do not possess virulence factors, but mimic the behavior of pathogens. As surrogates may not react identically under all conditions, there is a particular interest in identifying microorganisms to be used as surrogates for Salmonella spp. in the fruit juice industry.
The objective of the present study was to investigate the heat resistance of five Salmonella enterica outbreak strains associated with fruit juices, namely S. Senftenberg, two strains of S. Typhimurium, S. Saintpaul, and S. Enteritidis, as well as of a Salmonella cocktail and three potential surrogate bacteria. Thermal inactivation was performed in biological triplicate and technical duplicate at 55°C, 57.5°C, 60°C, 65°C, and 72°C in the two matrixes phosphate buffered saline and strawberry nectar (12° Brix). The D- and z-values of single Salmonella and potential surrogate strains as well as the Salmonella cocktail were calculated. The results propose Escherichia coli ATCC 11229 as a good surrogate candidate for Salmonella strains, as its heat resistance at 60°C, 65°C, and 72°C in strawberry nectar is higher than that of the examined pathogens. The results also show the differences in D-values in the tested matrices, which indicate a clear influence of the environment on heat resistance of bacteria, which may be caused, among others, by changes in heat transfer. In conclusion, we propose Escherichia coli ATCC 11229 as a nonpathogenic surrogate for Salmonella spp., that can be used as biological indicator in the validation of thermal as well as novel treatments in the production of strawberry nectar.

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P6.22 Effect of combined high pressure and Chitosan on the inactivation of Listeria monocytogenes

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During the last decades, High Hydrostatic Pressure (HHP) has been studied and used, as a novel non-thermal technology, against various spoilage and pathogenic microorganisms such as L. monocytogenes. Meanwhile, nisin (a heat-stable polypeptide produced by specific strains of Lactococcus lactis subsp. lactis) and chitosan have been broadly investigated for their antimicrobial activity extending the shelf life and improving the quality of food products. Various studies have shown the effect of HHP, nisin and chitosan in the reduction of L. monocytogenes. Our work showed a high effectiveness of HHP (200–400 MPa) alone or in combination with nisin (50-200 ppm), chitosan (up to 2.5%) and other parameters against various strains of L. monocytogenes. Each treatment was performed in three independent biological replicates. The results of this study are expected to point out a high reduction of L. monocytogenes by using the most effective combination of high pressure and nisin or chitosan. Thus, this research aims to identify the strategies of the decontamination efficiency optimizing the HHP technology.

P6.23 Use of adjunct lactic acid bacteria protectives cultures to increase shelf-life and probiotic potential of Greek traditional sheep yogurt

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Yogurt is a very popular fermented milk product that has been consumed by humans for thousands of years. Nutritionally rich in protein, calcium, riboflavin, vitamins B6 and B12, it is considered to offer more nutritional benefits than milk, and is perhaps the most attractive and digestible dairy product in the world. This results from the symbiotic growth of Streptococcus thermophilus and Lactobacillus bulgaricus, two widespread species of lactic acid bacteria (LAB), which eventually lead to a desired taste gel. Today, many different types of yogurt are commercially produced, which vary in appearance, structure and taste, targeting a variety of different consumer groups, from children to the elderly. Among those, yogurt made from sheep milk following the traditional way is quite popular in Greece and several other countries all over the world. However, its limited shelf-life (approximately two-three weeks) hinders to date any export prospects. At the same time, the legislation applicable in many countries (Europe included) does not allow the addition of any chemical preservative in yogurt, besides those naturally produced through fermentation (e.g., lactic acid). In this study, we thus tried to increase the shelf-life of sheep yogurt following a biocontrol approach. For this, we initially isolated LAB strains (n > 100) from raw sheep milk samples, grouped them (through repPCR) and identified them to the species level (through 16S rDNA sequencing). At the same time, the dominant yeast species provoking the spoilage of this yogurt were also isolated and identified. Next, we screened a panel of ca. 50 of those LAB isolates for antimicrobial action against those dominant yeast isolates (n = 12) following an agar well diffusion assay. Those LAB isolates were also tested for survival under in vitro gastrointestinal conditions (exposure to: pH 2.5 for 2 h, 1% w/v bile salts for 3 h) to investigate potential probiotic abilities. In the future, we plan to study some more probiotic properties of those LAB isolates (e.g., antimicrobial action against human pathogens, adherence to intestinal cells, antibiotic resistance) and hopefully include the most promising as adjunct bioprotective and probiotic cultures during the manufacture of Greek traditional sheep yogurt.

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P6.24 Implementation of Multispectral Imaging for spoilage detection of sliced turkey ham (e-PLATON)

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Applying a rapid, non-destructive method as a tool for microbial spoilage assessment, has become an area of major interest in recent times.
The aim of this study was to investigate the correlation (via multivariate analysis) between conventional microbiological analysis and multispectral imaging (MSI) to predict the spoilage of sliced turkey ham.
Turkey ham (cooked and smoked) slices (n=120) were placed in polypropylene bags and stored aerobically at 5, 12, and 20 °C until spoilage, simulating the period from slicing at retail to consumer. During storage, samples were analyzed microbiologically for the enumeration of Total Viable Counts (TVC) and Lactic acid bacteria (LAB). In addition to microbiological analysis, MSI spectral data from 19 wavelengths (395-970nm; VideometerLab 2) were collected from the surface of the sliced ham samples. Partial Least Squares- Regression (PLS-R) models were applied to assess TVC and LAB counts during storage (software Unscrambler)., via MSI data MSI data were pre-treated either with Standard Normal Variate (SNV) transformation or used to calculate Area under spectrum (Area= width x height). Independent experiments at 12oC using different batches of turkey ham were used for model validation.
Microbiological experiments showed that storage temperature affected the growth of microorganisms. Regardless of sample type (cooked or smoked) the specific spoilage organisms for turkey ham samples stored aerobically were LAB. PLS-R models exhibited good performance and the values of correlation coefficient (r) and root mean square error (RMSE) for the prediction of LAB were 0.871 and 0.52, respectively, while for the prediction of TVC were 0.79 and 0.86. Furthermore, it was found that the wavelengths with the highest effect on model performance were associated with reflectance by myoglobin, oxymyoglobin and metmyoglobin.
The results are indicative of the potential application of multispectral imaging as a rapid and non-destructive technique to predict the microbiological quality of sliced ready-to-eat meat products.

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P6.25 Evaluation of Sparus aurata fish quality, using FTIR spectroscopy, microbiological and sensory analysis

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Introduction: The microbial development is the most important factor affected fish shelf life. The comparison of the microbiological methods with the innovative technique of Fourier Transform Infrared Spectroscopy (FTIR) for the prediction of microbial growth and fish spoilage presents significant research interest.
Purpose: The aim of the present study was to evaluate the microbial spoilage of gilthead sea bream (Sparus aurata) by using FTIR spectroscopy compared to microbiological and sensory analysis.
Methods: Aqua cultured gilthead sea bream was stored aerobically at 0, 4, 8 and 12 oC. Duplicate samples from the dorsal half were subjected to standard microbiological analysis for the enumeration of Total Viable Counts, Pseudomonas spp., H2S-producing bacteria, Brochothrix thermosphacta, Enterobacteriaceae, Lactic Acid Bacteria and yeasts. Sensory analysis of odor, gills color and skin color was performed. FTIR of fish skin spectra were measured and data analysis was performed using multivariate methods included in the Unscrambler program.
Results: Based on TVC results, fish samples were indicating as spoiled at 0, 4, 8 and 12oC, after 189, 123, 67 and 43 h, respectively. Pseudomonas spp. and H2S-producing bacteria were the predominant fish spoilage bacteria at all temperatures, while Enterobacteriaceae, Br. thermosphacta, yeasts and LAB populations were found at lower levels. Sensory evaluation was in accordance with the microbiological results for fish stored at 0 and 4oC in contrast to 8 and 12 oC. The estimated model based on the FTIR spectra of fish exhibited a partially credible performance as the coefficient of determination (R2) value was 0.83, whereas the values of the root mean square error (RMSE) and the slope were approaching 0.9.
Significance: Pseudomonas spp. and H2S-producing bacteria were the dominant spoilage microorganisms in Sparus aurata at all temperatures storage trials. FTIR spectroscopy seems to be promising for the rapid and non-invasive assessment of the microbiological quality of whole gilthead sea bream.

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P6.26 Microbial safety and sensory properties of cold-smoked salmon produced with salt replacers and organic acid salts

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Certain levels of added salt are required to obtain cold-smoked (CS) salmon with characteristic taste, functionality, processability, microbial stability and shelf life. However, excess dietary sodium intake is associated with an array of health complications, and CS salmon may contain high levels of sodium salt. CS salmon may also represent a food safety risk due to possible presence of the foodborne pathogen Listeria monocytogenes which may cause fatal human infections. To reduce the health burden, improve competitiveness, and meet the demands of consumers and authorities for healthy, safe CS salmon, there is a need for the salmon processing industry to reformulate CS salmon products to reduce the risks associated with content of both sodium and L. monocytogenes. The aim of the current study was to determine how the use of sodium-reduced salt replacers containing KCl and acetate-based preservative salts affect microbial safety, quality and sensory properties of CS salmon. CS salmon was produced by partial replacement of sodium with potassium using KCl and commercial sodium-reduced mineral salts. Overall, only minor sensory changes were obtained in sodium-replaced CS salmon using seven commercial salt replacers compared with a conventional product with NaCl. Growth of L. monocytogenes was temperature-dependent (4°C vs. 8°C storage) with similar growth in sodium reduced and conventional CS salmon. Addition of 0.9% of the acetate-containing preservative salts Provian K or Provian NDV gave up to 4 log lower L. monocytogenes counts in both sodium-reduced and conventional CS salmon during storage. Analyses of CS salmon produced with selected mineral salt and preservative salt combinations in an industrial salmon smokery showed marginal differences in sensory properties. Samples with the preservative salt Provian NDV provided L. monocytogenes growth inhibition and low-level total counts (<2.8 log cfu/g) dominated by Photobacterium and Carnobacterium during storage. Production of sodium-reduced CS salmon with inhibiting salts provides a simple method to achieve a healthier food product with increased food safety.

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P6.27 Listeria monocytogenes in a Norwegian Atlantic salmon processing environment – a study of antimicrobial resistance and efficacy of disinfectants

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The presence of Listeria monocytogenes is a continuous microbiological challenge in the salmon industry which invests a substantial effort to prevent the persistence of the bacterium in the food processing environment. L. monocytogenes is the causative agent of human listeriosis, and poses a potential risk for consumers, especially those belonging to risk groups. The popularity of raw Atlantic salmon as a ready-to-eat product, such as sushi and sashimi, applies pressure on hygienic practices during production, including efficient cleaning and disinfection of the processing environment. The use of antibiotics in Norwegian aquaculture is highly restricted. However, the extensive use of disinfective agents has been suggested as a potential driver of antimicrobial resistance (AMR) in bacteria.
The objective of the study was to assess the diversity of L. monocytogenes isolates (n=49) sampled from a Norwegian salmon processing facility over a period of eight months. The strains were screened for their molecular serotype, susceptibility to a panel of 16 clinically and veterinary relevant antibiotics and two commonly used disinfectants.
As observed in comparable environments, the serogroup diversity was low. Most of the isolates (86%) were assigned to serogroup 1 (1/2 a, 3a), and the remaining strains to serogroup 3 (1/2 b, 3b). Six distinct AMR profiles were observed. All isolates were resistant to clindamycin, oxolinic acid and trimethoprim/sulfamethoxazole and thus classified as multidrug resistant (resistant to ≥3 antibiotics of different classes). All resistance levels (susceptible, intermediate, and resistant) were observed for cefotaxime. In serogroup 3, all isolates (n=7) showed an identical AMR profile, whereas the AMR profile diversity was high in serogroup 1. A selection of strains belonging to different serogroups and AMR profiles were tested for their susceptibility to two disinfectants in a microscale minimum inhibitory concentration (MIC) assay at different temperatures. The results will be presented in more detail at the conference.
The presence of opportunistic pathogenic and multidrug resistant L. monocytogenes in salmon processing environments calls for a continuous effort to prevent these bacteria from establishing in the processing environment. Furthermore, knowledge about the efficacy of disinfectants on specific bacterial species is needed to optimize protocols for disinfection routines.

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P6.28 Increasing shelf-life of poultry

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Poultry meat has increasing popularity and is the most important meat consumed worldwide. Chicken processing involves a number of steps and is therefore exposed to microbial contamination, and the initial bacterial load on carcasses is often relatively high (4 - 5 log cfu/cm2). Fresh chicken meat is a highly perishable product due to high nutrient and water content. Deterioration can be caused by microbial growth, oxidative damage, discoloration, formation of off-flavours and off-odours, nutrient loss, texture changes, and pathogenicity. With the increasing demand for high quality, convenience, safety, and a fresh appearance, there is a great interest in technologies that extend the shelf life of poultry meat.
Fresh chicken wings, used as a model for poultry carcasses, were subjected to treatments with solutions of fermented vinegars, mixes of potassium acetate and potassium diacetate, and lactic acid at different pH (3.0 and 3.9). Samples were submerged in solutions of different concentrations (1 – 10%) for varying times (5 – 300s) and thereafter stored in vacuum and modified atmospheres with high carbon dioxide: CO2/N2 (60:40) or high oxygen (O2/N2) (75:25) for up to 20 days. Control samples stored in vacuum at 4 °C reached 7 log (considered as spoilage limit) after only 4 days. By immersion in 5 % vinegar fermentates for 30 s, bacterial growth of the chicken microbiota was reduced so that the vacuum samples reached 7 log after 9 days. By storing similar samples in high carbon dioxide gas, 7 log was reached after 13 days. When treating the samples successively with lactic acid and fermented vinegar 7 log was reached at day 20 for vacuum packed samples. High carbon dioxide packages reached 6 log at day 20. Sensory analysis after 6 days of storage showed that treated samples scored significantly lower on cloying, sour, and sulfuric smell compared with the controls. The microbiota after storage varied with the compounds used and the packaging conditions. In summary, the use of organic salts and acids provides a simple way to increase shelf-life of raw poultry products.

P6.29 Antimicrobial effect of different extracts from the plant guápala (Simira ecuadorensis)

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The objective of this work was to evaluate the antimicrobial effect of 4 extracts obtained from Simira ecuadorensis, a plant native to Ecuador and Peru, against 19 pathogenic and spoilage microbial strains that affect the food industry. The bacteria selected were: Salmonella enterica, Escherichia coli, Clostridium perfringens, Staphylococcus aureus, Bacillus cereus, Campylobacter jejuni, Listeria monocytogenes, Listeria innocua, Shigella sonnei, Yersinia enterocolítica, Vibrio alginolyticus, Shewanella putrefaciens, Enterococcus faecalis, Aeromona caviae, Leuconostoc mesenteroides, Weissella viridescens, Pseudomonas putida, Pseudomonas fluorescens and Brochothrix thermosphacta. The agar diffusion method was used to study the antimicrobial effect. Four extracts from Simira ecuadorensis were used, SE ETOH (extracted with ethanol), SE ETOH-H2O (extracted with ethanol and water) SE H2O (extracted with water and lyophilized) and SE ATOM (extracted with water and atomized). The concentration used was 80 mg of extract / mL of solution (H2O:DMSO, 1:1), and with the results obtained, the concentrations of 40, 20, 10 and 5 mg of extract/ mL of solution were tested for C. jejuni, L. mesenteroides and S. putrefaciens, to establish the minimal inhibitory concentration (MIC).
The main results obtained when testing 80 mg / mL, was the antimicrobial against C. jejuni, S. putrefaciens and L. mesenteroides, being the extract SE ETOH-H2O the one that had the greatest effect followed by SE H2O > SE ATOM > SE ETOH (the latest did not affect S. putrefaciens). Regarding to the MIC, 80 mg / mL was established for these 3 microorganisms in all the extracts, except for SE ETOH-H2O extract for L. mesenteroides which was 40 mg / mL.
Therefore, it could be concluded that extracts obtained from Simira ecuadorensis extracted with ethanol and water, were the ones with the greatest antimicrobial potential to improve food safety and quality.

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P6.30 User-friendly tools to assess the food safety impact of innovations in the formulations of dry-fermented sausages

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Dry-fermented sausages (FS) are traditional food products frequently subjected to innovations to meet consumer demands, e.g. towards clean label or nutritionally improved formulations. Potential implications on the microbial safety of these innovative FS should be assessed throughout the production processes and distribution chain, which can be done through the application of available mathematical models integrated in user-friendly tools. Stakeholders just need to introduce the values of intrinsic and extrinsic factors as inputs to simulate the microbial response in terms of growth or inactivation. The aim of this work was to evaluate the change of behaviour of foodborne pathogens due to modifications of FS formulation, including fat reduction, sodium chloride (NaCl) reduction/replacement and nitrite elimination, using user-friendly tools available at http://www.dmripredict.dk. According to the "Yersinia enterocolitica" tool, a fat reduction from 20% to 10% would hardly impact the pathogen inactivation, as only 0.3-Log10 units difference on the inactivation was predicted. "ConFerm" tool allowed to comparatively quantify the impact of added NaCl on the inactivation of Salmonella, Escherichia coli and Listeria monocytogenes, being the later the most resistant. The reduction of NaCl from 3.5% to 1% could impair the inactivation of L. monocytogenes (1.4-Log10 less), Salmonella (3.7-Log10 less) and E. Coli (5.1-Log10 less). However, from the technological perspective, NaCl is usually replaced with equimolecular concentration of other salts and minimal impact in the pathogen inactivation is predicted when assessing 2% NaCl substitution with 2.5% KCl (about 0.2-Log10 difference). The effect of reducing nitrite from 150 ppm to 50 ppm or total removal is stronger in L. monocytogenes than in enteric pathogens. Considering a low-acid (pH=5.4) process, no inactivation of L. monocytogenes would occur in a nitrite-free formulation, while in an acid (pH=4.8) process the predicted inactivation was 1.6-Log10 lower compared with 150 ppm. Based on "Staphtox predictor", at a fermentation temperature of 22°C, 100 and 150 ppm of nitrite are needed to prevent S. aureus growth in acid and low-acid FS, respectively. The outputs of the assessments performed with these predictive tools can support management decisions and guide the innovation strategies to control microbiological hazards in FS.

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P6.31 Development and application of formulated endophytic fungus for novel plant growth strategies

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Most beneficial endophytic fungi are part of the microbial communities of plants and are directly related to promoting plant health and resistance to the abiotic stresses and facilitating their nutrition. Fusarium solani K (FsK) strain is such a fungus which is capable of conferring biocontrol of pathogenic fungi in both roots and leaves and protecting tomato plants, including tomato fruits, from the negative effects of reduced water availability. It can therefore be used as a promising alternative microbial inoculum in agriculture. The characteristics of these inocula can be greatly improved by encapsulation systems and suitable properties of the biopolymers.
In this study, we aimed at the selective propagation of finely dispersed Fusarium solani K (FsK) strain mycelium in submerged culture and encapsulation in calcium alginate/starch beads to protect the fungus during drying, enable growth in different soils and cultivation media and promote endophytism in tomato plants.
We found that a combination of culture conditions promoted selective formation of finely dispersed mycelium reflected by 4.5-fold decreased pellet diameters, 10-fold increased mycelial biomass concentrations and low blastospore contents of 52 ×106 mL-1 after 48 h. Encapsulation of mycelium enhanced survival under drying by 29.14%. Co-encapsulated starch served as a nutrient source for growth media with best results on sterile and non-sterile peat substrate with 3.99 mm and 4.28 mm radial mycelial growth, respectively.
This study provides the first evidence that survival of FsK mycelium can be substantially improved by encapsulation and that encapsulated FsK is able to grow out of beads in non-sterile soils. These results may provide the basis for future work on increasing efficacy in plant protection strategies against pathogens, enhancing inoculum shelf-life and improving endophytism by formulation technologies.
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P6.32

Polylactic acid-based EMA Packaging containing a polyhydroxybutyrate sachet with carvacrol as an alternative packaging for cherry tomatoes (BIOSTROFI)

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Active packaging is an emerging technology that allows controlled release of antimicrobial compounds like essential oils; however, their intense flavor may limit direct application in foods. Thus, controlling their release via incorporation in sachets suggest a promising alternative. The replacement of difficult reusable plastics like polypropylene by biobased biodegradable materials i.e., polylactic acid (PLA) and polyhydroxy butyrate (PHB) is an increasing trend.
The aim of this study was to develop an active PLA-based Equilibrium Modified Active Packaging (EMAP) with a PHB-sachet containing carvacrol to extent the shelf-life of cherry tomatoes.
Cherry tomatoes were placed in paper-kraft trays and packaged in PLA-based micro-perforated EMAP system containing a PHB-sachet with carvacrol (PLA-PHB-CARV) and stored at 15 and 25°C for up to 40 days. Samples packaged either in macro-perforated polypropylene (PP) or PLA-based micro-perforated film without carvacrol were used as controls. Weight loss, visible decay, headspace gases, pH, titratable acidity (TA), total suspended solids (TSS), ripening index (TSS/TA), color, texture and total viable counts (TVC) were monitored throughout storage. Organic acids and sugars were quantified via HPLC-UV/RI, while sensory analysis was also performed.
Visible decay was 40% in PLA-PHB-CARV, while PP samples reached 97% at 25°C (day-20), rendering cherry tomatoes in PP not acceptable by the sensory panel on day 14. PLA-PHB-CARV showed lower weight loss compared to controls, while limited variation was recorded on firmness among treatments, at both temperatures. TSS and TA remained stable at 15°C, while, at 25°C, TSS measured in PLA-PHB-CARV remained stable, contrary to the increasing trend in PLA and PP, resulting in faster ripening. This TSS increasing trend in PP and PLA correlates with the increase of glucose and fructose concentration throughout storage, at 25°C. Carvacrol addition significantly inhibited TVC compared to PP and PLA by ca. 2.0 log CFU/g, at 15 and 25°C. Color change in PLA-PHB-CARV was higher compared to PP and PLA, however sensory evaluation based on color did not reveal significant differences among treatments.
Applications using bio-based biodegradable materials like PLA and PHB and essential oils vapors may raise new perspectives on active antimicrobial EMAP in order to extend shelf-life of cherry tomatoes.

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P6.33 Impact of Phytochemicals on Viability and Cereulide Toxin Synthesis in Bacillus cereus

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The emetic toxin cereulide, a 1.2 kDa small dodecadepsipeptide produced by Bacillus cereus, is responsible for food intoxications leading to illness, vomiting, and, in some cases, also to severe organ failures occasionally resulting in fatalities. The chemical structure of the cereulide toxin, which is usually pre-formed in contaminated foods, makes it resistant against heat-treatment, proteolysis, and hydrolysis. Because of its small size, cereulide cannot be removed from contaminated food and food ingredients by filtration. Thus, strategies to prevent cereulide formation in food production and processing are of utmost importance.

In frame of this study, we tested structurally related phytochemicals (n=40), including benzene derivatives, monoterpenes, hydroxycinnamic acid derivatives and vitamins, on their inhibitory effects on growth of B. cereus and production of cereulide. For this purpose, we developed a high-throughput method that allows to analyze B. cereus survival and cereulide production simultaneously by coupling a viability assay based on the tetrazolium-based redox dye AlamarBlue with ultraperformance liquid chromatography-mass spectrometry (UPLC-MS/MS). Our approach revealed a variety of substances that showed inhibitory effects of B. cereus growth at very low concentrations (<0.1 µg/mL). The highest growth inhibitor potential was found among substances belonging to the group of benzene derivatives and vitamins, respectively. Notably, certain compounds showed no or only a moderate effect on viability (> 85%), but led to a sharp decline in cereulide production (< 15%), indicating a specific inhibitory effect on cereulide biosynthesis.

In summary, our newly developed combinatory assay was successfully applied to investigate the inhibitory potential of phytochemicals, naturally occurring as food ingredients or commonly used as food additives, against B. cereus growth and cereulide synthesis in one workflow. The results of this work might contribute to food safety by enhancing the knowledge of the antibacterial effect of naturally occurring plant compounds, in order to minimize the risk of bacterial contaminations and food intoxications.

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P6.34 Bioactive compounds from Rhodiola rosea extract reduce Campylobacter jejuni quorum sensing

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Overuse of antibiotics in veterinary and human medicine is responsible for the development of antibiotic-resistant strains. Searching for novel strategies and antimicrobial agents to control Campylobacter jejuni, the most prevalent food-borne pathogen, is of utmost importance according to the regulatory agencies, including the FDA and the ECDC.

The aim of this study was to i) prepare extracts from Rhodiola rosea cultivated plant material, i.e. Mattmark (the first synthetic cultivar of R. rosea) and Rosavine, ii) separate biologically active compounds or compound groups from the initial extracts into different fractions, iii) investigate antimicrobial activity, with emphasis on the anti-quorum sensing (QS) activity, of the extracts and their fractions in C. jejuni.

Extracts from the plant underground organs were prepared by ultrasonic extraction using denatured 96% (v/v) ethanol. Fractionation of the extracts was done on DIAION HP-20 adsorbent resin or using Polyclar AT (polyvinylpyrrolidone, PVP). Different concentrations of methanol (0-70% (v/v)) were used for elution of the compounds. Extracts and their fractions were phytochemically characterized by UHPLC-PDA-ESI-MS analysis. The minimal inhibitory concentrations (MIC) of the preparations against C. jejuni were determined by broth microdilution method. Autoinducer-2 (AI-2) bioassays were performed to evaluate their influence on C. jejuni QS. Disruption of membrane integrity was determined using LIVE/DEAD BacLight Bacterial Viability kit.

Eighteen compounds were identified in "Mattmark" ethanolic extract and seventeen in "Rosavine" ethanolic extract. Five fractions, rich in salidroside, rosavins, proanthocyanidins (PACs) and/or flavonoids, were obtained from each of them. Fractions rich in PACs had the strongest antimicrobial effect against C. jejuni, according to the lowest MICs and the highest anti-QS activity, while the bacterial membrane remained intact. Fractions without PACs were less effective.

Compounds that influence bacterial QS are known to be able to decrease their pathogenicity. We found that PACs are crucial compounds responsible for previously reported anti-QS activity of R. rosea ethanolic extract. As far as quantification of salidroside, rosavin and rosarin is the key factor determining the quality of R. rosea pharmaceutical preparations, PACs could be separated from the initial extracts and used in control of food-borne pathogens, such as C. jejuni.

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P6.35 Effect of organic acids on the growth of yeasts and molds in shelf-stable fruit filling

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Shelf stable fruit fillings are generally subject to long shelf life and slow spoilage. Shelf life for 12 to 18 months is common at temperatures of 25°C. Shelf stable fruit fillings have a broad range of pH(3.5-5.5), water activity (0.86-0.97) and can be spoiled with yeasts or fungi. In this study the effect of organic acids within these environmental ranges was investigated. Lactic- and acetic acid were tested at three concentrations. A control without preservatives, as well as samples containing potassium sorbate and sodium benzoate were included as references. Microorganism cocktails were selected with an origin in low pH products. One yeast cocktail consisted of more organic acid resistant strains than the other cocktail. Furthermore, a fungi cocktail was tested against organic acids.
To have the possibility to also use the data outside the fruit filling application, Malt extract broth was chosen as a model. Strains for the cocktails were separately grown and mixed equally to a cocktail and inoculated ~log 2 CFU/g in the model. Samples in triplicate were stored at 25°C and at appropriate time intervals measured during 78 weeks. If an increase of more than 2 log CFU/g was observed samples were marked as growth.
A fruit filling at pH 3.5 and water activity of 0.97 was selected to validate the data from the "fruit filling broth model". Microorganism cocktails were inoculated and colony counts were measured for 7 weeks.
Data covering the complete range of fruit fillings with respect to pH, water activity, spoilage organism and organic acids. Data from the fruit fillings show that the observation in malt extract broth are similar as in the fruit filling and that the model data can be used for growth predictions in fruit fillings.
A better understanding of the effect of different acids on the different spoilage flora will lead to better and specific interventions to elongate the shelf life.

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P6.36 An improved model for the growth of Bacillus cereus sensu lato: an approach based on phylogenetic affiliation

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Introduction: Sym'Previus (www.symprevius.eu) is a decision support software for prediction microbiological data. It integrates models for predicting bacterial growth, growth limits and thermal inactivation for a number of pathogenic microorganisms, including Bacillus cereus. This microorganism is a common foodborne pathogen, and its broad diversity is difficult to assess and as a source of difficulty for risk assessment. The phylogenetic structure of the B. cereus Group was recently resolved, resulting in seven different phylogenetic groups that exhibit high differences in their response to temperature and their ability to cause food poisoning. The objective of this study is to integrate this classification system into the Sym'Previus models for Bacillus cereus to improve prediction performance.
Methodology: Growth models were developed for each phylogenetic group (from Group II to Group VII). The developed models are based on the Gamma concept (multiplicative effects of environmental factors) and account for the effects of temperature, pH and water activity. The (intra-group) strain variability is incorporated in the model by using probability distributions for these strain-dependent parameters (e.g., cardinal temperatures for growth). Several pH terms were also assessed for improved performance at pH levels below 5.5. Model predictions were compared with literature data generated in different products.
Results: In comparison with the original Sym'Previus B. cereus growth model, this new model integrates growth data on 33 additional strains of Bacillus cereus sensu lato. The classical cardinal pH model was not found to be the best performing model and an alternative pH term was suggested taking into account an increasing effect of the pH at value closed to the pH growth limits. The growth parameters based on clustering the B. cereus strains into phylogenetic groups improved the model performance compared to the classical approach.
Conclusion and relevance : The affiliation to phylogenetic groups enables improving the model predictions by taking into account the biodiversity encountered. It is expected that combining group specific heat inactivation models with the developed growth models will allows improved exposure assessment of the B. cereus in foods.

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P6.37 The effects of food processing factors on the growth kinetics of Aeromonas strains isolated from ready-to-eat (RTE) seafood

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Aeromonas spp. are ubiquitous in fresh, brackish and marine environments, and there is a growing concern over Aeromonas spp. as foodborne pathogens, particularly in connection with consumption of seafood. The aim of this study was to evaluate the effects of different food processing factors on the growth kinetics of Aeromonas strains isolated from RTE seafood. Growth kinetics of eight Aeromonas strains were investigated at different storage temperatures (4, 8, and 15 °C) in Tryptone Soy Broth (TSB) and a salmon juice model system. In addition, the growth at low temperatures in combination with NaCl contents (1.5, 3.0, 4.5, and 6.0 %) or with two atomized purified condensate smokes (PCS) (Red Arrow™ SmokEz VTABB and JJT01) at different concentration (0.026, 0.13 and 0.26 %) was studied. Specific growth rates, duration of lag phase and maximum population density were estimated based on primary model of Baranyi and Roberts. Most of the strains were able to grow in the salmon juice media at all temperature, although specific growth rate was significantly lower in this media compared to TSB ($p < 0.05$). In addition, refrigeration temperature (4 °C) was not sufficient to inhibit the growth of A. media, A. bestiarum, A. piscicola, and A. salmonicida. They were still able to grow up to 3.0 % NaCl content at 4 °C. On the other hand, the addition of PCS at maximal legal concentration (0.26 %) completely inhibited the growth of Aeromonas strains even at 8 °C, except A. salmonicida and A. piscicola. Moreover, the growth kinetics of three Aeromonas strains were further studied in vacuum-packed fresh and cold-smoked salmon stored at 4°C for 14 and 21 days, respectively. The result demonstrates that vacuum packing and cold storage at 4 °C was not sufficient to inhibit the growth of all strains, while cold smoking was able to inhibit the growth of two strains. This study suggests that commonly used food processing conditions to produce RTE seafood might not be sufficient to inhibit the growth of Aeromonas. On the other hand, addition of PCS could be a good alternative technique to prevent the potential growth of Aeromonas.A better understanding of the effect of different acids on the different spoilage flora will lead to better and specific interventions to elongate the shelf life.

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P6.38 Comparison of the protective effect in cold-smoked salmon of three Carnobacterium strains against Listeria monocytogenes and impact on microbiome, chemical and sensory quality

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Cold-smoked salmon (CSS) is a leading fish product in Europe. However, Listeria monocytogenes is frequently detected at the processing plant level and can grow during storage, sometimes overpassing the 100 CFU/g European regulatory limit. Biopreservation is a mild preservation technology that consists in introducing in food selected bacteria at high concentration to reduce growth of undesirable microorganisms. Three strains of lactic acid bacteria (Carnobacterium divergens Cd1 and C. maltaromaticum Cm2 and Cm3) with different bacteriocin encoding genes, were selected, produced as freeze-dried powder and tested (10^6 CFU/g) in CSS artificially contaminated by Listeria monocytogenes (10^3 CFU/g). The effect on microbial ecosystem, chemical and sensory quality was also evaluated. The 3 carnobacteria had a similar effect on L. monocytogenes and almost totally inhibited its growth all over the vacuum storage at 4°C (1 week) followed by 8°C (3 weeks). Cm3 was tested at 3 initial concentrations (10^6, 3x10^5 and 10^5 CFU/g) and the higher the initial concentration the higher the inhibition. At the end of storage when the control was rejected by the sensory panel, all the strains had a beneficial effect. However, Cm2 released off-odor and flavor (sulfur, acid, amine) at day 14, when the control was still acceptable and so cannot be retained for an industrial application. The link between sensory characteristics and endogenous microbiota was difficult to establish. The strains reduced Brochothrix and Enterobacteriaceae counts (known as spoilers in CSS) but Cm3 had the lowest inhibitory effect although it had the highest positive impact on the quality. Concentrations of histamine, tyramine and cadaverine were generally higher in bioprotected product, and sometimes histamine overpass the EU limit 100 mg/kg. To conclude, as L. monocytogenes contamination is generally far lower than the one used in this study, C. maltaromaticum Cm3 at 10^5 CFU/g can be used to limit the risk of L. monocytogenes without organoleptic or histamine side effect. This study highlights the complex relation between bioprotective strains and global ecosystem, leading to results sometimes different than those observed in sterile CSS. For the first time a C. maltaromaticum strain was selected to improve simultaneously quality and safety of CSS.

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P6.39 Isolation and Characterization of Aeromonas hydrophila lytic Bacteriophages Obtained from Selected Aquaculture Farms in Uganda as a food safety biocontrol agent

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Aeromonas hydrophila is commonly found in various ecosystems including the aquatic environments, sewage, and foods. It is a food and water-borne zoonotic pathogen associated with diarrhoea, wound and various systemic infections among immunocompromised humans. It causes aeromoniasis in fish, an important disease to the aquaculture sector. Risks to human infections include handling infected fish, ingestion of contaminated water and food; and contact with contaminated soil. Aeromonas spp are prevalent on fish farms in Uganda and high disease cases as well as drug resistance have been reported. Antibiotic use in fish farms is discouraged, however there are no alternative methods for managing Aeromonas contamination/infection. This study aimed at providing a safe bio-control decontamination solution against Aeromonas hydrophila. Specific objectives included the determination of the drug susceptibility of Aeromonas isolates from diseased tilapia; and establishment of a stock of Aeromonas hydrophila bacteriophages. Aeromonas spp isolates were tested for antimicrobial resistance against 10 antibiotics Chloramphenicol, Penicillin, Tetracycline, Nalidixic acid, Nitrofurantoin, Streptomycin, Trimethoprim / Sulphamethoxazole, Ampicillin,Ciprofloxacin and Gentamycin, using the Kirby Bauer disk diffusion test. The double agar overlay method and spot assay were employed for phage isolation, purification and characterization. Susceptibility of the isolates to various antibiotics ranged from 0% to 100% with Ciprofloxacin and Gentamycin being more effective. For bacteriophages, three lytic bacteriophages with the highest host range (78.6% to 92.8%, n=28) were selected. The phages had a burst period of20–40 minutes, latent period varied from 10 to 40 minutes; with a burst size of 98–171 virions per infecting cell. For stability at various temperatures, there were no significant changes in phage titres on exposure at 40°C and 50°C for 60 minutes. However, gradual decrease in the titres was observed at 60°C while the fast decline was noted beyond 60°C. At storage temperatures, percent reduction in phage titres was by 30% and 40% at 4°C and -20°C respectively. The high phage host range, stability at temperatures where they are likely to be applied in the management of the Aeromonasis; and the high occurrence of drug-resistance support the need for developing the phage isolates for management of the contamination in fish.

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P6.40 Selection of bacterial strains combining probiotic and bioprotective activities: from fish farm to consumer

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Probiotic bacteria are included in aquafeeds for beneficial outcomes on fish health and growth. Furthermore, biopreservative bacterial strains are added to fish fillets after food processing to improve food safety and quality by inhibiting the growth of human pathogens and spoilage bacteria. Prior to use, probiotics and bioprotective strains require the same analysis approach: strain identification and characterisation, as well as estimation of safety and antimicrobial abilities. In the EU project MASTER, we aim at following the impact of probiotic strains both on fish farming and on fillet quality.

Twenty-one bacterial strains belonging to nine genera (Carnobacterium, Lactobacillus, Lactococcus, Pediococcus, Vagococcus, Weissella, Arthrobacter, Enterococcus) were selected from strain collections at Ifremer and Matis for screening of potential use. The strains were tested for their probiotic potential (simulated gastric juice and fish bile resistance, antimicrobial activities against human and fish pathogens and seafood spoiling bacteria, auto-aggregation potential). Their safety characteristics were estimated assessed by hemolytic activity tests, antibiotics resistance and histamine production capacities.

According to these results, ten potential probiotic strains were selected for a fish growth trial with juvenile Arctic charr (Salvelinus alpinus) over an eight weeks period. Strains were included in aquafeeds containing three alternative protein sources and/or three prebiotic substances. Growth rates and gut and skin microbiomes were analyzed during the trial. Two lactic acid bacterial strains, (Carnobacterium divergens and Lactobacillus plantarum), improved the growth rate of the fish, whereas the C. divergens strain also inhibits growth of Listeria in seafood. The two strains will be further tested in a whole food chain system of Atlantic salmon (Salmo salar) farming, where their persistence and bioprotective effects, will be evaluated on the end product of cold-smoked salmon.

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P6.41 Isolation and Characterization of Aeromonas hydrophila lytic Bacteriophages Obtained from Selected Aquaculture Farms in Uganda as a food safety biocontrol agent

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Background: Nowadays, there are 600 million cases with diarrheal diseases per year and 420,000 deaths associated with these illnesses. In this context, the most common foodborne pathogens which are major contributors to these diarrheal infections are Salmonella enterica, Campylobacter spp. and Escherichia coli. The epidemiological data highlight the importance of robust sanitization methods in the food industry. Thus, there is a need for non-thermal sanitization technologies that are also free of dangerous chemicals. Plasma-activated water (PAW) has emerged as one of the most promising sanitizing agent for both clinical settings and food industry plants. The goal of this study was to comparatively evaluate the ability of PAW and plasma-activated saline (PAS) to inactivate the major foodborne pathogens.
Materials and methods: PAW and PAS were prepared using distilled water and saline solution (NaCl 1.5 % w/v) and a GlidArc reactor as previously described. Their chemical composition was evaluated using specific analytical methods. Suspensions of different strains of foodborne pathogens (10^9 CFU/mL) were treated with PAW and PAS respectively, for different periods of time (1, 3, 5 and 7 minutes) and known volumes of mixture were transferred on appropriate solid media in order to determine the number of residual viable bacteria.
As testing microorganisms, we used different type strains: Escherichia coli O157 CECT 4972, Salmonella Typhimurium ATCC 1866, Salmonella Enteritidis ATCC 13076, Listeria monocytogenes ATCC 19114, Staphylococcus aureus ATCC 6583, Campylobacter coli ATCC 33559, Campylobacter jejuni ATCC 33560. In order to assess the PAW / PAS interactions with bacterial cell structures, some instrumental analysis methods were used: UV Spectrophotometry, Atomic Force Microscopy (AFM), Fourier Transform Infrared Spectrometry (FT-IR), and Dynamic Light Scattering (DLS).
Results: A 7log reduction of viable bacteria was achieved after one minute for both PAW and PAS, excepting for S. aureus where the same level was observed after 3 minutes for PAS and 5 minutes for PAW. The results obtained by instrumental analysis are consistent with impairment of various bacterial structures.
Conclusions: PAW and PAS could be promising food grade sanitizers and their influence on various food subtrates remains to be investigated.

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P6.42 Antibacterial marinades can control Salmonella enterica in fresh chicken meat

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Poultry meat is increasingly popular among consumers, with over 137 million tonnes produced globally in 2020. During poultry processing of cross-contamination with broiler microbiome-associated pathogens such as Salmonella is commonly observed. Salmonella enterica can cause food poisoning if meat is not suitably prepared, and poses a particular risk to immunocompromised and vulnerable consumers. However, antimicrobial interventions intended to reduce this risk may not appeal to consumers, who may prefer minimal or natural interventions. Citrus juices and essential oils are natural ingredients which have antimicrobial effects against Gram-positive and Gram-Negative bacteria. This study explored the incorporation of these ingredients into effective and appealing combinations in order to minimise the risk of salmonellosis from raw chicken.

Three marinades were prepared using optimised concentrations of essential oils; 0.5% (v/v) thyme oil in lemon juice with black pepper (M1), 1.0 %(v/v) lemongrass oil in lime juice with chilli flakes (M2), and 0.5% (v/v) oregano oil with 1.0 % (v/v) basil oil in olive oil with garlic flakes (M3). Chicken wings were inoculated with Salmonella enterica serovar Typhimurium ACTC14028 at a concentration of 106 CFU/g, then stored in one of the three marinades at 4 °C. Throughout the product shelf-life, the abundance of Salmonella, Total Viable Counts, Lactic Acid Bacteria, and psychrophiles were enumerated. Chicken stored overnight in these dips was also assessed for sensory acceptability using a consumer panel (n=50).

M1 and M2 caused significant reductions ($p < 0.05$) in Salmonella recovery over the product shelf-life while increasing the shelf-life of the product. These marinades were based on citrus juices, suggesting that low pH marinades have the potential to increase product safety, particularly when incorporated with essential oils. M3 did not contain a citrus component and showed no significant difference from untreated chicken. In preliminary trials, a focus group found that these dips were broadly acceptable to consumers as formulated with many liking the idea of antimicrobial marinades. The results of this study indicate that the field of antimicrobial marinades represents a promising product to mitigate the risk posed by meat-associated bacteria.

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P6.43 Light-emitting diodes to inactivate foodborne bacteria associated with the fresh produce industry

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Foodborne contamination causes more than 200 diseases, ranging from diarrhea to cancers, and is expected to affect 600 million people worldwide every year. On the other hand, every year, 1.3 billion tonnes of food, approximately one-third of the food produced for consumption, is lost or wasted globally, which also represents a huge waste of resources such as land, water, and energy.
The development of effective disinfection treatment processes will be crucial to help the food industry cope with the inevitable challenges resulting from the increase in the human population and climate change. This project focused on exploring the effectiveness of inactivation of ultraviolet (UV) light-emitting diodes (LEDs) that recently emerged as an alternative to UV mercury lamps. Optimizing inactivation of pathogenic and spoiler bacteria was performed in water and fresh vegetables.
Washing water collected at a food industry was tested unspiked and spiked (with a cocktail of bacteria isolated from ready-to-eat packaged salads as well as Salmonella, Listeria monocytogenes, and Escherichia coli) before and after exposure to LEDs that emit at 255 and 265 nm. Both UV-C LEDs were found to be effective to achieve inactivation of the target bacteria. The results show that two to four log reductions were reached with an extremely low UV fluence of 2 mJ/cm2. Unspiked lettuce and arugula leaves were also exposed to UV-C LEDs and up to two log reductions were achieved after 10 minutes of exposure to three small LEDs. The time needed to achieve a certain level of inactivation will decrease if a higher number of LEDs, higher intensity, and/or shorter distance between the matrix and LEDs is used. No visual damage of the leaves was observed after 10 minutes of exposure to the UV-C LEDs.
The results obtained show that this disinfection system is a promising approach that could be applied to different food matrices to achieve effective inactivation. These results could be of interest to pharmaceutical companies and hospitals to sterilize surfaces and medical devices.

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P6.44 Effect of selected Lacticaseibacillus casei and Lactococcus garvieae against Listeria monocytogenes in traditional soft cheese

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Listeria monocytogenes is a worrying pathogen in ripened foods including traditional ripened cheeses because of its great ubiquity and adaptation to processing conditions. It is necessary to develop control strategies for this pathogenic bacterium, such as the use of autochthonous lactic-acid bacteria (LAB). The objective of this study was to evaluate the antagonistic effect of two selected LAB strains, Lacticaseibacillus casei 116 and Lactococcus garvieae 151, isolated from soft cheeses against L. monocytogenes in "Torta del Casar" cheese. For this, 130 cheeses were made and inoculated with different levels of L. monocytogenes, Lc. casei 116 and Lco. garvieae 151 and divided into different batches. Subsequently, the cheeses were ripened for 90 days following a standard industrial procedure. Moisture content and water activity decreased throughout ripening, with no differences between the batches analyzed. Also, the LAB addition did not affect the color, texture, and sensory characteristics of the cheeses. LAB counts were always greater than 8 log CFU/g during the ripening. L. monocytogenes was reduced during the ripening of "Torta del Casar" cheese. Reductions in L. monocytogenes counts ranged from 1.2 to 1.1 log CFU/g when Lc. casei 116 or Lco. garvieae 151 was co-inoculated together with L. monocytogenes at the lowest concentration (4 log CFU/g) and a reduction of 2.61 and 3.55 log CFU/g when the pathogen was inoculated at the highest concentration (7 log CFU/g), reaching reductions in pathogen counts of up to 5 log CFU/g when Lc. casei 116 was inoculated. From these results, cheese processing itself does not allow the growth of the pathogenic bacteria and the presence of Lc. casei 116 and Lco. garvieae 151 causes a significant reduction of L. monocytogenes without affecting the physicochemical and sensory characteristics of the product. Both strains could be proposed as protective cultures in traditional soft cheeses to avoid the presence of L. monocytogenes from microbial contamination.
This work was supported by INIA through projects RTA-2017-00027-C03-03 and the Junta de Extremadura and FEDER by IB16149, GR15108 and GR18056. I. Martín has received a predoctora (FPU 16/05303). Irene Martín has finished her postgraduate studies in March 2022

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P6.45 Growth of E. Coli as affected by temperature and NaCl addition

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Description of the growth dynamic of E. Coli PSII in UHT milk depending on the incubation temperature as well as growth dynamics in PCA broth depending on temperature and water activity modified with NaCl addition in temperature range from 6 to 46°C was performed. Primary growth parameters were obtained by using Baranyi D-model and they were further analysed in secondary phase of predictive modelling. At 6°C, E. Coli PSII was able to survive for 329h, however at 6.5°C it was able to multiply with time to double 79.1h. Further increase of incubation temperature led to faster growth of the isolate until optimal temperature was reached. By using CTMI model, the cardinal temperatures were calculated as Tmin = 5.05°C, Tmax = 47.03°C and Topt = 40.07°C. They were also confirmed by using Ratkowsky model (Tmin = 4.73°C, Tmax = 49.2°C). In next part, the effect of NaCl addition (expressed by water activity values of 0.991 ±0.002; 0.970 ±0.002; 0.950 ±0.002 and 0.930 ±0.002) on the growth of E. Coli PSII was analysed at temperature range from 6 to 46°C. Based on obtained results it can be concluded that 1.72% addition of NaCl led to E. Coli growth stimulation at 7, 8, 43 and 46°C. In temperature range 8 to 40°C the inhibition of growth was observed depending on increased NaCl addition. The minimum water activity for the growth of E. Coli PSII isolate was 0.95 in the temperature range 18 to 37°C, at aw 0.93 the growth of the isolate at all the incubation temperatures was completely inhibited. Also in this case, the cardinal temperatures for growth were calculated. Besides that, the combined effect of temperature and water activity on the E. Coli PSII growth dynamic was described. At optimal aw values (0.997 – 0.998) and based on CTMI, Ratkowsky model and equations describing the combined factors effect, the minimum temperature for isolate growth is Tmin = 4.82 ± 0.42°C, optimum Topt = 41.1 ±0.80°C and Tmax = 48.03 ±0.94°C will be the maximum temperature that will allow the growth of the isolate.
The work was supported by APVV-19-0031.

P6.46 Characterization of an anti-Listeria bacteriocin produced by Leuconostoc carnosum DH25

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Lactic acid bacteria (LAB) are known to produce strain-specific bacteriocins with highly specific activity against the food pathogen Listeria monocytogenes and show significant promise for applications in biocontrol concepts. In a previous study, Lc. carnosum DH25 was isolated from salted beef and showed high activity against relevant serovars of L. monocytogenes (1/2a, 1/2b, and 4b). A first characterization of the nature of the compound indicated the presence of an anti-Listeria protein of the class of bacteriocins. In this study, the activity of the anti-Listeria bacteriocin of Lc. carnosum DH25 was investigated in a meat simulation medium after incubation at 4°C, 8°C, 12°C, 24°C, and 30 °C and a well diffusion assay (WDA). At 4°C the anti-Listeria activity was first visible after 15 days, at 8°C and 12°C after three days, and at 24°C and 30°C after 20 h. The inhibition zone reached a maximum of 4 mm on day 21 at 4°C, 6 mm on day 10 at 8°C and 12°C, and 5.5 mm after 48 h at 24°C and 30°C. To verify the stability of the bacteriocin, the cell free supernatant (CFS) produced from a 48-h culture of Lc. carnosum DH25 at 30°C was incubated at 4°C, 8°C, and 12°C for 24 h, at 60°C and 100°C for 30 min, and at 120°C for 15 min. The pH of the CFSs was adjusted to pH 2, 3, 4, 6, 8, and 10 and incubated at room temperature for two hours before it was re-set to pH 7. The CFSs incubated at 4°C, 8°C, 12°C, and 60°C did not show reduced inhibition compared to non-treated CFSs, while incubation for 30 min at 100°C reduced the inhibition zone from 5.5 mm to 2 mm, and incubation for 15 min at 120°C resulted in a complete loss of anti-Listeria activity. In terms of pH, the anti-Listeria bacteriocin was stable over a broad range from 2 to 10. The findings of this study demonstrate the ability of Lc. carnosum DH25 to inhibit Listeria even at lower temperatures and that the corresponding anti-Listeria bacteriocin is stable over a broad range of temperatures and pHs.

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P6.47 The Effect of Atmospheric Cold Plasma on Aspergillus chevalieri inactivation and stress responses to sublethal treatments

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Filamentous fungi are important spoiling agents in food commodities. Over time, different preservation techniques have been developed with the aim of discouraging fungal development in food products. Among of new technologies to reduce fungal growth Cold Atmospheric Clasma (CAP) is gained a high importance. This work was aimed to evaluate in vitro, the effects of CAP under NO regime, at different exposure treatment time (5,10, 20, 30 min) in Aspergillus chevalieri, one of the most xerophilic and xerotolerant molds that cause spoilage in nuts, dried beans, spices, etc. We focused our attention on the direct inhibitory effects of CAP treatments on conidia germination, mycelium reduction, and on the responses of A. chevalieri PSJ144 to CAP stress. The results revealed that while the 80% of spore germination was inhibit after 5 min of treatment, 30 min were necessary to inhibit the 70% of the A. chevalieri mycelia. In addition, CAP induced a significant cell membrane depolarization immediately after 5 min of CAP treatment and an increase of intracellular calcium levels during the first 6 hours of incubation at 28°C. Other stress responses of A. chevalieri were related with a high increase of the synthesis of osmolites trehalose, glycerol and synthesis of chitin. Our results suggested that under lethal ACP treatment a variety of specific and highly regulated adaptive responses were provoked, which could protect the filamentous fungi from the stress, in addition our work underline the good potential of cold plasma for fungal inactivation.

P6.48 Effect of lipopolysaccharide truncation on Salmonella Typhimurium membrane fluidity and resistance to stress conditions

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The cell envelopes are considered to be target structures of multiple preservation agents used by the food industry. Therefore, it has been suggested that cell envelopes properties, such as fluidity, could determine the bacterial resistance against several stresses. Lipopolysaccharide (LPS), which is composed of 3 regions (lipid A, core oligosaccharide and O-antigen polysaccharide), is the main component of the outer membrane and is essential for various cellular functions in most Gram-negative bacteria. The aim of this work was to study the effect of LPS truncation on Salmonella Typhimurium membrane fluidity and resistance to heat, pulsed electric fields (PEF) and bile salts. To achieve this objective, membrane fluidity (Generalized Polarization of Laurdan), resistance to heat (58°C), to PEF (25 kV/cm) and to bile salts (0-4%) of S. Typhimurium ATCC 14028 and its isogenic ΔrfaG mutant (not displaying the O-antigen polysaccharide) were determined. Results obtained indicate that LPS truncation has a great impact on membrane fluidity, as the LPS deficient strain showed a significatively higher membrane order, which is correlated with lower membrane fluidity, than the wild type strain. Despite the fact that it could be expected that the ΔrfaG mutant displayed higher resistance to heat than the parental strain, as its membrane was more rigid, results obtained showed that significant differences between both strains could not be found neither with heat treatments at pH 4.0 nor at pH 7.0. Similarly, in the case of the PEF treatments at pH 4.0 and pH 7.0, significant differences between the two strains were not observed. In regard to the bacterial resistance to bile salts, results showed that the ΔrfaG mutant cells were notably more sensitive than the cells of the parental strain. To conclude, all these results suggest that the LPS structure would play a major role in S. Typhimurium envelope properties (membrane fluidity and resistance to bile salts). Nevertheless, the O-antigen polysaccharide does not seem to exert a great impact on S. Typhimurium resistance to heat and PEF.

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P6.49 Fungal and Aflatoxin Progression in Nixtamalized Maize Using Activated Charcoal

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Aflatoxin causes non acceptability of farm produce such as grains at global markets. The aim of this study was to investigate the fungal and aflatoxin load of fermented maize treated with Activated charcoal. Twenty samples (n=20) of dry maize moisture level was adjusted from 12% safe moisture level to 17%, incubated at 28C and assessed for fungi and aflatoxin levels using standard microbiological procedures and High Performance Liquid Chromatography (HPLC) respectively. The result was statistical analysed by one way ANOVA using SPSS version 18.0. Four fungi isolates were isolated from the maize and identified as Aspergillus flavus (2%), Alternaria spp (15%), Saccharomyces cerevisiae (43%) and Rhizopus spp (40%). Aflatoxin quantification at the end of the experiment revealed samples treated with Activated Charcoal had no detectable aflatoxin and the control maize samples (fermented without Activated charcoal) had minimal aflatoxin (3µg/kg) which are less than the permissible limit (4µg/kg) recommended by European Union (EU). The study revealed Nixtamalization has 100% efficiency in the control of aflatoxin in fermented maize even though fermentation ordinarily controls aflatoxin without the use of activated charcoal but it is variety dependent. To ensure food safety, nixtamalization should be incorporated into maize fermentation processes which could also help in the management of aflatoxicosis.

P6.50 Natural bioactive compounds as antimicrobial agents against Campylobacter

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Campylobacter spp. is the leading cause of foodborne gastrointestinal infections in humans worldwide. Chemical food preservatives are widely used in the food industry to prevent spoilage and pathogen bacteria. However, they have become a source of controversy as they can cause serious health issues. Thus, there is a great need to find new compounds to combat bacteria, mainly Campylobacter spp., due to the high prevalence of this bacteria along the food chain.

The most suitable compounds to reduce C. jejuni contamination in poultry are those derived from natural sources. Four antimicrobial agents were extracted from food-by products and plants and tested in vitro against 5 clinical C. jejuni isolates (H249, H518, H529, H660 and H661), that were selected out of 33 clinical C. jejuni isolates regarding relatedness of multilocus sequence types (MLSTs) among individual strains with defined clonal complexes (CC) using a minimum spanning tree. Strains were clustered in ST-904/CC607 (H249), ST-354/CC354 (H518), ST-677/CC677 (H529) and ST-148/CC21 (H660 and H661). Those extracts were onion skin, olive leaf and moringa seed, that were extracted using supercritical fluids, and red wine pomace extract, whose extraction method is patented. Antimicrobial activity of extracts was evaluated in Mueller-Hinton broth, due to the loss of viability of C. jejuni to the organic solvents DMSO and ethanol, using 6-well plates inoculated at 7 log10 CFU/mL and incubated in microaerophilia for 48 h at 37 °C under orbital agitation. Colony Forming Units (CFU) were counted to determine the Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC) for each extract. Onion skin and moringa seed extracts were needed in lower concentrations (MBC: 0.0625-0.125%, MIC: 0.03125-0.0625%) to control C. jejuni isolates, compared to olive leaf and red wine pomace extracts (MBC: 1-2%, MIC: 0.5-2%). Red wine pomace extract was the only one that affected bacteria viability by reduction of pH of cell culture media. H529 was the most resistant isolate to all extracts, except for moringa seed.

These antimicrobial substances are promising natural compounds, especially onion skin and moringa seed extracts, that could be used as natural ingredients to combat C. jejuni in favour of clean label products.

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P6.51 Meta-analysis and regressions as tools for the development of microbial inactivation models by non-ionizing irradiation technologies (UV-C light, pulsed light, LED)

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The use of non-thermal methods for processing food products is a current trend for microbial inactivation . In this sense, ultraviolet non-ionizing irradiation technologies are steadily stablishing as a safe to use, easy to implement and free of by-products procedure that could help improving whole processing lines from an economic and environmentally friendly perspective. Low-pressure mercury lamps are currently the main technology used for microbial inactivation because they continuously emit 85–95 percent of their energy at 254nm, which falls between the range in which the germicidal effect is the highest. However, the use of mercury UV lamps is being restricted since 2020 and, therefore new alternative UV emission technologies are required by the industry. Potential alternatives are the use of pulsed light, which entails the application of brief flashes of intense light in a broad spectrum in short treatment times; the use of light emitting diodes is another alternative which stand out for being environmentally friendlier due to the absence of mercury and can also emit light in the UV-C spectrum. In this work, a meta-regression analysis of 102 studies reporting the inactivation achieved by different dosages and sources of non-ionizing irradiation was carried out. The analysis of the 102 datasets concluded that Weibull model, characterized by shape and scale factors, described accurately most of the data. However, according to the results obtained by the meta-analysis, the heterogeneity between the studies was high. Further categorization of the results by technologies, food matrix or microorganism were applied, revealing that in terms of the shape factor, the heterogeneity was low and models sharing this parameter among studies could be suitable. A global and by technologies shareable shape factor was obtained allowing to accurately describe the inactivation by non-ionizing irradiation, while the scale factor must remain variable and dependable of the microorganism resistance and the characteristics of the food surface.

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P6.52 A culturomic approach to evaluate the impact of ozone gas on Listeria monocytogenes and resident microbiota on surface of Gorgonzola cheese

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The occurrence of Listeria monocytogenes on Gorgonzola cheese (Italy) surface has been frequently reported. Risks to consumers arise from the dispersion of the pathogen inside the cheese during cutting operations. As a powerful antimicrobial, ozone could be applied in ripening rooms to mitigate L. monocytogenes contamination on cheese rind. Anyway, its impact on resident microbiota should be investigated. Culturomics is an approach for characterising food microbiota based on the use of multiple culture conditions and identification of colonies by MALDI-TOF MS. Our study was aimed at understanding the effect of ozone on L. monocytogenes and resident microbiota of Gorgonzola cheese surface through a culturomic approach.
Rind portions (5 g) obtained from cheeses at the end of ripening were inoculated with L. monocytogenes (3-4 Log CFU/g) and treated with ozone gas at 2 (O2) and 4 (O4) ppm for 10 min. Controls were not exposed to ozone. Microbial loads (L. monocytogenes, total viable count, enterococci, coagulase-positive staphylococci, mesophilic lactobacilli, mesophilic cocci, yeasts and moulds), aw, and pH were evaluated weekly until 63 days of storage (4°C). At each sampling point, colonies collected from the different media were identified by MALDI-TOF MS to obtain the relative abundances (RA) of microbial genera.
A less pronounced decrease of L. monocytogenes was observed in ozonized rinds with higher final loads (3.7±0.3 Log CFU/g) compared to controls (2.6±0.3 Log CFU/g). This behaviour coincided with a decrease of lactobacilli in treated samples. No significant differences were detected for the other microbial enumerations, aw, pH, and resident microbiota composition among ozonized and control samples. The dominant genera were Candida (RA 22.6-27.4%), Carnobacterium (RA 12.6- 18.4%), Staphylococcus (RA 10.8-13.8%), Penicillium (RA 6.6-12.0%), Saccharomyces (RA 5.5-9.4%), Aerococcus (RA 4.4-7.2%), Yarrowia (RA 2.8-4.2%), Enterococcus (RA 1.3-4.0 %).
Ozone was ineffective against L. monocytogenes inoculated on Gorgonzola rinds and affected the growth of lactobacilli. Since lactobacilli are usually antagonists of the pathogen in food, their growth dampening could explain the higher final L. monocytogenes loads in ozonized samples. Outcomes of culturomic analysis demonstrated the efficiency of this approach for studying the microbiota of complex matrixes, such as the surface of blue-veined cheeses.

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P6.53

Investigating the impact of natural anti-microbials on Listeria monocytogenes in processed meat products for enhanced safety and shelf-life

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Control of L. monocytogenes, a pathogen which can proliferate at chilled temperatures is crucial for the safety of processed meat products. Changing consumer demand for "clean label" products has created a need for natural anti-microbial agents, as an alternative approach to ensure the safety and prolong the shelf-life of processed meat products.

This study investigated the efficacy of 3 natural anti-microbial agents (carvacrol, thyme essential oil, cranberry extract) against L. monocytogenes (5 strain cocktail) in beef burgers. Initial studies focused on the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the above agents at 37˚C, pH 7.3, using micro-broth dilution assays. The anti-microbial agents were then added at different concentration levels (MIC, 2 x MIC, 4 x MIC) to beef burgers (20% fat) which were inoculated with 103 CFU/g L. monocytogenes. The beef burgers were vacuum packed and stored at 3˚C for 16 days. The total number of L. monocytogenes including injured cells were enumerated using an overlay method (tryptone soya agar incubated at 30˚C for 2 h followed by over pour with Oxford Listeria selective agar and incubation at 37 ˚C for 48 hours).

The MIC for carvacrol, thyme essential oil and cranberry extract against L. monocytogenes at optimum conditions (37˚C, pH 7.3) was determined as 1000 µg/ml, 0.125% v/v, 6.25 mg/ml respectively. The MBC value for carvacrol and thyme essential oil was the same as the MIC, whilst the MBC value for cranberry extract was higher (50 mg/ml).

In beef burgers, the addition of carvacrol and thyme essential oil at minimum inhibitory concentration had minimal impact on L. monocytogenes. However, the addition of higher concentration (4 x MIC) of carvacrol yielded a bactericidal effect with approximately 0.8 log10 reduction after 8 days of cold storage. The highest concentration of thyme essential oil tested (4 x MIC) showed lower anti-microbial activity compared to carvacrol. Moreover, burgers containing the lowest concentration (MIC) of cranberry extract had approximately 0.5 log10 lower count of L. monocytogenes after 12 days at 3˚C compared to control samples.

These results show that natural anti-microbials have potential use as natural preservatives in processed meats.

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P6.54

Natural fruit juices enriched with probiotic bacteria and other biofunctional constituents in encapsulated form

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Encapsulation of probiotics is applied to ensure the functionality and viability of the added cultures in the stressful environment of foods and the adverse conditions during processing, handling, storage and consumption of these products.

The project "FUNJUICE" aims to develop new biofunctional natural fruit juices containing probiotic bacteria and other biofunctional components, which will not affect their quality and organoleptic characteristics. Furthermore, the project aims to add value to these products by preventing pathological conditions in humans, promoting health and maintaining quality of life. Probiotics, functional microorganisms and other biofunctional components (e.g., omega3 fatty acids, vitamin D) will be encapsulated and added to fruit juices. Whey protein will be used as encapsulating material, which will enhance the protein content of the products. The shelf life of the products will be determined and an easy-to-use prediction software will be developed for the estimation of viability of probiotics in juices and in other products. Through "FUNJUICE": a) encapsulation systems will be developed along with the structured release systems, b) the effectiveness of the developed encapsulation systems to increase the viability and control of probiotic cell metabolism, and maintain the added biofunctional components during processing, distribution and storage of juices will be assessed, c) mathematical models to predict the survival of encapsulated probiotics in juices will be developed and validated to develop a prediction software that can be used to efficiently design products and determine their shelf life and d) the viability of encapsulated probiotics and their effect on health with clinical trials will be estimated. Development of microcapsules and application of the encapsulated probiotics/components in juices at a pilot-scale with simultaneous market research will follow.

The ongoing research includes studies of the effect of heat treatment and High-Pressure Processing on the efficiency of the whey-protein isolate-gum Arabic complex coacervate, to protect the encapsulated probiotic cells from these stress conditions. In addition, different encapsulation matrices are being studied, to evaluate the best matrix related to the target products of the project.

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Ozone was ineffective against L. monocytogenes inoculated on Gorgonzola rinds and affected the growth of lactobacilli. Since lactobacilli are usually antagonists of the pathogen in food, their growth dampening could explain the higher final L. monocytogenes loads in ozonized samples. Outcomes of culturomic analysis demonstrated the efficiency of this approach for studying the microbiota of complex matrixes, such as the surface of blue-veined cheeses.

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P6.55 Sensitivity of Listeria monocytogenes dairy strains to lytic bacteriophages in yellow cheese

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Listeria monocytogenes, causing life threatening listeriosis, is a significant concern to the food industry. Due to post-processing contamination, this pathogen can be present in factory equipment and the environment. Therefore, lytic bacteriophages have been suggested as natural antimicrobials or processing aids to control foodborne pathogens in processing environment and/or final products. To evaluate the influence of product matrices on bacteriophage performance, we investigated killing efficacy of two commercial phage solutions against 13 L. monocytogenes dairy isolates, in broth and on the surface of yellow cheese. 8 L. monocytogenes strains were sensitive to the phage solutions in broth, whereas 6 were phage-sensitive on the cheese surface. These results demonstrate that a significant level of phage-resistance is already present in L. monocytogenes dairy isolates. Using whole-genome sequencing, we found no correlation between phage-sensitivity and single-nucleotide polymorphisms (SNPs) in the tested L. monocytogenes strains.

P6.56 Functional characteristics of lactic acid bacteria strains isolated from quail and sheep meat to be used as starter cultures in the production of new fermented sausages

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Lactic acid bacteria (LAB) are the most important bacteria used in fermented foods. In this study nine LAB strains, isolated from quail meat and indigenous fermented sausages produced with quail and sheep meat, were evaluated for some functional properties of technological and probiotic interest to evaluate their potential application in the production of fermented sausages. Antibacterial and acidifying activities as well as survival to simulated stomach duodenum passage, in vitro adhesion capacity to Caco2-cells, BSH and amino acid-decarboxylase activities were evaluated. The isolated LAB strains were assigned to the species Pediococcus acidilactici, P. pentosaceus, Lactiplantibacillus paraplantarum, Lacticaseibacillus paracasei and Latilactobacillus sakei on the basis of carbohydrate fermentation profile and partial 16S rRNA sequencing. All the strains showed a good acidifying activity and were able to grow in presence of 8% NaCl. P. acidilactici strains demonstrated antagonist activity towards the food-borne pathogens Staphylococcus aureus and L. monocytogenes by both spot test and well diffusion method. All pediococci and L. paracasei 2MRS-SalQP1 strains showed a very good resistance to simulated gastric duodenum passage with only a 1-2 Log10 CFU/mL reduction with respect to the initial count (108 CFU/mL). All lactobacilli were able to adhere to Caco-2 cells to various extents (from 2.5 to 5 %), confirming that adhesion is a strain-specific property. Lower adhesion rates were detected in pediococci with the strain 3MRS-IQ showing the higher adhesion percentage (1.5%). Two Lactobacillus paracasei strains were not able to grow in the presence of 0.2% glycodeoxycholic acid after 72h of incubation but all LAB strains were found to weakly hydrolyze sodium taurodeoxycholate. None of the strains decarboxylated lysine, histidine and ornithine. These results indicate that autochthonous LAB strains analyzed possess some functional characteristics of technological and probiotic interest and could be used in the manufacturing of fermented quail-sheep meat products, but more in vitro and in vivo studies are needed to evaluate their probiotic and safety properties.

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P6.57

In vitro antimicrobial and antibiofim analysis of bioactive compounds as promising natural ingredients for the development of innovative eco-sustainable active food packaging

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Fresh fruit and vegetables are biologically dynamic foods, due to their metabolic activity and associated microflora, and therefore fragile in terms of integrity and hygiene. The intensity of degenerative processes depends on both biological factors and environmental conditions. In the framework of the "PACK-CHAIN" project, aimed at achieving the development of innovative and eco-sustainable bioactive packaging for extending the shelf- life of fruit and vegetables, this study focused on the characterization of antimicrobial and antibiofilm activity of bioactive compounds tested in vitro on bacterial and fungal type strains. Individual and synergistic effects of synthetic molecules (chitosan, citric acid), plant extracts and essential oils were tested in vitro. Traditional microbiological methods such as disk diffusion test, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), crystal violet assay and growth in microtiter plates, and Flow cytometry (FCM) were applied to evaluate effect on planktonic and sessile cells and to obtain information on the cellular physiological response following the applied treatments, respectively. Preliminary results obtained showed a higher efficacy of essential oils, in particular thyme and oregano, in reducing the microbial growth in vitro. The evaluation of the synergistic action of plant extracts combined with synthetic molecules like chitosan will be crucial for the development of innovative and compostable packaging. Indeed, further research on chitosan and its combinations with other materials is needed for improving the functional properties of packaging films.

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P6.58 Coridothymus capitatus hydrolate as a washing solution for controlling Listeria monocytogenes and spoilage bacteria on fresh-cut rocket salad

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Hydrolates are by-products deriving from the distillation of essential oils and consist of aqueous solutions that contain small quantities of oily molecules with antimicrobial properties. Their flavour is milder than the corresponding essential oils, compared to which they are also cheaper, and these factors make them suitable for applications as natural food preservatives. Among biopreservatives, essential oils have been extensively investigated for their antimicrobial activity in food, while for hydrolates only a few in situ studies have been carried out. Being hydrophilic, hydrolates could be used as washing solutions for foods, including fresh-cut vegetables. This ready-to-eat food normally supports the growth of Listeria monocytogenes, one of the main foodborne pathogens. Therefore, the aim of the present study was to investigate the antimicrobial activity of Coridothymus capitatus hydrolate on fresh-cut rocket salad (Eruca sativa L), stored in air at +4 °C. This hydrolate, like the corresponding essential oil, was found to be rich in carvacrol, a phenolic monoterpene with documented antimicrobial properties. Samples of fresh-cut rocket salad were inoculated with 10⍰ CFU/ml L. monocytogenes, dipped in a washing solution of 0.50 ml/ml of C. capitatus hydrolate for 5 minutes, and stored at +4 °C for 48 hours, to simulate domestic refrigeration. Microbiological analyses were performed to assess the evolution of the inoculum, together with other microbial groups representing the microbiota of rocket salads. In addition, chemical, physical, and sensory characteristics were evaluated. The treatment with C. capitatus hydrolate determined the major reduction for aerobic mesophilic count, total psychotrophic count, and Enterobacteriaceae (around 1 Log CFU/g reduction), and intermediate reduction for L. monocytogenes, lactic acid bacteria, Pseudomonas spp., and Bacillus spp. (between 0.5 and 1 Log CFU/g reduction). The greatest effects were observed after 24 hours of refrigerated storage. Analyses of pH, aw, colour, and sensory analyses did not show any decrease in the quality attributes and in consumer acceptability of the treated salad. Future studies are needed to optimize the potential of C. capitatus hydrolate as a washing solution for ready-to-eat vegetables, which could represent an environmentally friendly alternative to traditional chemical disinfectants.

Controlling and predicting microorganisms in food ecosystems

P6.59 Assessing the impact of fat on microbial dynamics of food related bacteria and their response to cold atmospheric plasma in novel triphasic 3D models

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Understanding and predicting bacterial behaviour in food systems is vital to ensure food safety. Although in the past most food microbiological research was conducted in liquid broths, it is now known that bacterial behaviour changes fundamentally when grown in structured environments. Furthermore, the bacterial behaviour is also affected by the natural microflora of foods and possible cross-contaminants. These can interact synergistically but can also be used as an antagonistic tool for food safety. The aim of this work is to perform a systematic study on the impact of fat on the bacterial kinetics, their inter-species interactions, and their sensitivity towards cold atmospheric plasma (CAP) in complex triphasic 3D systems.

Building on our previously developed biphasic protein/polysaccharide food model (FM), a third fat phase was incorporated into the system with fat concentrations (FC) in the range of 10-60%. Listeria monocytogenes, Escherichia coli, Pseudomonas aeruginosa and Lactococcus lactis were grown on the surface of the FM, as well as listerial co-cultures with each of the above listed bacteria. A multiscale analysis took place macroscopically (plate count) and microscopically (confocal laser scanning microscopy). Furthermore, the bacterial single- and co-cultures grown on the surface of the FM systems were treated with CAP.

Overall, the macroscopic analysis revealed an increased growth behaviour when grown in single culture in comparison to co-cultures, but no significant impact in respect to the different tested FC. However, on the microscopic scale, generally, differences between the FCs were observed. More specifically, the bacterial colony sizes and biofilm formation were increased with increasing FC, more significant in co-cultures than in single culture. Due to these microscopic differences, a different level of cell-to-cell and colony-to-colony interaction takes place. This was further demonstrated by the susceptibility and resistance of the tested single- and co-cultures to the mild preservation treatment of CAP.

In conclusion, our results indicate the importance of accounting for food biochemical composition and microstructural complexities when designing food decontamination treatments. More realistic model systems are necessary to ensure a better prediction of microbial interactions and resistance.

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P6.60 Disinfectants in food processing environments: impact on the diversity of bacterial communities and efficacy towards foodborne bacteria and biofilms

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Disinfectants are extensively used in the food production environment and play a key role for the prevention and control of pathogenic bacteria. However, the efficacy of disinfectants is generally defined based on susceptibility tests undertaken with bacteria in planktonic state, yet biofilms are the preferred form of bacterial life in most biological ecosystems. Therefore, increased knowledge on the efficacy of disinfectants against biofilms is needed considering their omnipresence.

Our study aims to bridge these knowledge gaps by investigating both the efficacy of disinfectants towards bacterial biofilms contra planktonic cells, as well as the impact of disinfectants on the diversity of bacterial communities associated with food processing environments.

A set of sampling points at a Norwegian poultry and a salmon processing plant were carefully chosen in cooperation with company representatives, and included locations hard-to-access by cleaning and disinfection (C&D). Surface samples were collected before and after C&D for characterization of bacterial communities in the production environments by next generation sequencing technologies. Additionally, culture dependent methods were used for selection of Enterobacteriaceae, Enterococcus spp., Pseudomonas spp., Aeromonas spp. and Listeria monocytogenes to monitor the direct effect of C&D on the bacterial composition, and for the isolation of single bacterial strains. Pseudomonas spp. were detected in nearly all sampling points (> 90%) after C&D, demonstrating their persistence in poultry and salmon processing environments and ability to survive the C&D routines. The bacterial isolates were identified by sequencing of housekeeping genes, and minimum inhibitory concentrations for the disinfectants will be determined using robotic high-throughput screening (HTS) protocols. Finally, biofilm assays (BacTiterGlo) will be performed in monoculture on representative isolates using already established HTS protocols to determine the disinfectants potential to eradicate biofilms. The results will be presented in more detail at the conference.

Our study will contribute to answer fundamental questions related to the efficacy of disinfectants and their impact on bacterial communities in food processing environments. Ultimately, such knowledge is needed to optimize the daily cleaning routines and the use of disinfectants to ensure safe and sustainable food productions with low environmental impacts.

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P6.61 Response of Parageobacillus and Geobacillus spores to germinating agents

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Geobacillus and Parageobacillus spores are frequent food spoilers of canned foods due to their ubiquity and extreme heat resistance. Improving stability of these products requires the development of spore eradication methods that do not compromise food quality, such as germination-inactivation approaches based on inducing germination to take advantage of the loss of resistance of germinating cells by mild inactivation methods. However, the large proportion of superdormant spores produced by Geobacillus and Parageobacillus spp. hampers activating germination efficiently. In this work, we studied the germination dynamics against different stimuli in G. thermodenitrificans (DSM 465), G. stearothermophilus (ATCC 12980) and P. thermoglucosidasius (DSM 2542) at different environmental conditions.
Unlike most sporulated species, Geobacillus and Parageobacillus spores did not germinate in the presence of any amino acid or inosine, but they did respond to a mixture of asparagine, glucose, fructose and potassium chloride (AGFK) in a concentration-dependent manner. Exposure to a potential activating treatment with heat or sodium nitrite did not induce or even impaired germination in all the strains. Although revival of spores in a rich nutrient medium was not affected by sporulation temperature, it did influence germination rate with AGFK and the magnitude of such effect depended on germination temperature. While spores obtained at 55°C germinated at a similar rate at different temperatures (55°C-65°C), spores obtained at the highest boundary value (65°C) germinated slower when decreasing temperature. Our results evidence the different germination behaviour of Geobacillus and Parageobacillus spores compared to most mesophilic spores and the need to further investigate it to design effective activating strategies.

P6.62 Influence of fat additives of black soldier fly (Hermetia illucens) larvae on the dynamics of microorganisms in model food systems

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The cultivation of black flies larvae (Hermetia illucens) has begun to be grown as one of the tools for the development and application of the circular economy in areas such as biological refining, waste management, treatment of industrial by-products and bioconversion of agricultural residues.
Fats, proteins, flours of farmed larvae can be used as additives for animal feed and cosmetics. In the future, it is likely to be applied to the production of natural medicine, including food.
The use of fat and protein fractions of the larvae of the black soldier fly larvae (Hermetia illucens) in model food systems has been little studied, with potential problems for consumer acceptability. By optimizing sensory parameters, acceptability problems for new food modeling systems should be eliminated. The biologically active substances of larval fat have antimicrobial activity. Using the agar diffusion method, larval fat was found to inhibit the growth of Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Candida albicans.
Modeling systems with larval fat were developed to optimize the composition and ratio of fatty acids. In model food systems with larval fat, the total number of microorganisms increases during storage at 5oC but a decrease in the number of microscopic fungi was observed. Therefore, the presence of larval fat in food systems not only optimizes the composition of fatty acids, but also protects against spoilage caused by microscopic fungi.

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P6.63 Biopreservation potential of Aerococcus sp. strains against common pathogenic and spoilage microorganisms of food

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Food preservation using microorganisms and/or their metabolites is a sustainable approach to enhance safety and extend shelf life of food. It aims to reduce human damage linked to food pathogens as well as global food waste and loss due to undesirable microbial contamination. We previously described the high potential of Lactic Acid Bacteria of Aerococcus genus as a source of bioprotective agents against Salmonella in dairy products. In this study, the activity spectrum of 23 Aerococccus sp. strains was evaluated against 24 representative pathogenic and spoilage bacteria of dairy, fish and meat products in conditions mimicking food matrices. The study was focused on dairy matrix with evaluation of activity against 11 common fungi and 14 yeasts contaminants. Biochemical parameters (pH, H□O□ and lactic acid production) and volatilome were studied for a selection of strains grown 48 hours at 30°C in Milk Synthetic medium. Antimicrobial activity of pure volatile compounds, identified by GC-MS, was assessed against Staphylococcus aureus CIP 53.154, Pseudomonas fluorescens UCMA 7405 and Salmonella enterica subsp. enterica serovar Dublin CIP 70.53. Interestingly, inhibitory profiles differed among strains and over food matrices for a same bacterial target. As example, 8 strains were found active against Brochotrix thermosphacta UCMA 18331 on fish or meat matrices, while only two were active on both. On milk agar, 4 strains inhibited at least 5 target bacteria over 13, with complementary activity spectrum covering Gram negative, Gram positive, pathogens as well as spoilage bacteria. No activity on yeasts was found but 3 Aerococcus strains had inhibitory activity on at least 2 fungi (Aspergillus fumigatus CBS 116887 and Penicillium crustosum CBS 115503). The strain UCMA 9817 displayed strong antifungal activity, with 6 inhibited fungi. The antibacterial activity of several volatile compounds was confirmed, indicating that the activity may be due to complex interactions of small organic molecules including succinic, valeric, lactic, 2-hydroxyisocaproic or phenyllactic acids. In conclusion, this study shows the high interest of screening approaches in conditions close to food matrices. It revealed promising Aerococcus sp. strains combinations as protective cultures, especially against bacterial and fungal contaminants for dairy industry.

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P6.64 Response of L. monocytogenes cells to the stress imposed by sublethal concentrations of Origanum vulgare essential oil and Corydothymus capitatus hydrolate in environmental conditions encountered in foods

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The increasing antibiotic resistance of microorganisms and the growing scepticism of consumers towards synthetic preservatives are driving research towards effective, innovative and possibly sustainable alternatives to traditional antimicrobials and food preservatives. For this reason, plant-derived compounds, such as essential oils (EOs) and hydrolates, have been increasingly studied in the last decades.
Generally, complex foods can interact with plant-based antimicrobials, thus reducing the effect of the dose applied. Therefore, to optimize the use of these agents in food preservation, it is fundamental to understand the microbial behaviour in response to sublethal concentrations of the antimicrobials. Thus, this study aimed to evaluate the behaviour of Listeria monocytogenes ATCC7644, exposed to sublethal concentrations of Origanum vulgare essential oil (OEO, 1.25 µL/mL) and Corydothymus capitatus hydrolate (CCH, from 250 to 500 µL/mL) for one hour at 30°C, by applying a phenotype microarray approach. In detail, after exposure to antimicrobials, the cells were washed, inoculated into Omilog GenIII microplates to monitor the cell growth in 94 substrates, at 30°C for 72h. Results were modelled to obtain growth parameters. A variable lag phase elongation was observed in treated cells. Moreover, the results showed a general reduction of the maximum growth value, which suggests a residual effect, particularly of OEO, in hampering microbial growth. The stressing effect of the antimicrobials was particularly clear when cells grew in presence of 4 and 8% NaCl and in acidic pH (6.0 and 5.0), conditions that normally allow the pathogen growth. Confocal Laser Scanning Microscopy revealed the aggregation of live cells, increasing at growing hydrolate concentrations and confirming the response to the stressing event, with cells trying to reduce the surface exposed to OEO and CCH. Interestingly, OEO also restored the pathogen sensitivity to lincomycin, rifamycin and vancomycin. The effectiveness of EOs in combination with antibiotics has already been reported, but in our case a simple pre-exposure to OEO was sufficient to restore sensitivity. Our data confirm the potential of OEO and CCH in strategies for controlling L. monocytogenes in food environments. In fact, even sub-lethal concentrations were sufficient to obtain a clear decrease of the growth potential of the microorganism.

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P6.65 Predictive models versus challenge studies to assess the safety of ready-to-eat foods: A study of growth of Listeria monocytogenes in composed deli salads with unequal distribution of sauce and ingredients

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Models normally show good correlation between predicted and observed growth in single ingredients, but there are concerns for composed salads, due to different growth conditions between ingredients. The objective of the present study was to investigate the growth of Listeria monocytogenes in cooked chicken meat, partly or fully covered by mayonnaise-based sauces. These ingredients have very different growth potentials, and were chosen to simulate maximum variation of growth conditions in a mixed salad.
Cooked chicken meat (2x2x2 cm) was placed in two kinds of mayonnaise-based sauces, w/wo sour cream added. The sauce and the top surface of the cubes were then inoculated with L. monocytogenes (100 cfu/g). Another layer of sauce was added to half of the samples to fully cover the cubes. The samples were incubated at 4 or 12°C for 56 or 21 days, respectively. The observed and predicted growth of Listeria were compared using a gamma model developed for deli salads.
At 4°C, the growth was minimal during the entire period independent of the kind and amount of sauce. At 12°C, the growth in chicken being partly covered with sauce exceeded a 100-fold doubling within 3 days for both sauces, and reached maximum concentrations of log 9 cfu/g within 2 weeks of storage. For fully covered pieces, the growth was slower, and the maximum concentrations were only 1.5-2.5 log units higher than the inoculated concentrations. The pH in the sauces alone were 4.5 and, in chicken alone app 6.5, and in homogenised chicken and sauces pH of 5.7–6.2. These preliminary results indicated that conditions measured in a homogenised salad will not predict Listeria growth correctly. In well mixed ingredients, the pH on the chicken surface will drop immediately. In chicken meat partly covered with sauce, however, the favourable conditions for growth in meat will remain for some time in the uncovered parts, and a 100 fold increase can be observed even if a growth inhibiting sauce is present.
The obtained results are particularly relevant for modellers and producers of mixed salads who place the main ingredient on the top as decoration.

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P6.66

Plant polyphenol formulations for bioactive chitosan-based coatings of food packaging foils

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The main concern of food sector is to ensure food safety, quality, prolonged shelf-life, and reduced food waste and food packaging waste. Microbial contamination is a major cause of food spoilage. To avoid the use of synthetic compounds, in research focus are new natural plant-based additives that can impart antimicrobial and antioxidant properties of food and food packaging materials.

The aim of this work was to i) prepare and characterize polyphenolic extracts from blackberry leaves and prickly juniper needles using novel "green" extraction methods, ii) optimise their antioxidative and antimicrobial activity in-vitro, iii) incorporate them into chitosan-based coating of biodegradable polylactide (PLA) foil to improve its functionality.

The polyphenols from blackberry leaves and prickly juniper needles were isolated by microwave-assisted extraction in aqueous ethanol (50%, v/v). The antimicrobial activity of the extracts was tested by broth microdilution method and evaluated by minimum inhibitory and bactericidal concentration, and quantified by growth inhibition kinetics at sublethal concentrations against food-borne pathogens and spoilage bacteria in-vitro. Further, chitosan as macromolecular solutions, (1%, 2%, v/v) and nanoparticles with embedded extracts were prepared in liquid formulations as coatings for PLA foils by »roll-to-roll« technique. Particle size and zeta potential as stability parameters of liquid formulations were measured. Antimicrobial activity of the foils was tested by standardized ISO protocol. Antioxidant activity was measured by DPPH and ABTS, whilst physico-chemical characteristics were followed by XPS, ATR-FTIR, goniometry and SEM.

Although both plant extracts showed moderate antimicrobial activity in-vitro, they contributed significantly to antimicrobial and particularly antioxidant activity of chitosan-polyphenol formulations in coatings. The impact of physico-chemical structure (e.g. type and concentration of chitosan, layering followed by the deposition of the extracts encapsulated into chitosan nanoparticles, as well as type of phenols) on physico-chemical characteristics and correlations with antimicrobial efficacy of formulations as foil coatings will be discussed in view of food packaging.

By developing functionalized and biodegradable PLA foils with chitosan-based polyphenol formulations, we aimed to contribute in reducing spoilage of perishable foods, reducing food waste and detrimental impact of plastic in our environment.

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P6.67 Insights into the behavioral heterogeneity of individual bacterial cells

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Most conventional bacterial growth studies are based on deterministic approaches utilizing large bacterial populations that are considered as a whole, rather than taking into consideration the individual cells. However, single cells can exhibit remarkable variability regarding their growth responses. Since stochastic models take into account the variation of various factors affecting microbial behavior, they are considered a proper tool for the description of the high level of variation characterizing microbial dynamics. The objective of the present study was to evaluate and describe the heterogeneity in the behavior of individual bacterial cells under growth conditions, as well as to evaluate population dynamics. For this, the colonial growth of Escherichia coli single cells on solid media was studied using a (phase - contrast) time lapse microscopy method. Images of the selected field of view were acquired every 5 min for 6-8 h. Individual final images were compiled to give a sequence of frames for the field of view showing the behavior of the same cell over time. The results showed a notable heterogeneous behavior regarding the fraction of the population that can divide. Some cells did not grow showing filamentation or lysis. Two stochastic approaches for describing microcolony dynamics were used. The first approach relied on the fitting the growth data of each microcolony to a primary model for the estimation of the growth kinetic parameters. The kinetic parameters were further described with appropriate distributions which were introduced to a stochastic model which taking into account heterogeneity. The model provided stochastic growth curves using Monte Carlo simulation. The second approach was based on the division times of single cells. The division times of 5 generations of single cells were estimated, and their variability was quantitatively described for the development of a stochastic birth model. The comparison of these approaches provided useful information in understanding the biological mechanisms behind microcolony growth dynamics while the stochastic models can enhance the accuracy in risk assessment studies.

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P6.68 The COM-Poisson process for modelling Listeria monocytogenes survivors under multiple hurdles

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Stochastic models are required for quantifying and integrating variation, since point estimates models can be insufficient for a realistic estimation of microbial survivors. The aim of the present study was to evaluate the responses of different strains of L. monocytogenes subjected to multiple hurdles and to describe the observations in a modelling approach that incorporated both the random (Poisson) and the non-random components of the total variation. The applied stresses were osmotic, acidic and from liquid smoke at low and permissive temperatures, resembling products, such as Italian dry sausages. Variation in the population distribution arising from the multi-hurdle treatment was captured by fitting the pathogen inactivation curves to a three-parameter Weibull model within the Conway–Maxwell–Poisson process, which contains the Poisson distribution as a special case and includes a dispersion coefficient c0 (Polese et al. 2021). Dilution uncertainty was minimized by determining the regression coefficients on cell counts. The distribution of survivors was characterized by various levels of dispersion, i.e under-, equi- and over-dispersion, described by c0, which was affected by the rate of inactivation. Simulation results were congruent with the experimental observations, supporting the idea that it is worth including the non-random component of variation for providing a more accurate estimation of survivors which certainly impacts intervention practices.

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P6.69 Temperature-dependent growth kinetic as a selection criterion for protective cultures of cold-stored seafood products

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Biopreservation is a powerful novel preservation method that uses natural or added microorganism and their metabolites to maintain the quality and safety of food products. Lactic acid bacteria (LAB) are selected as suitable for seafood preservation since they are naturally a part of fish microbiota, have antimicrobial properties, and are safe for human consumption. So far, LAB biopreservation is mainly studied in lightly processed seafood products such as cold-smoked salmon (CSS). Application of LAB biopreservation in fresh fish products is challenging due to their clean sensory profile and short shelf life. Thus, LAB must be carefully selected, and the ability to grow at low temperatures without producing metabolites causing undesired sensory effects in the product is the first requirement.
The present study aims to examine the temperature-dependent growth kinetic parameter of the selected strains in vitro and in a real product. In vitro testing was done at 4°C, 8°C, 12°C, and 16°C in a sterile salmon juice model system to mimic fresh salmon products. This study favours the fact that the strains were previously isolated from seafood products -sushi, gravlax, and CSS. Ten LAB (five Carnobacterium sp. and five Leuconostoc sp.) were selected according to demonstrated antimicrobial activity against L. monocytogenes and E. Coli in fish juice. All ten strains could grow at all four temperatures, with specific growth rates ranging from 0.010-0.036 h-1 at 4°C, reaching a maximum population density of 8.46-9.06 log CFUml-1. One strain from each genus (Carnobacterium divergens and Leuconostoc mesenteroides) was selected for application in vacuum-packed salmon loins at 4°C stored for 17 days. Both strains were able to grow, achieving specific growth rates of 0.23±0.4 h-1 and 0.41±0.7 h-1for L. mesenteroides and C. divergens, respectively. No observed colour changes nor off-odours were detected in inoculated samples compared to control samples. The results demonstrate promising results for applying these strains for biopreservation of raw salmon. However, full-scale sensory assessment, as well as their effect on the total microbial community structure of raw salmon should be studied in more detail.

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P6.70 Combined use of high-intensity pulsed light and different antimicrobial washing treatments against bacterial pathogens on spinach leaves

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Decontamination techniques applied to fresh vegetables seek to increase microbial safety and prolong shelf life. Removal of foodborne bacterial pathogens from vegetables during washing can significantly improve the inactivation of the bacteria. High-intensity pulsed light (HIPL) is a promising technology for the inactivation of pathogenic or spoilage microorganisms on the surfaces of vegetables.

In this study, the bactericidal effect of the combination of HIPL (4, 8, 12 J/cm²) and different antimicrobial washing pretreatments such as chlorine dioxide (ClO_2) (10 ppm) and peracetic acid (PAA) (80 ppm) against E. Coli O157:H7 (ATCC 43888), L. monocytogenes (NCTC 11994), and Salmonella Typhimurium (ATCC 14028) on spinach leaves was evaluated. In addition, the impact of individual and combined treatments on the texture and color of spinach was assessed immediately after treatment.

Spinach leaves were inoculated with a cocktail of three foodborne pathogens. Samples were spot inoculated using approximately 50 µL of inocula to achieve a cell population of about 10^6 CFU/g. Inoculated samples were dried for 1 h in a laminar flow safety cabinet in order to allow bacterial attachment. All sanitizer treatment solutions were prepared fresh prior to the experiment. Distilled water and chlorine washing (200 ppm) were used for comparison. An inoculated sample (100 g) was initially immersed in 400 mL of sanitizing solution under continuous agitation for 10 min at room temperature, and then they were gently dried under a safety cabinet to drain the excess water, followed by HIPL with the different energy doses.

The combination of antimicrobial washing with the most intense HIPL treatment resulted in the highest microbial inactivation rate. For example, by combining a PL treatment at 12 J/cm² with one of the antimicrobial washing, a microbial reduction >3 log cycles was reached. Thus, it can be concluded that antimicrobial washing combined with HIPL is a promising method for decontamination of spinach leaves, which could be exploited to ensure their microbiological safety.Acknowledgement: DiTECT Grant agreement ID: 861915

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P6.71 Antimicrobial effect of natural antimicrobials on C. difficile

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Clostridium difficile is known as a nosocomial pathogen causing gastrointestinal diseases. However, the presence of spores has been detected in environmental sources (water, soil, fertilizer), animals (domestic and food animals) and foods (sea products, vegetables, milk and dairy products, prepared foods). C. difficile spores are highly resistant to heat, pH, alcohol, and disinfectant treatments. Therefore, this study aimed to contribute to the prevention of C. difficile-associated infections by reducing the spores of environmentally originating C. difficile with antimicrobial agents. For this purpose, the antimicrobial activity of Nisin, Epigallocatechin gallate, borax, green tea extract, trans-cinnamaldehyde acetic acid, clove oil, tea tree oil, mahaleb oil and the natural disinfectant which was formulated in our laboratory on four different C difficile spores were investigated. The Minimum Inhibition Concentration (MIC) values of the substances used in the study ranged from 0.15 mg/mL to > 493mg/mL. The natural disinfectant solution was determined as the most effective substance, followed by Nisin and trans-cinnamaldehyde. No synergy was detected between the substances tested with the checkerboard synergy test, but an additive effect was observed. As a result, it was determined that the developed natural disinfectant was effective against C. difficile and could be used for the disinfection of food processing surfaces. However, considering the cost and physical properties (color, odor, etc.) of green tea extract, it could be an alternative disinfectant for food contact surfaces, especially in removing possible C. difficile spore contamination from vegetables.

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P6.72 Heterogeneity in resistance of L. monocytogenes and E. Coli strains exposed to High Hydrostatic Pressure

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High Hydrostatic Pressure (HHP) is a mild preservation method, effective against pathogen and spoilage microorganisms for production of safe and nutritious food with extended shelf-life. However, species and strain variability may influence microbial resistance towards HHP and molecular mechanisms promoting survival have not been extensively characterized. Therefore, the aim of this study was to investigate the resistance of a variety of L. monocytogenes and E. Coli strains towards HHP treatment.

The effect of HHP treatment for selected holding time intervals on the viability of L. monocytogenes and E. Coli K12 strains were investigated. WT L. monocytogenes strains of different origin were included as well as E. Coli WT and mutants were considered in order to identify factors that contribute in piezotolerance. The applied pressure level was set at 300 MPa and the strains were grown in rich medium to stationary phase since higher resistance was expected. Fixed volume bacterial suspensions were placed in sterile plastic stomacher bags before exposure. Biological triplicates were obtained and viability of stress exposed cultures was determined by spot plating on non-selective medium.

Inter species variability in resistance to HHP was noted for Listeria monocytogenes WT strains with some displaying higher sensitivity to the treatment while others marked an enhanced survival. Furthermore, various genetic determinants were identified as important for piezotolerance in E. Coli. This research can form the basis for prediction of HHP decontamination efficiency, development of risk assessment models, relevant validation studies and evolution of hurdle technology.

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P6.73 Influence of different packaging materials on the shelf life of tomatoes

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Especially in the fruit and vegetable sector, a very large part of the food intended for human consumption is discarded throughout the supply chain, resulting in large amounts of food waste. Therefore, the aim of our work is to develop an innovative sustainable packaging for tomatoes, which saves resources and extends shelf life of the product at the same time to reduce food waste and enhance resource efficiency in the supply chain. In order to generate reference values for the shelf life of tomatoes, storage trials were first carried out with tomatoes in different packaging so that the influence of innovative sustainable packaging could later be measured. For this purpose, 72 tomato packages (24 per each packaging type) were stored at a constant temperature of 20°C for 23 days. The tomatoes were packed in commercial rPET packaging with lid, packaging made of wood pulp and packaging made of cardboard with perforated film tube. The tomatoes were examined for their microbial, sensory and physicochemical quality during storage on eight consecutive examination days. During the microbiological examinations, the total viable count, yeasts, molds, and Enterobacteriaceae were determined and enumerated. A trained sensory panel determined the sensory quality. In addition, physicochemical parameters such as pH and Brix, as well as texture and color were measured and weight loss over storage time was determined. The total viable count at the beginning of storage for tomatoes was 3.54 log10 CFU/g (± 0.18). However, the growth of spoilage bacteria does not follow a typical pattern. Thus, other factors, such as sensory characteristics, color or texture, were combined to a parameter set determining the shelf life. The results can be used to model storage stability, suitability for consumption and quality of tomatoes stored at 20°C in conventional packaging. A comparison can thus be made with the innovative packaging and the possible shelf life extension associated with this packaging can be calculated. In addition, the individual packages can be compared in terms of life cycle assessment and the environmental impact of the packages can be calculated.

Controlling and predicting microorganisms in food ecosystems

P6.74

Application of encapsulation matrices for the enhancement of probiotic survival under simulated gastrointestinal conditions

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Probiotics are considered as health-promoting live microorganisms that can fit on the people's daily diet. However, their survival through the gastrointestinal track (GIT) could be negatively affected by pH variations, bile salts and enzymatic reactions. Microencapsulation is considered as the most efficient method that protects probiotics against external adverse environment. The aim of the present study, was to study the survival of 7 potentially probiotic LAB strains (Lacticaseibacillus paracasei subsp. paracasei E93, E94, Lactiplantibacillus pentosus E104, E108, Lactiplantibacillus plantarum E10, Lacticaseibacillus casei Shirota and Lacticaseibacillus rhamnosus GG), microencapsulated in a whey protein isolate (WPI)-gum arabic (GA) complex coacervate matrix, under conditions simulating the human GIT (pH=2.5, 0.5% bile salts and their combination).. Free cells of the 7-strains were also used as controls. The cell survival was evaluated by enumeration on MRS agar. Results showed that the encapsulated cells exposed to low pH (2.5) for 3h at 37°C (simulating the food passage through the stomach), exhibited a ca 2.5-3 log CFU/ml reduction, in contrast to free cells that showed a higher decline of ca 4 log CFU/ml depending on the strain (initial population in all cases ≈9 log CFU/ml). The viability of the encapsulated probiotics in 0.5% bile salts showed a 0.5 log CFU/ml reduction after incubation for 4h at 37°C (simulating the food passage through the small intestine), in contrast to free cells that showed a higher decline (≈1.5 log CFU/ml). At the sequential combination (exposure to low pH and then to bile salts), the population of the encapsulated cells was reduced by 2 log CFU/ml, while the population of the free cells was reduced by 5 log CFU/ml. It can be concluded that the microencapsulation can protect the potential probiotic cells against GIT stress conditions and to allow them to reach and colonize in adequate amounts the large intestine. Future studies are needed to evaluate the cell viability and functionality of the coacervate on a real food system.

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Controlling and predicting microorganisms in food ecosystems

P6.75 Development of a predictive model for raw pork sausage to apply dynamic shelf life and increase resource efficiency along the supply chain

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Food waste of perishable products – although still in a good condition – occurs often due to a lack of information concerning the real-time remaining shelf life at different supply chain steps. Predictive modelling is known as a reliable tool to improve cold chain and logistics management on each chain level. Especially for highly perishable products, it can be used to implement dynamic shelf life and therefore, lead to an optimized food quality, increased food safety and resource efficiency.
The aim of the study was the development of a dynamic shelf life criterion for raw pork sausages based on predictive microbiology. For this purpose, laboratory storage tests of 289 samples packed under modified atmosphere were conducted under constant and dynamic temperature conditions. The development of the total viable count and the growth of typical spoilage microorganisms (Lactic acid bacteria, Pseudomonas spp., Enterobacteriaceae, Brochothrix thermosphacta, yeasts and moulds) was investigated. Additionally, physicochemical parameters such as gas composition, pH-value, aw-value, meat color and texture profile and an analysis of sensory parameters were determined to characterize the quality loss.
The results of the microbial growth analysis identified Lactic acid bacteria as the main spoilage organism. Based on that, the predictive shelf life model was developed using the modified Gompertz model and the Arrhenius model. The end of shelf life was determined on a bacterial count of log10 7 CFU/g. The temperature effect on the bacterial growth rate was calculated as the activation energy EA by using the Arrhenius model. Primary and secondary models were combined to predict shelf life under dynamic temperature conditions. Additionally, the investigation of physicochemical parameters showed a high correlation between microbial growth and sensory index, color value, texture and oxygen level dependent on individual storage temperatures.
The predictive model for raw pork sausage allows the application of a dynamic shelf life at all steps of the supply chain. Combined with digitalized temperature monitoring and data exchange systems, additional information can be provided to involved stakeholders. Furthermore, dynamic shelf life enables the implementation of dynamic pricing, the optimization of resource efficiency in logistic processes and the prevention of food waste.

Controlling and predicting microorganisms in food ecosystems

P6.76 Efficacy of bacteriophages FO1a and S16 in the reduction of Salmonella on chicken carcasses in a South African poultry processing environment

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Foodborne illness due to Salmonella is a prominent problem with millions of cases reported every year. Many of these incidences are linked back to poultry meat. The high throughput volumes of chicken through processing facilities to meet consumer demands - coupled with previous antimicrobial abuse - has allowed for the survival of multi-drug resistant Salmonella. A possible solution for this potentially devastating problem is the use of bacteriophages (phages). This study investigates the efficacy of phages in-line in the processing setting, highlighting the persistence of Salmonella, the challenges of the factory conditions, and the use of a suitable spray application method for safe delivery of the phages to the chicken carcasses. The study was conducted in a poultry processing facility as a 4-week factory trial, whereby PhageGuard S™ (a commercial cocktail of FO1a and S16 phages) (1%) was applied using a spray machine to a moving line of carcasses (three per second) after the chlorine (3 ppm) spin chilling step. Daily neck skin samples were collected at evisceration (before any microbial control methods were applied), and after the combined treatment of chlorine spin chilling and subsequent phage application. At evisceration 81% of samples (n=80) collected were Salmonella positive while after phage application only 40% were positive (n=160). The Salmonella positives were identified phenotypically using the EN ISO 6579/A1(02/2006) method and characterized for bacterial identification using VITEK® 2 COMPACT technology. The surviving 40% were then screened for antimicrobial resistance (using disc diffusion – EUCAST criteria) where resistance to tetracycline, penicillin and sulfonamide was found. Kill assays using PhageGuard S™ were successful on the surviving antibiotic resistant isolates. Due to surviving isolates being susceptible to PhageGuard S™, careful consideration should be paid to optimization of the application method, ensuring the accessibility of the Salmonella for the phages – as well as the safe delivery of the phages to the carcasses. In conclusion, phages for Salmonella reduction on chicken carcasses in the processing environment have a high efficacy and can be incorporated into the large-scale hurdle concept after chlorine spin chilling.

Controlling and predicting microorganisms in food ecosystems

P6.77 Functional analysis of two newly isolated lytic bacteriophages directed at inhibition of Escherichia coli in a plant food matrix

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The decrease in the quality of minimally processed food is related mainly to the growth of saprophytic bacteria. The food industry is still searching for novel solutions to effectively ensure the microbiological safety of food. Nowadays, the use of bacteriophages as potential biological control agents is a promising strategy. Despite the lack of approval of phage biopreparations for contact with food in the European Union, many research centers conduct research on the use of bacteriophages in food industry. The aim of the study was the isolation and functional analysis of two novel bacteriophages with lytic activity against saprophytic bacterial microflora of minimally processed plant‐based food products. Two phages were isolated from municipal sewage - namely Escherichia phage KKP 3266, and Escherichia phage KKP 3267 have lytic activity against Escherichia coli KKP 3824, and Escherichia coli KKP 3825 strains, respectively. Phages showed activity against most Escherichia coli and changed the growth kinetics of the tested bacterial hosts. Results showed that infection of bacterial hosts at MOI 1.0 more strongly inhibited cell divisions compared to the lower infection coefficient (MOI 0.1). Transmission electron microscopy (TEM) and whole‐genome sequencing (WGS) identified Escherichia phage KKP 3266, and Escherichia phage KKP 3267 as members of the Myoviridae family. Genome sequencing revealed that these phages have linear double-stranded DNA with sizes of 151,120 bp (KKP 3266), and 150,343 bp (KKP 3267). No antibiotic resistance genes and virulence factors, which are the main markers of lysogenic viruses, were annotated in phage genomes. The phages retained their lytic activity in a wide range of temperatures (from -20 °C to 40 °C). There was no significant effect of active acidity (in the range of pH 3 to 12) on the inhibition of the lytic activity of the tested bacteriophages. The exposure of phages to UV radiation significantly decreased their lytic activity in proportion to the exposure time. The results indicated that KKP 3266 and KKP 3267 phages could be a potential biological control agent against saprophytic bacterial microflora of minimally processed plant‐based food products, also in combination with other physicochemical methods of food preservation.

Controlling and predicting microorganisms in food ecosystems

P6.78 Effect of lemon balm and spearmint extracts on the survival of S. aureus in goat's raw milk cheese

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Previous investigation by our research group has revealed that hydroethanolic solid-liquid extracts from lemon balm and spearmint present high inhibitory capacity against S. aureus.
Cheese produced from raw milk has shown moderate prevalence of S. aureus in the past, therefore imposing a health safety issue for consumers. Thus, the aim of this study was to evaluate the antimicrobial effect of lemon balm and spearmint extracts against S. aureus in goat's raw milk cheeses during maturation.
Lyophilised extracts of lemon balm and spearmint aerial parts were obtained using ethanol 70% (v/v) as solvent in a shaking water bath. Milk was inoculated with S. aureus to reach 5 log CFU/g and 1% (w/w) of each extract was added to the curd, while a non-inoculated control was kept. Cheeses were kept in a chamber at 10 °C/98% RH for 15 days. Water activity, pH and S. aureus counts were determined at specific days.
For every treatment, a dynamic model was adjusted that consisted of a log-decay function with tail in differential form as primary model (with varying D-value), coupled to a secondary model Bigelow equation of D-value as a function of pH (with parameters log D* at pH 7.0 and zpH).
The dynamic models adequately fitted the survival curves with root mean square errors (RMSE) of 0.1172 and 0.0633, for spearmint and lemon balm, respectively, producing significant parameter estimates. The parameter log D* was affected by the addition of extracts (0.621 [SE=0.061] for spearmint and 1.189 [SE=0.200] for lemon balm) versus the controls (0.932 [SE=0.166] and 0.996 [SE=0.056]); whereas zpH tended to be higher with the addition of extracts (3.172 [SE=0.660] for spearmint and 2.339 [SE=0.835] for lemon balm).
In this sense, the addition of plant extracts significantly decreased the time to achieve one log reduction, which in practical terms corresponded to up to 1.36 log CFU/g reduction by the end of maturation.
Using a dynamic predictive microbiology model, this work characterised S. aureus survival parameters in goat's raw milk cheese and demonstrated that lemon balm and spearmint extracts can have a beneficial effect in controlling S. aureus during the maturation stage.

Controlling and predicting microorganisms in food ecosystems

P6.79

Purification of natural antimicrobial peptides from mantle tissues of Pecten maximus

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Despite the cleaning and disinfection procedures in the food plants, the presence of microbial biofilms in the environment remains an important source of food contamination. Indeed, the repeated use of chemical biocides may impact the antibiotic resistance profile of bacteria. The emergence of multi drug resistant pathogenic strains leads to find antimicrobial alternatives, mainly natural ones. In this context, bivalve molluscs appear to be a source of many antimicrobial compounds due to their primitive but also effective immune system. Previous studies carried on clams, mussels, oysters and even scallops show the production of some antimicrobial peptides (AMP) by bivalve haemocytes as defensins and others cationic cysteine-rich AMP. The great scallop Pecten maximus is an economically important species, representing almost 80% of European wild harvested scallops. However, only adductor muscle and gonads are mainly consumed, causing heavy losses of other parts of this mollusc. In this study, we investigated the presence of AMPs produced in the mantle of P. maximus by bioassay-guided purification. Acid extraction followed by ammonium sulphate fractionation allowed to obtain active fractions against Staphylococcus aureus NCTC 6571. Peptides with molecular weight between 6.5 and 10 kDa were purified by using reversed-phase high-performance liquid. They were analysed by LC-MS/MS to be identified. Some putative ubiquitin and histones were highlighted and their role of antimicrobial should be confirmed. Nevertheless, the presence of a big defensin as suggested by the results of a previous transcriptomics survey is not excluded.
P. maximus by-products may be a new source of AMP to be characterized that could be used as biopreservatives in food or coated on surfaces to improve food safety.
Keywords: Antimicrobial peptides (AMPs), Pecten maximus, Staphylococcus aureus

Controlling and predicting microorganisms in food ecosystems

P6.80

Microbial Electrocution and Electrostimulation using low intensity electric current: a novel approach for controlling the pathogenic, spoilage and fermentation microbiota.

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Low intensity electric current (LIEC) or electric pulses (LIEP) have been used for therapeutic and rehabilitation purposes in recent years, as they can affect membrane permeability and cell apoptosis. This technology was transferred and tested in liquid synthetic media and beverages (cider and beer) by four collaborating research groups, aiming to either inactivate food pathogenic or spoilage bacteria and enteroviruses, or to stimulate the growth of beneficial microbes involved in fermentation. This new approach is of low cost and uses low intensity cunductive electric current, in contrast to the (high intensity and expensive) inductive Pulsed Electric Fields (PEF).

For the purpose of microbial inactivation LIEF were used as single treatment, or in combination with low temperature pasteurization (65°C). Different combinations of electric current frequency (Hz), intensity (mA) and time (min) were applied. The in vitro studies showed that a LIEF of 800 Hz, with either 1 or 10 mA, was effective against E. coli, S. typhimurium, C. jejuni, S. aureus, L. monocytogenes, C. perfringens and high titers of Enteroviruses, when applied for 10 or 30min. When combined with pasteurization, LIEC could significantly improve the destructive effects of thermal treatment and cause a further decrease of microbial counts by up to 3 log cfu/ml, when applied in combination with pasteurization. In contrast, low intensity LIEC (2Hz) and short time of application (1 or 10 min) could stimulate microbial growth.

In fermented cider and beer, a LIEC of 800 Hz could reduce the counts of spoilage bacteria (Lactic acid bacteria, Acetobacter, Zymomonas) to an extend that was comparable to mild pasteurization. The microbiocidal effect of pasteurization was significantly improved by 2-3 log cfu/ml when combined with LIEC. Yeasts were more resistant to the destructive effects of LIEC, and interestingly, a short application (2-15 min) of low frequency LIEC (2 or 90 Hz) could stimulate the growth and metabolism of S. cerevisiae in vitro.

This research shows that this novel approach of LIEC can be used as a single or combined treatment for control of pathogenic, spoilage or beneficial food microbiota and modified according to the targeted microbes and the purpose of application.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.1 Phenotypic and molecular characterization of Listeria monocytogenes isolated from Greek Myzithra soft cheese and related food processing surfaces

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Listeriosis is a serious infectious disease with one of the highest case fatality rates (i.e., 20-30%) among the bacterial pathogens encountered in foods. In this study, we characterized 54 Listeria monocytogenes strains isolated from a Greek traditional whey cheese ('Myzithra') (48 isolates) and swabs sampled from cheese processing surfaces (6 isolates) of a milk industry in Epirus region, Greece. All but one strain belonged to serotypes 1/2b, 3b, 7 (81.5%; 44/54) and 1/2a, 3a (16.7%; 9/54), while the latter was identified as a serogroup 4 strain (1.9%; belonging to serotypes 4b, 4d, 4e). A total of 50 strains (92.6%) were susceptible to the seven antibiotics tested, the six surface isolates also included, while 3.7%, 1.9%, and 1.9% of the cheese isolates were resistant to ciprofloxacin, erythromycin, and meropenem, respectively. Thus, no multiple antibiotic resistance was revealed. Whole-genome sequencing (WGS) was applied to determine the phylogenetic positions of the strains and their genetic variability, whereas stress response and virulence gene analysis for the isolates was also studied. Findings of this work should be useful for the food industry as they could be used for epidemiological investigations, revealing possible contamination scenarios of food products.

P7.2 Five years of monitoring of Campylobacter in broilers in Sweden by whole genome sequence typing

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Campylobacter is the most reported zoonotic bacterial cause of gastroenteritis in Europe. Sweden has, since 1991, monitored the majority of broiler flocks at slaughter for presence of Campylobacter. Most cases of human Campylobacteriosis and the highest prevalence of Campylobacter in broilers in Sweden are reported during the summer months.
In 2017, the National Veterinary Institute (SVA) started performing whole genome sequence (WGS) typing of all Campylobacter isolates collected in the broiler monitoring programme from 2.5 specific weeks in the winter and in the summer. During the first four years, the collecting periods corresponded to the weeks preceding the collecting of the human isolates typed by the Public Health Agency in Sweden. This WGS monitoring programme has been applied from 2017 until present.
During the five years 2017–2021, 291 isolates from broilers have been sequenced and typed within this monitoring programme. Of these, 281 isolates were C. jejuni, 9 C. coli and 1 was C. lari. 85 isolates were from the winter weeks and 206 from the summer weeks. Among the C. jejuni isolates, 48 different sequence types (STs) were detected. Most of them were detected in Campylobacter isolates from one breeder only. The most commonly detected STs were ST-45, ST-918, ST-19, ST-48 and ST-257. ST-918 and ST-19 were both linked to outbreaks in Sweden in 2017 and 2020, respectively, and isolates of these STs were almost exclusively found in those years. ST-45 was represented by a total of 38 isolates during these five years and is the only ST that was detected every year. It was not linked to any large outbreaks during these years, but it seems to be the most prevalent ST in Swedish broiler flocks. ST-257 and ST-48 were detected all years except 2020 but very few isolates were detected each year.
The results of the monitoring by typing of Campylobacter isolates have regularly been shared and discussed with the poultry industry in Sweden and used as a measure to follow-up on Campylobacter prevalence and distribution between producers.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.3 Antibiotics resistance profiling of pathogens isolated from selected ready-to-eat fruits and vegetables

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Fruits and vegetables are sources of dietary nutrients of great importance. However, they have been identified as vectors for the transmission of pathogenic antimicrobial-resistant (AMR) microorganisms harboring AMR genes. This is of food safety concern, thus a need for continuous surveillance. This study aimed to profile antibiotics resistance bacteria in selected fruits and vegetables obtained from markets; commercially sold (CS) and home garden (HG) sources. Fifty-three (53) samples of watermelon, cucumber, tomatoes and garden-eggs were collected from the South-Western region of Nigeria and subjected to microbiological analysis using standard procedures. Antibiotics sensitivity test was carried out on all isolates using Kirby Bauer with selected antibiotics; amoxicillin-clavulanic acid, ampicillin, ceftazidime, ceftriaxone, cefuroxime, ciprofloxacin, cloxacillin, erythromycin, gentamicin, nitrofurantoin, ofloxacin. Multi-drug resistant (MDR) isolates (Bacillus cereus, Listeria monocytogenes, Salmonella enterica and Staphylococcus aureus) were screened for detection of AMR genes using molecular techniques. A total of 53 pathogenic bacteria were isolated and identified within the genera Bacillus (24), Corynebacterium (13), Listeria (3), Staphylococcus (2), Aeromonas (3), Enterobacter (1), Erwinia (2), Salmonella (1), Serratia (2), Shigella (1) and Vibrio (1). Forty-eight (91 %) of the isolates were multidrug-resistant strains, however, resistance to 5 out of 8 antibiotics was the most predominant pattern observed. AMR genes: blaTEM and blaCTX-M were detected in Salmonella enterica isolated from CS tomato, blaTEM, blaSHV, blaCTX-M and ERM(B) were detected in Listeria monocytogenes from CS watermelon, blaSHV and blaCTX-M were detected in Bacillus cereus isolated CS tomato, blaTEM, blaSHV, blaCTX-M and ERM(F) were detected in Staphylococcus aureus isolated HG garden-egg. The presence of multi-drug resistant (MDR) pathogens harbouring antibiotic-resistance genes which could be chromosomal or located on the mobile genetic elements hence responsible for the spread of the resistant gene is of clinical concern. Therefore, the occurrence of MDR in ready-to-eat commercially sold and home grown fruits and vegetables is a pointer to public health risks and food safety threats.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.4 Exposure to lethal doses of citral could lead to stable antibiotic cross-resistance in Salmonella Typhimurium

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Essential oils (EOs) and their constituents have been proposed as an alternative to food preservatives or biocides in cleaning and disinfection systems due to their great antimicrobial properties. Furthermore, several studies support that the use of these natural compounds as antimicrobials can prevent the generation of antimicrobial resistance. However, recent studies have shown that continuous exposure to EOs or their constituents enables the emergence of direct-resistant variants (RVs) against these natural antimicrobials. In this regard, the questions arise whether these natural compounds may also lead to the emergence of cross-resistance to others antimicrobials, such as clinical antibiotics.

Therefore, the objective of this study was to assess whether resistant variants of S. Typhimurium selected by citral, a major constituent of citrus essential oils, can show stable cross-resistance to antibiotics and to identify the responsible mutations.

For this purpose, wild-type strain (WT) was exposed to lethal doses of citral (400µL/L) for 4.30h, and the surviving cells were recovered and incubated at 37°C for 24h. This protocol was repeated 30 times until resistant variants to citral were isolated (RVs). Minimum inhibitory concentration (MIC) was determined against citral and agar disk diffusion assay was performed to test the antibiotic susceptibility. Finally, whole genome sequencing (WGS) of RVs was performed by Illumina technology, and subsequently compared to SeWT's genome.

MIC of RVs against citral (>2500µL/L) increased more than 2 fold in comparison to WT (1250µL/L). Agar disk difusssion showed no increased resistance ($p>0.05$) of RVs against cephalexin, kanamicyn or rifampicin. However, RVs showed a higher resistance ($p<0.05$) against ampicillin, chloramphenicol, nalidixic acid, norfloxacin, novobiocin, tetracycline and trimethoprim, compared to WT. WGS revealed nine single nucleotides polymorphisms (SNPs) in resistant variant located in several transcriptional regulators, including the redox sensitive transcriptional regulator SoxR.

These results support that the use of natural antimicrobials may allow the emergence of resistant genetic variants, not only against the natural compounds themselves but also against other types of antimicrobials such as clinical antimicrobials. Moreover, the SNPs were located in transcriptional regulators related with bacterial response to oxidative stress and to the resistance of many different antimicrobials.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.5 Bacteriophage for detection and biological control of Mycobacterium avium subspecies paratuberculosis

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Mycobacterium avium subsp. paratuberculosis (MAP) is the causative agent of Johne's disease in ruminants. Several studies have reported the presence of MAP in milk and dairy products and all agree that current detection methods are problematic. There is also accumulating data suggesting a possible link of exposure to MAP with the development of Crohn's disease in humans. Hence, there is an increasing need for rapid detection methods in addition to the arising need to monitor milk and if possible assure, that milk entering the human food chain, is from MAP-free herds or MAP-free. A combined phage-PCR assay, using the lytic D29 mycobacteriophage, was developed and evaluated for the detection of MAP from raw milk in less than 24 hrs. The assay was evaluated against the current standard method for detection, demonstrating better sensitivity and specificity. Phage was also applied into colostrum in an attempt to biologically control the disease and the results revealed the ability of the phage to lyse MAP and other mycobacterial cells in artificially and naturally contaminated samples under different experimental conditions. The fact that bacteriophage can distinguish between live and dead cells make them a very promising tool for the detection of food borne pathogens. Finally, the survival and working ability of phage in food matrices and environments, point to a new challenge with great perspective towards the biological control of food borne pathogens.

P7.6 Antimicrobial resistance and geographical distribution of Staphylococcus isolated from fish and sea water in the English Channel and North Sea

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Aims: Staphylococcus is a major food safety issue. The marine environment is a source of food for humans and is subject to numerous anthropogenic discharges in which Staphylococcus may be found as well as antibiotic residues and antibiotic resistance genes. This can lead to the appearance of antimicrobial resistant (AMR) Staphylococcus strains. The objective of this study was to evaluate the occurence and geographical distribution of AMR Staphylococcus strains in areas subject to heterogeneous human impacts and various environmental conditions by sampling seawater and food fish from the English Channel and North Sea sectors.
Methods: We isolated and identified Staphylococcus community associated with 460 whiting (Merlangius merlangus) and 46 sea water samples from 46 areas mapping the English Channel and North Sea sectors. AMR was determined by disk diffusion, following the CLSI standards (CLSI, 2015). In addition, we collected the environment data and the anthropogenic influences (i.e. integration of riverine inputs, potentially carrying ARBs) may drive the occurrence of AMR Staphylococcus
Results: Twelve coagulase positive Staphylococcus strains were identified and 100% of these strains showed at least one antibiotic resistance. Two hundred and thirty-three coagulase-negative Staphylococcus strains were identified and 53% had at least one antibiotic resistance. Seven Multi-drug resistant strains (MDR) were found (i.e. remaining to at least 3 classes of antibiotics). In these 7 strains, we identified the AMR genes that confirmed the phenotypic resistances data. We demonstrated that rivers had local influence, especially on the English coast, on the occurrence of AMR Staphylococcus. Moreover, the measurement of several marine environmental factors showed that turbidity or the phosphate concentration was involved in the occurrence of AMR Staphylococcus.
Conclusions: These results showed for the first time the occurrence of antibiotic-resistant Staphyloccus in captured fish and their associated marine environment (seawater). This study also showed the importance of taking into account multiple parameters in addition to bacterial occurrence to study AMR in the marine environment, and paves the way for further research before developing monitoring plans.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.7 The preservative propionic acid differentially affects survival of conidia and damages germ tubes of feed spoilage fungi

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The weak organic acid propionate is an important preservative in food and feed and inhibits the growth of various spoilage bacteria, yeasts and fungi, including mycotoxigenic fungi. The mode of action of this compound on fungal survival structures (conidia) and germ tubes of xerophilic feed-spoiling fungi is scarcely studied.
We have isolated and identified fungal strains from nine samples of poultry feed originating from different countries using a shelf-life test and molecular methods. Xerophilic Aspergillus were present at very high predominance. We assessed the sensitivity of a panel of isolated fungi for propionic acid and evaluated the viability of treated conidia and germ tubes. MIC values were measured by means of a microtiter plate assay. Survival of conidia was tested after a 24-hour exposure to 31 mM propionic acid. To evaluate if propionic acid damaged germ tubes, a novel method was developed in which young biofilms of the fungi were tested for 30 min with 31 mM propionic acid in Erlenmeyer flasks using the live-dead fluorescent dye TOTO-1.
The MIC values of 4.6 to 32.1 mM of these poultry-feed-specific fungi were well in the range as described in the literature. Propionic acid prevents outgrowth of conidia (spores) in a species-dependent manner. Twenty percent of Asgergillus chevalieri and 71% of Penicillium lanosocoeruleum conidia germinated after exposure. Dependent on the species, cell damage was visible after incubation with propionic acid. Germ tubes of P. lanosocoeruleum in a biofilm showed extensive (85 %) cell death after a 30 min treatment with propionic acid and slightly lower sensitivity was observed with A. proliferans (62% cell death). Microscopic analysis of these fungal biofilms revealed extensive damage to the cell membrane and showed distorted intracellular structures. Fluorescent life-dead staining of the germ tubes showed a clear dose response of propionic acid indicating a fungicidal effect on these growing cells. These results show that conidia can be inactivated by propionic acid, but that germ tubes show a much higher sensitivity. These observations shed new light on the mode of action of this important preservative to prevent fungal contamination of feed.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.8 Antimicrobial resistance profile of Enterococcus spp. isolated from retail lamb meat

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Introduction: Enterococci are organisms widespread in nature, they are present in the gut and often found in contaminated meat. Furthermore, they operate as source of antimicrobial resistance and virulence genes, having the potential to easily transfer these genes to other bacteria presents in the same microbiome through conjugated transposons and plasmids. This study evaluated the antibiotic resistance of Enterococcus spp. present in retail lamb meat.

Material and Methods: First, suspected colonies of Enterococcus spp. isolated from 21 lamb meat samples were identified by MALDI-TOF/MS. Then, antimicrobial susceptibility tests were performed using the disk-diffusion and the resistance was determined against ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), doxycycline (30 µg), enrofloxacin (5 µg), gentamicin (120 µg), imipenem (10 µg), levofloxacin (5 µg), linezolid (30 µg), minocycline (30 µg), nitrofurantoin (300 µg), norfloxacin (10 µg), teicoplanin (30 µg), tetracycline (30 µg), tigecycline (15 µg) and vancomycin (30 µg). The results were classified as sensitive, intermediate, or resistant.

Results: Thirty-nine isolates of enterococci were identified as follows: E. faecalis (23), E. hirae (6), E. faecium (5), E. gilvus (2), E. gallinarum (2) and E. casseliflavus (1). Out of the 39 isolates, 29 (74.35%) were resistant to at least one of the antibiotics tested. Moreover, the highest frequency of resistance was observed for tetracycline, 27 isolates (69.23%). Regarding E. faecalis strains, four isolates were resistant to ciprofloxacin, enrofloxacin, levofloxacin and norfloxacin, whilst 2 isolates were resistant to tigecycline. Meanwhile, only one isolate of E. faecium was resistant to nitrofurantoin. 5 of E. hirae isolates showed resistance to tetracycline (83.33%). Totally, 5 tested isolates of the present study (12.82%) were resistant to 3 or more antibiotics, being all of them E. faecalis. Finally, all the Enterococcus spp. isolates were sensible to ampicillin, gentamicin, linezolid and vancomycin.

Conclusion: The presence of multidrug-resistant enterococci in lamb meat warns about the risk of potential resistance gene transmission between the microbiota of this food.

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Food Microbiology in the context of One-Health (animal, plant and human)

P7.9

Validation of an alternative method for the detection of Escherichia coli O157:H7 in sprouts in comparison with U.S. FDA Bacteriological Analytical Manual (BAM) protocol

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Consumption of raw sprouts were associated with numerous outbreaks caused by Shiga toxin-producing E. Coli (STEC). Detection of the pathogen from sprouts is challenging. This study aims to validate an alternative method for detecting E. Coli O157:H7 from sprouts in comparison to the United States FDA BAM method. Four outbreak-associated strains H3482, TW14584, ESC01485, and ESC01482 were tested with mung bean sprouts in four independent trials; and H3482 were tested with alfalfa sprouts in one trial. Each trial consists of 60 test portions, with 20 fractional (low inoculation levels to achieve 50 to 75% positive rate) test portions each for the alternative and reference methods, as well as 5 positive and 5 negative controls for each method, respectively. Sprouts were inoculated with the strains at 1.3 to 1.9 CFU/25g for fractional levels, and 13-16 CFU/25g for positive controls. For the alternative method, 25 g of sprouts were incubated in 225 ml modified Buffered Peptone Water with pyruvate (mBPWp) and Cefsulodin-Vancomycin (CV) Supplement (C, 10 mg/L; V, 8mg/L) for 24 h at 42 ± 1°C static; for the BAM method, Acriflavin-Cefsulodin-Vancomycin (ACV) Supplement (A, 10mg/L; C, 10 mg/L; V, 8mg/L) were added to mBPWp after 5 h static incubation at 37 ± 1°C before incubation to at 42 ± 1°C for another 19 h. After enrichment, immunomagnetic separation (IMS) was performed using anti-E. Coli O157 Dynabeads® before analyses by real-time PCR and on selective media. All enrichment samples were also screened for the presence of O157:H7 with Atlas® STEC EG2 Combo Detection assay for comparison. Altogether, all 50 uninoculated test portions tested negative and all 50 positive controls were positive for E. Coli O157:H7 with the three assays. Although 54 of 100 test portions were positive for E. Coli O157:H7 for the alternative method and 63 of 100 test portions were positive for the reference method, there were no statistical differences (⍰ = 0.05) by Fisher's exact test. Nevertheless, the alternative method recovered 1 to 2 logs more target bacteria (CFU/µL IMS beads, $p<0.001$) than the BAM method from enriched inoculated sprouts in the test portions that were tested positive. These results show that conidia can be inactivated by propionic acid, but that germ tubes show a much higher sensitivity. These observations shed new light on the mode of action of this important preservative to prevent fungal contamination of feed.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.10 The prevalence of Salmonella and Escherichia coli in fresh leafy salad vegetables, across the food chain, in Cyprus

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Salads containing raw leafy vegetables have been widely consumed in Cyprus as a traditional side dish of daily meals. As a result of recent dietary trends, they are also increasingly being consumed as a main course. It has been reported that fresh leafy greens can be a source of food poisoning by Salmonella and other pathogens with contamination occurring at different stages of the food chain.

Official controls and the European regulation 2073/2005 generally focus on the microbiological safety of ready to eat (RTE) products like salads and pre-cut (washed) vegetables. A relevant EFSA Scientific Opinion in 2014, on the risk posed by pathogens in leafy greens eaten as salads, highlighted the lack of any microbiological criteria for primary production and packing stages. It also summarized results from studies on unwashed leafy vegetables at various stages of the food chain. In Cyprus, fresh (unwashed) vegetables like lettuce, cabbage, rocket, coriander, parsley etc, are sold unpackaged in markets, groceries and supermarkets and are used by consumers to prepare salads.

Here, we present aggregate results from official controls and surveys during 2015-2021, regarding the prevalence of Salmonella and Escherichia coli in salads from catering establishments, RTE pre-cut vegetables from retail and non-RTE (unwashed) vegetables from the field and from retail.

The results show that the microbiological quality (regarding the presence of Salmonella and E. Coli) of unwashed vegetables from the field is exceptionally good, whereas there is a small proportion of Salmonella positive samples in salads at retail level (<1%). On the other hand, unwashed vegetables from groceries and supermarkets have a significantly higher prevalence of both Salmonella and E. Coli, which indicates cross contamination at the initial processing (washing tanks) or packing (they are normally sold in open-top plastic boxes) stages. More focus is therefore necessary on the process hygiene at these stages. In addition, considering the difficulty in elimination of any present pathogens by the consumer through washing, it may be reasonable to consider microbiological criteria for fresh unwashed salad vegetables at retail level.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.11 Characterization of antibiotic-resistance phenotypic and genotypic traits in new isolates of Akkermansia muciniphila

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Akkermansia muciniphila is a commensal bacterium that accounts for 1-5% of human intestinal microbiota. The presence of this bacterium is commonly associated with a healthier status in humans. The type strain, A. muciniphila MucT, has been extensively studied and is considered a next-generation beneficial bacterium. Although the species has not yet been granted qualified presumption of safety (QPS) status, recently the EFSA panel has declared that pasteurized A. muciniphila strain MucT can be considered safe as a novel food, opening the door to its commercialization as a food supplement. Information on antimicrobial resistance (AMR) for bacteria deliberately introduced into the food chain is of paramount importance. However, there is little information regarding the AMR patterns of this species and reference cut-off values to distinguish strains with acquired resistance from susceptible strains.
In our work, we determined the antibiotic susceptibility profile of five human A. muciniphila isolates through the search for AMR genes and phenotypic testing based on the determination of minimal inhibitory concentrations (MICs) for selected antibiotics. Overall, the MICs obtained were found to be similar between new A. muciniphila isolates and the type strain. Specifically, only one strain harboring tetW gene showed reduced sensitivity to tetracycline, but all A. muciniphila strains showed low sensitivity to ciprofloxacin and aminoglycosides. Interestingly, all strains present adeF gene encoding a subunit of the resistance-nodulation-cell division (RND) efflux pump system and potentially involved in the resistance to ciprofloxacin. Conversely, only two strains showed a possible genetic determinant for resistance to aminoglycosides, indicating that poor sensitivity to this class of antibiotics could be due to a more general intrinsic mechanism. To better investigate the role of efflux pump ciprofloxacin reduced susceptibility, we determined MIC in presence of sub-inhibiting growth concentration of efflux pump inhibitors (CCCP and PA□N). Our results revealed that the susceptibility towards ciprofloxacin was not affected by the presence of inhibitors, thus indicating no evidence of active drug efflux. In conclusion, further studies on a larger number of strains are needed to better assess antibiotic resistance in this species.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.12 Composition and survival of spore-formers along the milk powder production line

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Spore-forming bacteria are a continuous threat to the dairy industry due to their ability to withstand processing conditions, such as pasteurization. These ubiquitous microorganisms have ample opportunity for multiple points of entry into the milk chain, creating issues for food quality and safety. Certain spore-formers, namely Bacilli and Clostridia, are more problematic due to pathogenicity and the ability to spoil dairy products. This project aimed to explore how the composition and survival of spore-formers along the milk production line is affected by various processes, as well as what significance their presence may have on product quality and food safety. Samples were obtained during the production line of dairy powder and examined in both a culture independent and dependent manner. The culture dependent method involved inoculating samples in reinforced Clostridium broth (RCM) in anaerobic conditions. The bacterial composition of samples from gas-producing tubes, as well as all culture independent samples, was assessed by high-throughput 16S rRNA amplicon sequencing and metagenomic analysis. Results revealed that spore-former presence and bacterial composition is highly affected by separation. Clostridial spores were not present in cream to any great extent, indicating that the cream is a less central gateway for Clostridia in cheese. A total of 19 MAGs were recovered from nine tubes. Four near-complete and two medium-quality genomes were found in raw milk/milk powder samples and further assigned as C. tyrobutyricum and C. diolis, which may constitute a problem in the finished dairy product. Throughout the various points of the production line, the occurrence of species belonging to the B. cereus group was higher than that of Clostridia. This observation indicates that it is not just Clostridial spores that may be a quality problem in cheese. Additionally, several Bacillus species were detected in milk powder, including B. licheniformis, B. thuringiensis and B. cereus, with B. cereus as a known pathogen that can also cause spoilage. In conclusion, our findings may aid in the intervention and control of spore-former presence in the dairy production line and highlights the importance of spore-former impact on dairy quality.

P7.13 Assessment of the occurrence of Ochratoxin A in commercially available corn and corn-based products from the Greek market

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Ochratoxin A (OTA) is a mycotoxin, which is produced naturally from several species of the genera Aspergillus and Penicillium. OTA's ascertained toxicity has been linked with various adverse effects on human health rendering its occurrence in the food supply chain a major problem. This study aims to evaluate the safety of the corn-based products available in the Greek market with regards to the biological hazard of OTA. A total of seventy (70) corn-based products, were purchased from local stores. Samples were grouped to the following categories: corn starch, corn flour, corn flakes, canned sweet corn, frozen corn, and corn cakes with even distribution. The occurrence of OTA was assessed with High-Performance Liquid Chromatography equipped with a fluorescence detector (HPLC-FLD). Pretreatment of the samples was performed through immunoaffinity columns (IACs) to assure the detection of the OTA even in negligible concentrations reinforcing the capability of HPLC. The limits of detection (LoD) and quantification (LoQ) for the certain method were 0.2 μg/kg and 0.6 μg/kg, respectively. According to those indicators, six out of seventy (6/70) samples were detected positive for the presence of OTA. OTA's concentration in positive samples ranged between 0.229 to 0.452 μg/kg, values significantly lower than the strictest acceptable levels (<3.0 μg/kg) as presented in the Commission Regulation (EC) No 1881/2006. These results demonstrate that the tested commercially available corn-based products from the Greek market could be considered as safe regarding OTA. Nevertheless, future studies include the evaluation of other significant harmful mycotoxins, such as aflatoxins, in these products.

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Food Microbiology in the context of One-Health (animal, plant and human)

P7.14 Effects of environmental stress on expression of virulence factors among Staphylococcus aureus isolated from cheese chain production

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Staphylococcus aureus is considered the most effective food-borne bacterial pathogen that has ever evolved. Methicillin-resistant (MRS) strains are of great importance in public health owing to their opportunistic ability as a cause of mastitis, source of zoonotic infection, and reservoir of antimicrobial resistance genes in dairy farms. Each of the food processing elements during the production may be a stress factor for a cell which induces an increase in enterotoxicity and antibiotic resistance. Stress conditions result in metabolic changes in bacterial cells through the expression of specific gene sets. S. aureus exhibits several stress response mechanisms to survive under different environmental conditions.
Therefore, the aim of this study was to evaluate determine the frequency of virulence factors such as the antibiotic resistance, enterotoxicity and virulence-related genes among S.aureus strains isolated from cheese chain production. Moreover, changes in the expression of selected virulence-related genes in response to food environmental stress factors were checked. he number of 50 isolates were analyzed.
Results showed that after the onset of stress, a marked increase in the minimum inhibitory concentration values against erythromycin, oxacillin and tetracycline was observed in most of the isolates tested. The obtained results demonstrate that stress that can occur during processing can can affect changes at the cellular level and induce the changes in the virulence-related genes expression.

P7.15 The anti-hypertensive character of traditional Feta cheese and its indigenous microbiota

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Feta, a white brined cheese, is the most prominent PDO (Protected Designation of Origin) Greek cheese and one of the most important export products of the Greek secondary dairy sector. The microbiome of Feta cheese has been thoroughly studied by classical microbiological analyses in the past, while in a recent study performed by our group, both amplicon based and shotgun metagenomics analysis were used for the first time to investigate four artisanal homemade Feta cheese samples from different regions of Greece, providing an in-depth overview of their microbiome. In parallel, 130 non-starter lactobacilli and 27 enterococci were isolated and identified. Feta cheese is characterized by its rich, piquant and salty taste, with a salt-in-moisture content of 3.5-5.5 %. Taking this into account, the aim of the present study was to determine the presence of anti-hypertensive peptides, namely angiotensin-converting enzyme inhibitory (ACE-I) peptides, in the above four Feta cheese samples as well as the ability of the Feta isolates to produce ACE-I peptides. Interestingly, the four Feta cheese samples exhibited ACE-I activity higher than 87%. Regarding Feta cheese isolates, initially 41 of the above-mentioned isolates, 33 lactobacilli and eight enterococci, were selected depending on their species and rep-PCR group. Their proteolytic activity towards milk proteins was determined and the strongest proteolytic strains were tested for ACE-I activity. The results revealed that 37 isolates were proteolytic, all except one exhibited ACE-I activity, while 15, namely isolates belonging to the species Levilactobacillus brevis, Lacticaseibacillus paracasei, Loigolactobacillus coryniformis, Lactiplantibacillus plantarum, and Enterococcus faecium/durans, had an ACE-I activity higher than 50%. Additionally, all isolates were also tested for their ability to produce gamma-aminobutyric acid (GABA), which is known, among others, to possess anti-hypertensive properties, and a total of six strains, belonging to the species of Latilactobacillus curvatus, L. brevis and L. paracasei were found positive. To conclude, this is the first study focusing on the anti-hypertensive potential of Feta cheese as such, as well as its indigenous microbiota.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.16 Occurrence of enterococci in raw goat and sheep milk and cheese, pheno- and genotypic analysis of isolated strains

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Enterococci are one of the groups of bacteria of increasing importance as an etiological factor of bacterial infections. Their ability to acquire resistance present a significant challenge for therapeutic measures. While the prevalence and transmission of antibiotic resistance among bacteria associated with food animals has been well documented, research regarding bacteria isolated from raw products is lacking. An example is goat and sheep milk and cheese, which is becoming more popular among consumers.

A total of 173 raw milk and cheese samples were analyzed, including 126 raw milk samples and 47 cheese samples. Isolated strains were identified biochemically using the VITEK 2 Compact System. The identification of Enterococcus faecium and Enterococcus faecalis species using PCR protocol was based on the amplification of specific genes for each of the species. Determination of minimum inhibitory concentration (MIC) for a panel of 12 antibacterial substances was performed using the broth dilution method (EUVENC). Isolates were classified as sensitive (S) or resistant (R) according to EUCAST or CLSI recommendations. Selected antibiotic resistance genes were detected using PCR.

Enterococcus spp. were isolated in 47 samples (26.7%). E. faecalis (35.3%), E. hirae (29.4%) and E. faecium (11.8%) were most frequently isolated. Other species constituted 23.5%. There was no resistance to ampicillin, teicoplanin, tigecycline, vancomycin and penicillin among isolated strains. Resistance to quinupristine/dalfopristine (18 strains), lincomycin (18 strains) and tetracycline (10 strains) predominated within E. faecalis. Within E. faecium, most isolates showed resistance to lincomysin (6 strains) and quinupristine/dalfopristine (5 strains). The multiple resistance patterns were observed only within E. faecium and E. faecalis strains. The resistance patterns of two and three substances were most frequently identified in E. faecalis strains. Vancomycin resistance was not detected among E. faecium and E. faecalis isolates. Strains were analyzed for vancomycin, MLSB and tetracycline resistance genes. The tet(M) gene was detected in the genetic material of nine E. faecalis isolates. The tet(L) and ermB genes were detected in the genetic material of single strains. The genes VanA, VanB, tet(W), vatD and vgaA were not detected in any of the tested isolates.

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Food Microbiology in the context of One-Health (animal, plant and human)

P7.17 Antifungal and Anti-Mycotoxigenic Properties of Lactic Acid Bacteria: A Review

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Filamentous fungi occur in a wide range of foods, crops, and environments. The ubiquitous presence of fungi is due to their versatile nutritional and environmental requirements. The beneficial effects of certain fungi are well known. Fungi have not only served to synthesize antibiotics, but also to produce some foods. Fermented foods, such as some cheeses, soy sauce, miso, tempeh, and other products are prepared with the help of molds or in combination with lactic acid bacteria and/or yeasts. However, molds are also major spoilage agents of foods and crops. In addition to economic losses, molds growing on foods and crops present a potential health hazard to humans and animals by producing mycotoxins. Toxigenic molds have caused food safety problems for as long as foods have been harvested and stored.

To control the growth of these fungi in the food supply physical methods and chemical preservatives have been widely used. However, due to the increasing concerns about the safety of chemical preservatives, and increasing demand by consumers for fresh, and "natural" food products, development of biological and safe preservation methods is warranted. The Lactic Acid Bacteria (LAB) which are Generally Recognized as Safe (GRAS) microorganisms, have been widely investigated during the last few decades for their antifungal and anti-mycotoxigenic properties. Numerous investigations have shown that LAB produce a wide variety of metabolites that inhibit fungal growth and prevent the production of mycotoxins. These compounds include proteinic compounds, various organic acids, reuterin, phenolic antioxidants, diacetyl, and other compounds. LAB have also been shown to adsorb or break down mycotoxins such as aflatoxins, ochratoxins and some Fusarium toxins. This paper presents a current and concise review of the antifungal properties of LAB, and outlines research questions that need to be answered in the future, to fully understand the mechanisms by which these LAB affect fungal growth and mycotoxin production.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.18 Is Hi-C feasible to link antibiotic resistance genes and species in mixed bacterial communities?

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Antimicrobial resistance genes (ARGs) are mainly disseminated within microbial communities via mobile genetic elements (MGEs). When environmental samples are analyzed with metagenomics the link between the extrachromosomal elements and their hosts is lost. Even for ARGs located in the chromosome the inverted repeats that flank the MGEs cause de novo assemblies to fail and thus to correctly determine their genomic context.
A technique called Hi-C, also known as chromosome conformation capture, can preserve the links between ARGs and the bacterial genome by covalently linking DNA located in the same cell. Combined with high-throughput sequencing, Hi-C enables the exploration of ARG context in complete environmental samples. In our study, we constructed an artificial bacterial community consisting of bacteria with known complete whole genome and plasmid sequences to evaluate the feasibility of this method using a commercially available kit. Following NGS the resulting data were analyzed with two different pipelines – a commercially available and our own developed pipeline that was designed to assign ARGs to species present in microbial communities. In contrast to the analysis of usual next-generation sequencing (NGS) data the resulting data require a different type of analysis, since the read pairs are often derived from different DNA molecules or from distant chromosome segments. We present the two different types of analysis workflows and the comparison of their results. We show the influence of parameters and the choice of databases for our own pipeline on the specificity, sensitivity and accuracy of the method. For the constructed microbial community we successfully recovered the expected links between most of the ARGs and their host species. While these associations are often inferred in metagenomics studies by bioinformatics tools and frequently lack experimental evidence, we show an opportunity to actually link this information.

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Food Microbiology in the context of One-Health (animal, plant and human)

P7.19 Resistance mapping in a poultry production process line by robotic HTS technology

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A potential route of transmission of antimicrobial resistant (AMR) bacteria and genes from animals to humans is consumption of food. The occurrence of AMR bacteria in poultry has been acknowledge for several years, and the extended spectrum ?-lactamase (ESBL) producing Enterobacteriaceae is believed to be triggered by the use of feed additives such as Narasin. Bacteria's response to antibiotics is generally defined based on susceptibility tests with bacteria in planktonic state, yet biofilms are the preferred form of bacterial life in most biological ecosystems. Biofilm formation is known to be a contributing factor to development of AMR and biofilm may be challenging in food processing. However, knowledge on the extent of biofilm formation within different sites in poultry production is limited, as is the presence and properties of resistant bacteria in such biofilms. Standard methods like the disk diffusion test involves high degree of manual labour, which is time consuming and restricts both the number of strains and antibiotics to be tested. The aim of the present study was to increase the knowledge on the occurrence of ESBL and quinolone resistant E. Coli (QREC) in a Norwegian process line for poultry production. Important factors for resistance development, such as biofilm formation, variation in resistance spectrum and presence of multi-drug resistant bacteria were studied by use of advanced robotic high-throughput screening (HTS) technology.
Samples were collected from the poultry process line a total of five times during a one-year period and resulted in a large strain collection (>800) of ESBL and/or QREC. Further, a large-scale and detailed study of the generated culture collection was performed. HTS susceptibility assays were conducted on the whole strain collection with 3 different concentrations of 10 different antibiotics simultaneously, and several strains were characterized as multidrug resistant. Robotic biofilm assays confirmed that there exist hot spots for biofilm and AMR phenotypes in the processing plant. Our study contributes to answering fundamental questions related to the occurrence of AMR in poultry processing, and robotic screening of large strain collections has the potential to provide important information on phenotypic characteristics with relevance for AMR treatment, spread and development.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.20 Consumers exposure to ochratoxin A from traditional meat products in Croatia

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Ochratoxin A (OTA) is mycotoxin produced by certain species of Aspergillus and Penicillium genera. It can be found in feed and food, such as cereals, spices, dried fruit, but it can be also found in meat from animals intended for human consumption, coming from feed contaminated with moulds. Tradition of meat production in Croatia has a long history, and is particularly related to some parts of the country. It is common that lot of small pig producers or even households produces pork meat products intended for their consumption or for smaller amount sale. The food technology of this product is simple, since pig meat is just cured in salt and afterwards dried on smoke or wind, or in case of sausage production, minced meat is mixes with salt, red paprika powder, paper or garlic added, and also dried or smoked. In this research, samples of traditionally produced meat products were collected and analysed during period of two years, to obtain results of OTA mycotoxin contamination. The samples were collected from households in geographically different parts of Croatia. They were analysed using liquid chromatography-tandem mass spectrometry method (LC-MS/MS) with the limit of detection (LOD) for OTA of 0.18 µg/kg. The OTA concentrations above LOD were found in 31 out of 288 samples. Average result was obtained using "lower bound" (LB), "middle bound" (MB) and "upper bound" (UB) scenario, because of cases when result was lower than LOD. For exposure calculation average result for OTA occurrence was used and pared with individual result of product intake, obtained from food consumption data base for adults in Croatia, for all three scenarios. Calculated body weight weekly exposures for LB, MB and UB scenario were respectively 0.0003, 0.0006 and 0.0008 µg/kg. Sausage influenced the most on consumer's exposure, among six traditional meat product category. Although OTA occurrence was rare, it is recommended to monitor its presence in TMPs to observe the trend of human exposure. The results obtained in this paper will help to determine the main source of OTA in meat products, and to consider opportunities to reduce its presence as low as possible.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.21 Detection of carbapenem resistance determinants in environmentally isolated Gram-negative bacilli in the Western Cape, South Africa: Subsections of a One-Health approach to AMR

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Carbapenem antibiotics (a class of β-lactams usually reserved for multidrug-resistant bacterial infections) are essential to human health, and resistance from environmental origin is a major concern. Carbapenem resistance (CR) determinants in Gram-negative bacilli (Enterobacteriaceae, Pseudomonas spp., Aeromonas spp.) were studied in the environment (irrigation water, hydroponics) and animals, within the same geographical area. A total of 48 samples (water = 33, and chicken (faeces) = 15) were collected (2020-2022) and subjected to phenotypic resistance screening. Presumptive resistant colonies (according to EUCAST breakpoint guidelines) were streaked on CHROMID® CARBA SMART agar for screening of carbapenemase-producing Enterobacterales (CPEs). Isolates were selected for confirmatory bacterial identification and antibiotic susceptibility testing (AST) using the VITEK® 2 COMPACT automated platform. Genotypic assays included PCR analysis for a subset of environmental isolates, for the detection of carbapenemase genes (KPC, NDM, VIM, IMP, GES, and OXA-48), and whole-genome sequencing (WGS) of an Aeromonas sobria isolate. Genomic DNA was purified and sequenced as pair-ended reads on the Illumina HiSeq X platform (CosmosID). The webserver, ResFinder 4.1, was used to identify acquired antimicrobial resistance (AMR) genes. From the 119 Gram-negative bacilli isolates obtained, CR was identified in 60% and 40% of water and chicken isolates, respectively. On CPE selective media, 55 (46%) isolates were identified as presumptive carbapenemase producers. Furthermore, 60 isolates (water = 48, and chicken = 12), were selected for VITEK® 2 processing, where results showed 9 (19%) water, but no chicken isolates showed a resistant profile. Bacterial identification by VITEK® 2 showed Aeromonas sobria (5), Pseudomonas fluorescens (2), Pseudomonas putida (1), and Aeromonas hydrophilia/punctata (caviae) (1). AST indicated resistance to imipenem (44%), meropenem (44%), both imipenem and meropenem (11%), but no resistance to ertapenem. None of the environmental isolates harboured any of the carbapenemase genes. However, WGS identified two chromosomally-mediated resistance genes, one a class B metallo-β-lactamase (cphA7), and the other a class D penicillinase (ampS), both with β-lactam resistance phenotypes. This study confirmed the prevalence of environmental CR organisms in the Western Cape, further emphasizing the significance of all sectors of a One-Health approach to AMR.
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Food Microbiology in the context of One-Health (animal, plant and human)

P7.22 Characterisation of antibiotic and disinfectant resistance profiles of Enterobacteriaceae isolated from a Norwegian salmon processing facility

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From a One-Health perspective, antimicrobial resistance in humans, animals and the environment cannot be viewed separately, and the World Health Organization (WHO) has identified Enterobacteriaceae to be of critical importance due to the dissemination of extended spectrum β-lactamases (ESBLs), ampicillin hydrolysing (AmpC) β-lactamases, and carbapenemases. Members of the Enterobacteriaceae family are regularly detected in food processing environments, including salmon processing facilities, due to their natural presence in the environment and animals. Salmon products are often consumed raw. Therefore, strict cleaning- and disinfection regimes are of utmost importance to maintain the food safety of such products. However, indiscriminate use of disinfectants can contribute to the development of resistant microorganisms, and potential cross-resistance to antibiotics has been suggested. Thus, correct application of disinfectants is crucial.

The study aimed to assess the diversity of presumptive Enterobacteriaceae isolates (n=69) from a Norwegian salmon processing facility and to examine their resistance to a panel of 10 antibiotics and two disinfectants. The isolates were also tested for AmpC and ESBL enzyme production. The strains originated from different parts of the processing environment and were collected after cleaning and disinfection of the facility over a period of eight months.

The presumptive Enterobacteriaceae isolates were assigned to the families of Yersiniaceae (38), Hafniaceae (10), Enterobacteriaceae (15) and Aeromonadaceae (6) based on sequencing of the 16S rRNA gene. The housekeeping genes dnaJ, gyrB and rpoB were applied to identify the isolates on genus and species level, revealing Serratia (25) as the dominant genus, followed by Hafnia (10) and Yersinia (13). Fourteen out of 69 isolates displayed a combination of resistance to ampicillin, cefotaxime and ceftazidime. Notably, two Serratia isolates displayed additional resistance to meropenem, trimethoprim/sulfamethoxazole and chloramphenicol accompanied by co-expression of ESBL and AmpC. These isolates are currently being screened for disinfectant resistance. The results will be presented in more detail at the conference.

The high diversity of Enterobacterales and isolates with noticeable AMR profiles demonstrates the need of more knowledge to optimize the cleaning and disinfection regime to prevent their presence in the processing environment.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.23 Elucidating the ecological function of the emetic toxin cereulide produced by the opportunistic pathogen Bacillus cereus

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Most bacterial species produce an arsenal of toxins, which often function in interspecies competition. The foodborne pathogen Bacillus cereus is an endospore-forming bacterium displaying a wide array of phenotypic and pathogenicity traits, depending on the strain background. It can cause different types of food-associated diseases, manifested in diarrheal or emetic symptoms. Emesis is triggered by the heat- and acid-stable cyclic peptide toxin cereulide. The genetic elements facilitating its biosynthesis are encoded by the ces gene cluster (cesPTABCD) located on the megaplasmid pCER270. Cereulide is a highly lipophilic ionophore that interferes with cellular and mitochondrial membrane potentials, with high cytotoxic potential. Besides, cereulide shows antimicrobial activity against certain fungi and bacteria. However, the ecological role of cereulide remains largely unexplored.
Here, we assess the toxic action of cereulide on bacterial and fungal species, as well as on higher eukaryotes. We identify a subset of gram-positive bacteria and yeasts showing growth inhibition in response to cereulide, which additionally displays nematocidal and insecticidal properties. This implies that cereulide action may depend on the ecological context and the interaction partner. To test whether B. cereus modulates expression of the ces gene cluster in response to other species, we further employed a reporter strain harboring a luciferase cassette regulated by the ces promoter. Indeed, transcriptional kinetics of ces gene cluster expression is altered upon co-culture with fungal species. Moreover, B. cereusactivates cereulide biosynthesis genes during saprophytic growth in an insect host. These data suggest that the ability to produce cereulide may function in interspecies interaction and competition. Given that emetic B. cereus is increasingly recognized as an opportunistic human pathogen, dissecting the biological function of cereulide will aid in better understanding of its ecological sources and hence, leading to improved food safety strategies.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.24 Antibiotic resistant coliform bacteria in food of animal origin

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The objectives of this study were to assess the occurrence of selected antibiotic resistant coliform bacteria in raw cow milk, and ready-to-eat foods of sushi and poke.
In case of unpasteurized milk was assessed 6 samples, collected during winter season (in February-March 2017) and summer season (in August-September 2017) from vending machines in Slovakia. Number of total coliform ranged from 2,45 log CFU/mL to 4,18 log CFU/mL. Raw milk collected during summer season contains less total and resistant coliform bacteria than winter milk. Resistant isolates were consequently identified and characterized in term of their antibiotic sensitivity and biofilm production. Overproduction of efflux pumps as one of mechanism of resistance was also determined. According to results, Escherichia coli formed 43 % of all identified resistant coliforms. Resistant bacteria were registered in each sample and all isolated bacteria were sensitive to ciprofloxacin, meropenem and none of them were multidrug resistant. Overproduction of efflux pumps was detected in 17 % of cases and 68 % of resistant bacteria were strong producers of biofilm, which can represent a risk in milk industry increasing bacterial potential for microbial contamination.
On the other hand, samples of sushi and poke were also monitored for presence of antibiotic resistant coliform bacteria (in March 2019-December of 2020). Number of total coliforms range from 2,3 log CFU/g to 5,3 log CFU/g. In one sample of sushi was not detected any coliform bacteria. Predominant genus of identified antibiotic resistant coliform bacteria was Enterobacter spp. and in one case was observed multidrug resistance among this bacterium. Sensitivity to gentamycin, ciprofloxacin and meropenem was detected in all resistant coliforms and only two strains have the ability to overproduction of efflux pumps. Predominant was weak ability to form biofilm.
The findings have showed that food chain is also a source for antibiotic resistant bacteria and can contribute to spreading of resistance among population.
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Food Microbiology in the context of One-Health (animal, plant and human)

P7.25

Genomic diversity and fitness of Listeria monocytogenes in the pig manure recovery sector

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LListeria monocytogenes (L. monocytogenes) is a foodborne pathogen and the causative agent of listeriosis, a rare but severe disease. Ubiquitous, L. monocytogenes is a genetically heterogeneous species and most of the strains are grouped into either hypervirulent or hypovirulent Clonal Complexes (CC). Some of these CCs are more prevalent in specific ecological niches.

Because pig manure is an excellent source of nutrients (nitrates, phosphorus), it is widely used for crop production. However, the recent intensification of livestock production in Europe also concentrated manure production, raising eutrophication issues. Consequently, manure treatment techniques were developed in order to reduce environmental impacts and to produce energy and fertiliser.

Nitrification-denitrification is an aerobic/anoxic manure treatment limiting soil pollution by nitrates. Anaerobic digestion allows energy recovery by producing methane, promoting a circular economy. The endpoint of these processes is land spreading of the processed manure as fertiliser and amendment. This can potentially reintroduce pathogens in the environment and the food chain. Pathogens flows between pig manure disposal and food sectors are currently poorly understood, in particular for L. monocytogenes.

This project aims to analyse the genomic diversity of L. monocytogenes occurring throughout the pig manure management chain. In the "One Health" context, CCs found in the manure treatment chain were compared to the overall diversity found in food and agricultural environments, to acquire a systemic and innovative picture of pathogen fluxes from farm effluent management to the food system.

We assembled a collection of 430 strains isolated from pig faeces and slurries, before and after denitrification and anaerobic digestion treatments. All isolates were characterised (serotype, PFGE profile) and CCs were inferred from Whole Genome Sequencing.

The distribution of CCs was compared along the treatment chains in order to investigate the impact of aerobic and anaerobic processes on L. monocytogenes survival and population dynamics. Further experiments will be performed to investigate intrinsic factors underlying survival throughout the value chain from farm effluent to agronomic land spreading. Pan-Genome-Wide Association Study (GWAS) will be used to identify genetic markers linked to soil survival, which could be further used to develop diagnostic tests for public health surveillance.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.26 Rapid detection of Mycoplasma bovis, Staphylococcus aureus and Streptococcus agalactiae in cattle bulk tank milk in Cyprus and relations with somatic cell counts

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One hundred and seventy-seven (177) bulk tank milk samples were analyzed with a commercially available real-time polymerase chain reaction kit and 11 (6.21%), 41 (23.16%), and 58 (32.77%) tested positive for Mycoplasma bovis, Staphylococcus aureus, and Streptococcus agalactiae, respectively. Statistical analysis revealed a significant relationship between the presence of S. aureus and S. agalactiae. Enumeration of somatic cells was performed in the same samples by flow cytometry. The somatic cell counts were found higher in S. aureus and S. agalactiae positive samples. No association was found between M. bovis presence and somatic cells counts. Low internal assay control Ct values were found to be related with high somatic cell counts. Noticeably, this is the first report for the presence of M. bovis in Cyprus. Therefore, its presence was confirmed by bulk tank milk culture, conventional PCR, and next generation sequencing. Furthermore, M. bovis was typed with multilocus sequencing typing and was allocated to sequence type 29 (ST 29). Real-time PCR in bulk tank milk samples is a useful tool to detect mammary infections, especially for neglected pathogens such as M. bovis

Food Microbiology in the context of One-Health (animal, plant and human)

P7.27 Detection of Sars-CoV-2 on environmental surfaces and animal foodborne matrices

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Introduction
During Sars-CoV-2 pandemic, the interest in research of sources virus spread increased and different studies are performed to understand surfaces involvement.
There is a lack information regarding the presence of this virus on food; wastewater contamination and poor hygiene practices could be the sources of viral contamination in handled food surface products.
The use of swabs has been widely demonstrated to be effective in the clinical diagnosis of viruses contamination. Recently, this tool has been introduced in the research of pathogens in food.
The aim of this work was to evaluate the presence of Sars-CoV.-2 on the different environmental and food surfaces.
Methods
During 2021 and 2022, a total of 522 environmental-, 250 meat- and 260 dairy products-surfaces were tested.
The flocked nylon swabs (FLOQSwab™, Copan®, Italy), coupled with eNAT® or SRK® buffer were used, given their ability to improve detecting viruses and bacteria with molecular amplification assays.
The preparation and nucleic acids extraction were performed according to ISO 15216-2, which involves the use of swabs on the surfaces and using Mengovirus as a process control. Real-time RT-PCR were applied to detect the Sars-CoV-2 virus, testing 4 targets: ORF 1ab (nsp14), E-gene (envelope structural protein), N1- and N3-gene (nucleocapsid structural proteins).
Results
A total of 1032 samples were analyzed to evaluate the presence of Sars-CoV-2 RNA. Results showed a positivity in a single sample of environmental surface; in particular, for ORF 1ab and N3 genes.
The totality of the samples analyzed reported an acceptable recovery efficiency, making the result reliable; indeed, Mengovirus was detected in every sample.
Discussion and conclusion
Detection of Sars-CoV-2 genome it may not necessarily indicate vitality of virus in the sample; although, literature reported, the persistence of Sars-CoV-2 in the environment, for days.
Nylon flocked swabs are designed to improve sample absorption and to release an high quantity of the collected sample; therefore it could be a valid tool to be used for the research for viruses on environmental or food surfaces.
This surveillance has highlighted an evident low risk of virus transmission on the tested surfaces, linked to the consumption of food, even manipulated.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.28 Detection of Mycobacterium avium paratuberculosis in dairy herds in Cyprus

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Mycobacterium avium subspecies paratuberculosis is the causative agent of Johne's disease, a chronic debilitating enteritis in cattle and small ruminants. The route of transmission is faecal-oral and the bacterium can be excreted in faeces, colostum and milk. The source of infection for new-born animals is by consumption of contaminated colostrum, while fully grown animals can be infected via contaminated water and feed. In humans, the bacterium has been related with Crohn's disease with possible source of transmission the consumption of contaminated milk and dairy products. A survey was contacted analyzing 239 bulk tank raw milk samples (BTM) from cow herds and 47 samples from sheep and goat herds in an attempt to estimate the prevalence of the bacterium in BTM in Cyprus and compare the results with previous similar surveys which were conducted over a decade ago.
Notice: *Results are currently analyzed and will be presented in the conference.

P7.29 Antiviral natural compounds: a comparison between the use of essential oils and hydrolates on Murine Norovirus infectivity

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As co-products of Essential Oils (EOs) extraction, deriving from steam-distillation or hydro-distillation of fresh medical plants, hydrolates retain their antimicrobial, antiviral and antifungal properties, making them suitable for food safety purposes. The aim of this study was to evaluate the antiviral effect of EOs and hydrolates, obtained from C. limon and T. serpyllum, on Murine Norovirus (MNV-1), a human norovirus surrogate.
Preliminary assay demonstrated that different concentrations, from 0.01 to 1% v/v, of EOs (emulsified in 33% sunflower oil, 0.1% tween 80 and physiological solution) and hydrolates did not have cytotoxic effect on MNV-1 permissive cell line RAW 264.7. The highest concentration (1%) was chosen to evaluate the effect on viral infectivity. At this aim, a MNV-1 suspension (approx. titer of 2.7×105 TCID50/ml) was treated with hydrolates solutions and EOs emulsions, individually. Treated virus aliquots were stored at -80°C immediately (t=0) and after 24 h of incubation at 20±2°C (t=24). Untreated MNV-1, hydrolate solutions and EOs emulsions were used as positive and negative controls, respectively.
To assess the antiviral effects of hydrolates and EOs, the reduction of viral infectivity was estimated by comparing the TCID50/ml of untreated viral suspension and the viral suspension treated with hydrolates and EOs. The results showed a natural loss of infectivity of untreated virus after 24h of approx. 1 log. With regard to T. serpyllum, both EO and hydrolate caused a reduction of MNV-1 infectivity of about 1.83 log immediately (t=0), but did not provide a further significant decrease after 24 h. Instead, the EO and hydrolate of C. limon exerted an immediate (t=0) reduction of the viral infectivity of about 1.33 log and 1 log respectively, followed by a further reduction of infectivity of 1 log after 24h for the hydrolate.
In conclusion, the results obtained with C. limon and T. serpyllum showed a quite comparable antiviral activity, both for EOs and hydrolates. This opens promising perspectives in the use of hydrolates, easier to use, especially in the field of food technology and food safety, as they support their efficacy at low concentrations and with the use of short contact times.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.30 Antimicrobial activity of natural products against foodborne pathogens

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The rise of antimicrobial resistance alongside the shift in the consumers' demand for safer and healthier products highlight the pivotal need to investigate natural products and their potential antimicrobial activity against foodborne pathogens. The aim of this study was to investigate the antimicrobial effect of pure phenols as carvacrol, eugenol, catechin, quercetin, gallic acid, and extracts namely carob leaves, pomegranate peels, Capparis spinosa leaves, Geranium purpureum leaves, Glycyrrhiza glabra leaves and roots vine and liquorice industry by-products as well as Pistacia lentiscus essential oil against 10 different strains of Listeria monocytogenes, Salmonella Enteritidis, Escherichia coli and Staphylococcus aureus. Agar well diffusion assay and the broth microdilution method were performed to determine the MIC and MBC of each substance tested. Results revealed significant antimicrobial activity from Liquorice extracts against all gram-positive organisms tested ranging from 31µg to 62.5 µg mL-1. Furthermore, a strong antimicrobial activity was evident from carvacrol, eugenol, vine by-product, catechin and quercetin, in some Gram+ and Gram- bacteria with MIC values ranging from 125 to 2000 µg/ mL -1. The remaining plant extracts tested alongside the Pistacia lentiscus EO exhibited a lower antimicrobial activity to Gram+ bacteria to none towards Gram- bacteria. Results highlight the need to investigate further some of the more promising natural products tested and their potential application in food matrices as alternative for food processing and preservation aids.

P7.31 Microbial survey of poultry drinking water reveals the diversity of bacterial residues in the waterlines

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The quality of poultry drinking water has a significant effect on broiler health and performance. The effect of waterline sanitation approaches on microbial drinking water quality and microbial diversity have not been extensively studied. For this reason, this study aimed to perform a comprehensive microbiological survey of poultry drinking water on 20 different poultry farms in Austria. The effect of three different waterline sanitation applications on poultry farms, namely (APP), APP1 (4.0 ppm ClO2), APP2 (3.0% peroxyacetic acid (PAA) with 4.0 ppm ClO2), and APP3 (3.0% PAA and 4.0 ppm ClO2 combined with mechanical treatment) were evaluated. While chemical water treatment alone (i.e., APP1 and APP2) did not significantly reduce microbial load in the poultry drinking water, a substantial effect was observed after combined chemical and mechanical (APP3) treatment. Microbial diversity analysis revealed that the majority of the bacteria isolated both before (APP1 49.3%, APP3 43.4%) and after (APP1 42.0%, APP2 42.0% APP3 38.7%) waterline sanitation were opportunistic pathogens. The most frequently isolated bacterial species from poultry drinking water were Pseudomonas aeruginosa and Stenotrophomonas maltophilia. The biofilm-forming capacity of these organisms is indicative of high level of biofilm presence in the poultry drinking waterlines, which represent a potential source of waterborne pathogen transmission and is a risk to animal health. Both, Citrobacter freundii and Enterobacter cloacae complex indicated resistance phenotypes to enrofloxacin, tetracycline and trimethoprim/sulfamethoxazole. Lack of proper water hygiene management programs on poultry farms may lead to increased use of antibiotics and selection of bacteria resistant to antibiotics and disinfectants, which could contribute to their spread in the environment.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.32 Inhibition of Fusarium spp. by selected essential oils

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Fusarium spp. are ubiquitous plant pathogens affecting grain. They can cause several plant diseases in grain resulting in reduced grain size and quality and crop losses. Furthermore, in stored grain Fusarium is able to grow and to produce mycotoxins. These can affect human and animal health after consumption of contaminated food and feed, respectively. Therefore, the precautionary prevention and control of Fusarium infestation and in stored grain is of great importance.
So far, numerous natural inhibitors with an antimicrobial effect are known including some essential oils (EOs) and their active components. Most of these EOs have been tested inhibiting bacteria, very little is known about their ability to inhibit moulds like Fusarium spp.
A variety of essential oils, their active components and conventional preservatives were analyzed for their minimum inhibitory concentration (MIC) by broth macro-dilution. F. graminearum CBS 110263 (CBS Utrecht, Netherlands), F. culmorum DSM 62191 (DSMZ, Braunschweig, Germany) and F. sporotrichioides (isolate) were used. All essential oils and their active components are obtained from Vögele (Lauffen, Germany.) Each MIC is presented as the median of at least three duplicates. Tested substances: Carvacrol, eugenol, thymol, trans-cinnamaldehyde, octanoic and propionic acid and oils of Origanum vulgare (origanum), Cimbopogon citratus (lemongrass), Syzygium aromaticum (clove), Cinnamomum zeylanicum (cinnamon bark), Curcuma longa (turmeric) and Zingiber officinale (ginger).
MICs range from 40 µg/mL (C. zeylanicum for F. culmorum and trans-cinnamaldehyde for F. sporotrichioides) up to 800 µg/mL (propionic acid for F. culmorum).
The efficacy of the selected substances results in the following ranking:
F. graminearum: C. zeylanicum = trans-cinnamaldehyde > O. vulgare = thymol = octanoic acid > carvacrol = C. citratus > eugenol = S. aromaticum > propionic acid
F. culmorum: C. zeylanicum > trans-cinnamaldehyde = octanoic acid > carvacrol > O. vulgare = C. citratus = S. aromaticum = thymol > eugenol > propionic acid
F. sporotrichioides: trans-cinnamaldehyde = octanoic acid > C. zeylanicum > carvacrol > eugenol = O. vulgare = C. citratus = S. aromaticum = thymol > propionic acid.
C. longa and Z. officinale do not show antifungal activity (>4500 respectively >800 µg/mL).

Food Microbiology in the context of One-Health (animal, plant and human)

P7.33 Coagulase-negative staphylococci – potential microbiological hazard in goat's milk

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Coagulase-negative staphylococci (CNS) include many species with different pathogenic and biochemical properties. As opportunistic microorganisms CNS can cause hard infections, especially among immunocompromised people, which are often difficult to treat because of the high prevalence of multiresistant strains. Some CNS also have the ability to produce toxins the same as those produced by coagulase-positive Staphylococcus aureus.
The aim of the study was to specify the species of CNS, determine their antibiotic resistance and enterotoxigenicity to estimate the health risk for people consuming raw goat milk.
A total of 128 CNS strains isolated from goat's milk were tested. The identification of Staphylococcus spp. was performed by morphology of colonies using Baird-Parker agar with rabbit plasma fibrinogen and mass spectrometry method (MALDI-TOF MS). Antimicrobial and methicillin resistance of the isolates were determined using Minimal Inhibitory Concentration (MIC) test on a Sensititre EUST plate (Thermo Scientific) and PCR methods, respectively. The detection of enterotoxic genes was performed using two multiplex PCR.
The most frequently isolated species were: S. lugdunensis, S. xylosus, S. simulans, S. chromogenes and S. haemolyticus. Antimicrobial resistance analysis showed that 23.4% of CNS were susceptible to all antibiotics tested and 76.6% were resistant to at least one of them. The highest resistance was observed for tiamulin (35.9%), fusidate (35.9%), penicillin (30.5%) and tetracycline (18.0%). 33.7% of CNS showed resistance to only one antibiotic, resistance for two drugs was found in 33.7% of isolates and 32.7% of them were multiresistant (3 to 6 antibiotics). On the other hand, all tested strains were sensitive to chloramphenicol, vancomycin, gentamycin, linezolid and synercid. No methicillin resistant strains were detected. Only two strains identified biochemically as Staphylococcus haemolyticus and Staphylococcus lentus showed the presence of enterotoxic genes: seg, seh, sei and sec, respectively. The sea, seb, sed, see, ser, sej, sep genes were not detected.
The results obtained in this study indicate the presence of various species of coagulase-negative staphylococci in raw goat milk. The consumption of raw milk and traditional dairy products made from unpasteurised milk may pose a potential risk of infection caused by these bacteria, especially by multiresistant or enterotoxigenic strains.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.34

Identification of highly virulent S. aureus strains circulating in the dairy value chain in Zambia

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Staphylococcus aureus is an opportunistic pathogen common in humans and animals. By producing various virulence factors, S. aureus can induce several diseases, e.g. soft-tissue infections, food intoxication, and the toxic shock syndrome. Staphylococcal mastitis infections are a frequent cause of raw milk contamination with S. aureus. If hygiene is poor, the pathogen can also be transmitted from humans to food/animals and vice versa.
The dairy chain in Zambia was investigated for S. aureus using a one-health approach. On-farm samples were collected from humans (milkers' nasal/hands swabs), environment (farm water, surface of clean milk buckets), and food (pooled raw milk). In addition, raw milk, processed milk, and/or dairy products were sampled along the chain at milk collection centres, traditional markets, informal traders, milk processing plants, and supermarkets/shops.
Fifty-seven S. aureus isolates were selected for detailed characterisation by whole genome sequencing (WGS). Eight of those carried a toxic shock syndrome toxin (TSST) gene. Two pairs of strains each showed a close relatedness by core genome multi-locus sequence typing (cgMLST). One pair comprised strains of spa type t355 isolated from a milker's nasal swab and a milk bucket swab from the same farm. They additionally carried genes for the Panton-Valentine leucocidin (PVL) associated with severe disease progression in humans. The other pair were isolates from raw milk belonging to the novel spa type txAA (r35:r13). One derived from a milk collection centre, the other from a traditional market sampled four months later. These isolates carried genes for staphylococcal enterotoxins (SEI, SEM, SEN, SEO, SEU), which might cause food poisoning.
Our study identified the Zambian dairy chain as reservoir for highly virulent S. aureus that can be transmitted along the dairy value chain and may persist in the chain. Improved hygienic practices are needed to limit transmission and spread of such strains.
This work was supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) (grant number: 2815DOKP04) and was co-funded by the German Federal Institute for Risk Assessment (BfR).

Food Microbiology in the context of One-Health (animal, plant and human)

P7.35

Behaviour of Staphylococcal Egc Enterotoxins during bacterial growth and under food production-like stress conditions

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According to the European Food Safety Authority (2019), 77 out of 114 outbreaks caused by staphylococcal enterotoxin (SFPO) are weak evidence outbreaks. However, only five out of 27 enterotoxins can be analyzed using commercially available kits. Especially the presence of the so called "new enterotoxins", that have been described being involved in SFPOs, cannot be determined. A group of these new enterotoxin genes (seg, sei, sem, sei, seo and seu) is located on the same enterotoxin gene cluster (egc).
The aim of the present study is to improve the understanding of the parameters and conditions in which egc enterotoxins are produced to better control their expression during food production and storage.
For this purpose, a selection of eight strains from different origin were chosen according to their genetic diversity (genetic structure) and origin (human, animal, environment, food). For them the enterotoxin expression (mRNA) of seg, sei, sem, sen and seo was measured using RT-qPCR at three different points during the bacterial growing phase (start, mid-log and end-log). Based on these results three strains were selected to study the enterotoxin gene expression under stress conditions: salt concentrations up to 100 g/l, higher temperature (45 °C). In addition each sample was tested on Staphylococcal enterotoxins G and I expression using an in-house sandwich ELISA method.
The results show that egc enterotoxins are mostly expressed in the mid-log phase of bacterial growth and seem to switch off at the end of the log phase. SEG and SEI are already produced at an early stage of the growing phase. Interestingly, common salt and temperature stress, mimicking conditions found in food production, seem not to affect the expression of egc enterotoxins, even though differences between strains were observed.
The study gives insights on the production of egc enterotoxins under stress conditions. This information will enhance the availability of methods controlling egc enterotoxins in food production and storage.The results obtained in this study indicate the presence of various species of coagulase-negative staphylococci in raw goat milk. The consumption of raw milk and traditional dairy products made from unpasteurised milk may pose a potential risk of infection caused by these bacteria, especially by multiresistant or enterotoxigenic strains.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.36 Mushroom Mycelia as a Nutraceutical Food Supplement

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Mushrooms are good source of vitamin D2 (ergocalciferol). They contain ergosterol (provitamin D2, a precursor for vitamin D2) in their cell membrane. Ergosterol can be transformed into vitamin D2 by UV-B exposure. Vitamin D helps in maintaining the calcium and phosphorus homeostasis in the body. Vitamin D deficiency has been reported to be associated with cancers, heart diseases, obesity, diabetes, and arthritis. The deficiency of vitamin D can lead to osteoporosis. Mushrooms are also a good source of many nutraceuticals. Currently, commercial production of medicinal mushrooms is mainly obtained through the field cultivation of the fruiting body, which is labor-intensive, time-consuming and prone to contamination. Submerged cultivation of the mushroom mycelia has received much attention as a promising alternative for the efficient production of the biomass of medicinal mushrooms and their active metabolites. The present study focuses on large-scale production of nutraceutically enriched edible Pleurotus eryngii mushroom mycelia under submerged culture conditions in bioreactors and vitamin D enhancement strategies in harvested mycelia through optimized UV irradiation conditions. Further, the effect of UV irradiation on the other nutraceutical qualities (beta-glucans, phenolics, antioxidants) was also studied.

P7.37 Microbiological quality, antibiotic resistance and virulence genes in Escherichia coli isolated from chicken samples of Ouagadougou

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Introduction: In Burkina Faso, flamed/grilled chickens is very popular and well-known to consumers. The aim of this study is to evaluate the microbiological quality, the virulence gene from E. Coli isolated of these chickens in Ouagadougou and these antibiotics resistance.
Methods: A total of 102 grilled, flamed and fumed chickens were collected in Ouagadougou and analysed, using standard microbiological methods. All E. Coli isolates were checked with the antimicrobial test and also typed by 16-plex PCR.
Results: The mean of Aerobic mesophilic bacteria (AMB) and Thermo-tolerant coliforms (TTC) were found respectively between 6.90 ± 0.12 107 CFU g-1 to 2.76 ± 0.44 108 CFU g-1 and 2.4 ± 0.82107 CFU g-1 to 1.27 ± 0.9108 CFU g-1. Forty of samples (38.24%) were unacceptable based on the AMB load. Fifty nine samples (57.85%) were contaminated with TTCs. E. Coli strains were found to 27.45%. Diarrheagenic E. Coli (DEC) strains were detected in 21.43% of all samples. Only STIa, stx2A, invE, astA, and aggR virulence genes were detected belonging EAEC and ETEC (in two samples each) and STEC and EIEC (in one sample each). No EPEC was detected. Low resistance was observed with antibiotics of Betalacmins family.
Conclusions: This study showed that flamed/grilled chickens sold in Ouagadougou could pose health risks for the consumers. Need of hygienic practices or system and good manufacturing practices are necessary to improve the hygienic quality of flamed/grilled chickens and also to avoid antibiotics resistance. Slaughter, scalding, evisceration, plucking, bleeding, washing, rinsing, preserving, grilling and selling, may be the ways of contamination.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.38

Antifungal activity of biosurfactants against spoilage organisms associated with bakery products

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Microbial spoilage of bakery products occurs mostly due to the growth of filamentous fungi and yeasts. This does not only contribute to the world's food waste problem, but can also lead to negative health effects via mycotoxin production by some fungal strains. Furthermore, there is an increased demand for foods with more natural ingredients. Therefore the food industry is urged to limit the usage of chemical preservatives and make the shift to natural, more sustainable and less toxic alternatives. The aim of this study is to investigate the antifungal activity of different biosurfactants against spoilage strains relevant in bakery products and define their minimum inhibitory concentrations (MIC).

The antifungal susceptibility testing of biosurfactants was performed using broth microdilution assays in 96-well plates. A range of concentrations of the compounds were screened against Aspergillus niger D-02906, Penicillium paneum CBS 302.97, Penicillium roqueforti CBS 174.87, Rhizopus stolonifer CBS 819.97, Eurotium rubrum D-061178 and Hyphopichia burtonii C-00349. The test medium was yeast extract sucrose, and calcium propionate was used as a control at concentrations according to the maximum permitted levels established by the European Commission for bread and rolls. The effects of the lipopeptides in the baker's yeast strain Saccharomyces cerevisiae NCYC 77 were also assessed using the same methodology.

The broth microdilution assays allowed the determination of MIC values of the biosurfactants for each of the fungal strains tested. Additionally, the antifungal activity of biosurfactants against the studied spoilage strains is compared to that of calcium propionate, a food additive commonly used as a preservative in the baking industry. This research is vital to better understand the possibility to use biosurfactants as natural preservatives in bakery products.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.39 The environment shaping our salad – an NGS perspective

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Main contamination sources transferring antibiotic-resistant bacteria (ARB) and resistance genes (ARG) to fresh produce are irrigation water, soil, and fertilizers (e.g. manure). In a real-practice field study, we investigated the contribution of irrigation water, soil, and manure to lettuce microbial communities and resistome. Lettuce was grown using: (A) river water and untreated soil; (B) river water and manured soil; (C) UV-treated river water and manured soil; or (D) UV-treated river water and untreated soil. Plant, water, soil, and manure were sampled from planting to harvest for all treatments. Relative bacterial abundance (phylum level) showed (1) stable soil communities irrespective of treatment; (2) higher Proteobacteria abundance in UV-treated as compared to untreated river water; and (3) increasing proportions of Proteobacteria from young to mature plants. Lettuce communities at harvest resembled soil communities, possibly due to overhead irrigation-induced soil splashes accumulating on the leaves over time. Diversity analyses for lettuce showed that with increasing proportions of Proteobacteria, ?-diversity dropped significantly. No significant differences were observed overall between treatments. Only for young lettuce, treatments A and D (untreated soil) had higher Shannon and inverse Simpson indices as compared to B and C (manured soil). Accordingly, UniFrac-based ordination of lettuce yielded good separation of young and mature lettuce. Linear regression identified Time as the main driver for the observed community variation. Preliminary resistome analysis showed a clear impact of UV-treatment in water and higher ARG abundance and diversity in young compared to old lettuce plants.
In a BSL2-greenhouse, lettuce was treated with water or manure containing ESBL-producing E. Coli (106 or 108 CFU/mL) with a blaCTX-M-15 multidrug-resistance plasmid. ESBL-producing E. Coli was cultured from soil and lettuce and blaCTX-M-15 was detected by qPCR. In summary, blaCTX-M-15 was shown to persist in the pot-lettuce system until the end of our experiment, that is, up to 4 weeks on lettuce and up to 9 weeks in soil, which is long after culture-based detection (incl. enrichment culture) of the introduced ESBL-producing E. Coli was negative. Whether blaCTX-M-15 persisted in non-culturable E. Coli, in other bacteria, or as extracellular DNA is matter of current investigation.

P7.40 Probiotics Lactiplantibacillus plantarum J-15 reduced calcium oxalate kidney stones by regulating intestinal microbiota in rats

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Probiotics are playing an increasingly important role in functional food and disease treatment. As an important probiotic, Lactiplantibacillus plantarum shows an increasing prevention role against the formation of kidney stones. However, its mechanism has mainly been focused on inhibiting the inflammation in the colon in the gastrointestinal (GI) system, and the intestinal metabolites from microflora has not been revealed fully. In this study, we investigated the effect of L. plantarum J-15 on kidney stone formation in renal calcium oxalate (CaOx) rats induced by ethylene glycol, and monitored the changes of intestinal microflora and their metabolites detected by 16S rRNA sequencing and widely targeted analysis, followed by the evaluation of the intestinal barrier function. The results showed that L. plantarum J-15 effectively reduced renal crystallization and urinary oxalic acid. Ten microbial genera, including anti-inflammatory and SCFAs-related Faecalibaculum, were enriched in the J-15 treatment group. There are 136 metabolites from 11 categories significantly different in the J-15 supplementation group compared with CaOx model rats, most of which were enriched in the amino acid metabolic and secondary bile acid pathways. The expression of intestinal tight junction protein Occludin were decreased in the intestine, which further reduced the translocated lipopolysaccharide in the blood upon J-15 treatment. It suggested that L. plantarum J-15 might reduce kidney stone formation by restoring intestinal microflora and metabolic disorder, protecting intestinal barrier function. This finding provides new insights into the regulation of health and the diseases therapies for renal stones by Lactiplantibacillus plantarum, and L. plantarum J-15 has the potential to be developed into a probiotic food or food additive to assist in the clinical treatment of kidney stones and the general health promotion.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.41 Molecular and microscopical identification of resistant viable but non-culturable (VBNC) bacteria in commercially pasteurised milk

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Pasteurisation temperature is one of the adverse environmental conditions claimed to be a predisposing factor for the formation of the VBNC state in different resistant microorganisms. This study aimed to identify VBNC in three main mastitis related microorganisms (E. Coli, streptococci, and staphylococci) and their potential to retain some antimicrobial resistant genes in commercially pasteurised milk by molecular and microscopic assessment after pasteurisation. in this study 84 pasteurized milk samples were collected from commercial suppliers in Adelaide, South Australia. DNA extraction and PCR molecular identification of 16S specific for E. Coli, staphylococci and streptococci and plasmid mediated AMR genes (bla-TEM-1B, tetA, blaZ, ermC, mecC, tetK, ermB and tetM), coupled with viability testing using BacLight™ LIVE/DEAD® staining assay and screening electron microscopy (SEM). Molecular identification revealed prevalence of E. Coli, staphylococci and streptococci in examined samples of 61.9%, 86.9% and 95.5%, respectively, Prevalence of bla-tem-B1 (43%), tetA (55%) and tetM (31%) genes was also high. Viability testing and SEM confirmed the existence of the VBNC bacteria in the commercially pasteurised milk. The current study supports that the commercial pasteurisation inducing VBNC state in some of dairy resistant bacteria together with the persistence of the AMR genes related to these bacterial species raising a significant consideration for both dairy industry and public health sectors. Hence, further studies are required to estimate the potential horizontal gene transfer of plasmid-mediated AMR genes, resuscitation possibility of those VBNC bacteria, and their potential hazard levels.

P7.42 Comparative genomics of dairy-associated sub-Saharan Staphylococcus aureus identifies milk as reservoir for human- and animal-derived strains and a putative animal-related clade with presumptive novel siderophore

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Staphylococcus aureus is considered a neglected tropical disease with high impact on human and animal health alike. Dairy production in sub-Saharan Africa (SSA) relies heavily on various animals such as cows, goats and camels, depending on the region. S. aureus is known to cause mastitis and exhibits high prevalence in raw milk. The population structure including genotypic and phenotypic traits of dairy S. aureus in relation to animal and human isolates is, however little known for SSA. In this work, 20 S. aureus dairy isolates from East and West Africa were selected for comparative genomics and phenotypic analysis. Comparing their population structure revealed a large diversity of different origins suggesting milk to be a reservoir for human and animal strains alike. Furthermore, a novel putative siderophore was detected in multiple strains in a distinct animal-clade with strains of global origin that suggests horizontal gene transfer. These findings combined with the virulence genes harbored by these strains dairy-derived strains such as pvl, human evasion factor scn various enterotoxin, leucocidin and antibiotic resistance genes, stresses the need for an integrative One Health approach to tackle the problem of S. aureus infections in animals and humans in sub-Saharan Africa

Food Microbiology in the context of One-Health (animal, plant and human)

P7.43 Synergistic effects of Lentinula edodes and probiotics: Potential in improving health

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The burden of meeting the needs for high-quality food and ensuring nutritional security is being elevated by population growth. The importance of nutraceutical-enriched foods and natural food items with health-promoting properties has garnered public attention, in present circumstances. In view of the constant rise in health-related complications, there is an upward trend in demand for nutraceuticals with additional health-promoting attributes; thus, probiotic-mediated food fermentations can be an excellent prospective approach. Lentinula edodes are well-known for their wide range of medicinal qualities. In Lentinula, bioactive substances like flavonoids, minerals, proteins, ?-glucans, etc., are also present in a substantial amount, which can scavenge free radicals created in our systems and play essential roles in various diseases. L. edodes also contain non-digestible polysaccharides that act as possible prebiotics, allowing Lactobacillus bacteria to proliferate more expeditiously. Prebiotics provide sustenance for cell proliferation in bacteria that are considered nourishing and beneficial to human health. The objective of this study was to assess the role of nutraceutical-enriched mushroom powder (rich in bioactives) in probiotic bacteria growth, as well as to measure the quality characteristics supplemented by probiotic bacteria in fermented samples. In-vivo investigations on osteoporotic mice were also conducted to evaluate how the combination of probiotics and mushrooms affects osteoporotic bones. Outcomes from the analysis revealed that the growth of probiotic bacteria significantly ($p<0.05$) increased in fermented mushroom powder compared to control. The contents of flavonoids, phenols, and antioxidant amino acids are also considerably ($p<0.05$) improved in the fermented mushrooms powder. Results from in-vivo studies assessed using SEM unveiled that mushrooms and probiotic bacteria act synergistically to benefit osteoporotic bones.

P7.44 Genomic resistance characteristics of Listeria monocytogenes isolated from bivalves

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Listeria monocytogenes is one of the major microbiological hazards associated with food including fish and bivalves. L. monocytogenes is characterized by high resistance to antibacterial substances, increases the possibility of survival and cross-contamination of final food products, leading to possible outbreaks of listeriosis. In this study, whole genome sequencing (WGS) was used as a tool to characterize 20 bivalves origin L. monocytogenes isolates. The obtained WGS data from the isolates were analysed to assess the genomic diversity of L. monocytogenes and to detect the absence / presence of genes encoding antimicrobial resistance. The obtained results allowed to determine that the dominant number of strains belongs to Lineage II, the remaining strains belong to Lineage I. The genes coding tetracycline resistance, including the lmo0839, tetA_3 and tetC genes, were most often found in the genomes of the strains. The tetA_2 and tetA_1 genes were found in majority of strains. In addition to the tetracycline resistance genes, all strains had lincomycin, trimethoprim and daunorubicin resistance genes. Additionally, genes encoding resistance to cadmium (cadA and cadC) and aluminium (lmo1297) were observed in each of the strains. The presence of the Tn6188 transposon was observed in four strains, containing genes encoding resistance to benzalkonium chloride, tetracyclines and macrolides. The obtained results indicate that L. monocytogenes strains isolated from food are characterized by a wide variety of genes encoding resistance to antimicrobial compounds, and additionally they may transfer resistance via mobile genetic elements.

Food Microbiology in the context of One-Health (animal, plant and human)

P7.45 High pressure processing effect on conjugal antibiotic resistance genes transfer in vitro and in the food matrix among strains from starter cultures

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Food processing methods are associated with changes in environmental conditions, which may constitute a stress factor for food-related microorganisms, such as lactic acid bacteria (LAB) included in starter cultures. LAB are very valuable for the food industry, so it is important to thoroughly understand their stress response mechanisms. However, there are currently no studies available on the effect of food-related stresses on antibiotic resistance among LAB. One of the food-related stresses most recently applied into food industry is high pressure processing. It is considered as one of the most promising food preservation method. The LAB response to HPP is more difficult to identify because the HPP response systems are very similar to the response to other stress factors, potentially due to the presence of cross-protection systems. Some authors point to the possibility of resistance genes transfer from starter cultures microorganisms to pathogens in food, especially in food matrix. According to the literature data the horizontal transfer of antibiotic resistance plasmids can be promoted by food preservation stresses, although the mechanisms are as yet unknown. A variety of stressors can promote the acquisition of resistance genes.
The aim of the study was to assess changes in the frequency of resistance gene transfer in response to high pressure treatment among strains from starter cultures. Gene transfer possibility was checked in vitro and in the food matrix. The obtained results indicate changes in the frequency of resistance gene transfer after high pressure treatment. The frequency of gene transfer varied depending on whether the transfer took place in vitro or in the food matrix. The frequency of gene transfer changed depend on the value of the applied pressure and the duration of the process. Currently, there is no extensive discussion in the literature on this area, so it can be suggested that there is a need for further research in this area to assess changes in conjugal genes transfer in response to food-related stresses, which is important in the context of food processing and antibiotic resistance.
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Food Microbiology in the context of One-Health (animal, plant and human)

P7.46

Lactobacillus fermentum ACA-DC 179 administration modulates gut microbiota, metabolic profile and atherosclerosis progression in Apo-E-/- mice

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Atherosclerosis is an inflammatory disease of the medium and large arteries that is recognized worldwide as the leading cause of cardiovascular morbidity and mortality. Recently, the gut microbiota appears to play a crucial role in atherosclerosis development through several mechanisms, and probiotics have been proposed as an emerging solution for the disease control. In our study, Lactobacillus fermentum ACA-DC 179, a strain known for its anti-inflammatory properties, was examined for its effect on gut microbiota, metabolic profile and atherosclerosis progression in the Apo-E-/- murine model that is currently the most widely used pre-clinical model for atherosclerosis. Male and female ApoE-/- mice received intragastrically for 60 days either high (1.00E+09 cfu/day) or low (1.00E+06 cfu/day) dose of the strain, while control animal groups received water for injection. Classical microbiological analysis along with amplicon-based metagenomics approach were used to explore gut microbiota. According to the classical microbiological analysis of the feces (Day 0, 30 and 60) and the intestinal contents (after animal sacrifice at day 60), Lactobacillus spp. and Bifidobacterium spp. significantly increased, especially in the male animals receiving the high dose. Additionally, amplicon-based metagenomics analysis revealed a vast diversity of bacterial genera in both feces and intestinal contents' microbiota. In general, the bacterial microbiota of female animals was not as stable as the one of the male mice, since abundance changes were observed in several genera throughout the experiment. On the contrary, the genus Kazachstania was the predominant fungal genus identified in all mice groups. Among the biochemical markers tested in the blood serum (Day 60), only triglycerides were significantly lower in male and female mice receiving both low- and high-dose of L. fermentum compared to the control animals. The histological analysis of the aortic arch segments and the quantification of the lesion area revealed significant reduction of atherosclerosis in low- and high-dose animals compared to the control animals. Overall, the L. fermentum ACA-DC 179 administration to ApoE-/- mice seems to play an important role in gut microbiota modulation and atherosclerosis progression; however, our results need further investigation at the mechanisms' level.

Other

P8.1 Development and testing of herbal microemulsions for the management of Anthracnose disease of Capsicum annuum L.

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Chilli (Capsicum annuum L.) one of the most widely grown horticultural crops, is an essential ingredient of many celebrated cuisines worldwide. However, its production has been limited by many biotic and abiotic constraints. Anthracnose caused by the fungal phytopathogen Colletotrichum capsici is one such hurdle inflicting the pre and post-harvest losses of chilli fruits. Although this hemibiotrophic fungus causes latent infections in the aerial parts of the chilli plants, the major economic losses occur due to the degraded fruit quality. The use of natural alternatives such as those of plant origins have emerged as an efficient management tool against this phytopathogen. In the current study, we have developed microemulsion formulations using the thyme essential oil alone (TFO) and with licorice aqueous extract (TFO-10LAE) as the active phase. The addition of licorice aqueous extract in the microemulsion was observed to be enhancing its stability. Also, the TFO-10LAE microemulsion showed improved in-vitro antifungal efficacy against C. capsici compared to TFO. Microemulsion characterization using the FTIR and GC-MS analysis exhibited the preserved bioactive components of botanicals used. The developed microemulsion was further assessed for its efficacy to control anthracnose disease in the chilli plants and also for plant growth-promoting activities. The data pertaining to these experiments will be presented at the conference.

P8.2 Food safety monitoring within the household: The egg floating method

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Egg floating to monitor egg freshness/safety is a frequently observed behaviour among consumers in the individual household. Therefore, we aimed to scientifically demonstrate that there is no correlation between the presence of Salmonella and egg floating.
Fresh egg yolks (intact eggs) were inoculated with 20 ml of a five-strain cocktail of Salmonella enterica containing five different serovars, to achieve a contamination level of approximately 102-103 colony forming units (cfu)/g, by injection with a fine needle under sterile conditions, and immediately sealed with warm wax. Control eggs were inoculated with 20 ml of sterile saline solution and treated the same way as contaminated eggs. Eggs were stored at room temperature (22 °C) during 5 weeks, more than the entire shelf life period. Every week, three contaminated eggs and three control eggs were collected and immersed in water to investigate if they floated, followed by determination of Salmonella counts by spread-plating technique.
All eggs, control and inoculated, sank to the bottom and lay flat on their sides, until the third week of experiment. After the third week, the eggs passed the expiration date. On the fourth week, control eggs were still submerged, but by the fifth week the three eggs floated to the surface, 15 days after the expiration day. Interestingly the contaminated eggs were at the bottom of the bowl, but standing on one end, both in the 4th and 5th week.
It was proven that eggs inoculated with Salmonella do not float, after storage at 22 °C for five weeks. The pathogen was able to grow in inoculated eggs, showing a 7 log cfu/g increase in just one week. In the future, it will be necessary to replicate this experiment using a storage temperature of 4 °C.
This work has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 727580.

Other

P8.3 Evaluating the enteropathogenic potential of Bacillus thuringiensis isolates from soil, food and biopesticides

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Bacillus thuringiensis is frequently used as biopesticide. It is also closely related to Bacillus cereus, an opportunistic human pathogen that causes foodborne diseases. B. thuringiensis also harbours genes encoding enterotoxins, which are responsible for the diarrheal type of food poisoning. Routine outbreak investigations do usually not differentiate between the two species, thus, the role of B. thuringiensis as cause of disease might be severely underestimated. In 2016, the European Food Safety Authority (EFSA) made clear that new data on the hazardous potential of B. thuringiensis are urgently needed. Hence, in this study a set of 24 isolates gained from foodstuffs, animals, soil and biopesticides was comparatively analysed. All tested B. thuringiensis strains showed the genetic prerequisites necessary to provoke the diarrheal type of food poisoning caused by B. cereus. Moreover, all isolates could grow under simulated intestinal conditions and produce significant amounts of enterotoxins. By performing WST-1 bioassays on CaCo-2 cells, 14 isolates were classified as highly, and eight as medium toxic. Only two strains exhibited low cytotoxicity. Additionally, growth inhibition by essential oils (EOs) and washing of lettuce were investigated as preventive measures against putatively enteropathogenic B. thuringiensis. Cinnamon Chinese cassia showed the highest antimicrobial activity, followed by citral, oregano and winter savory. Washing of lettuce in water with citral oil led to an average reduction of bacterial counts by 88 %, for vegetative cells as well as spores.
Altogether, the present study shows a non-negligible pathogenic potential of B. thuringiensis, independently from the origin of isolation. Generally, biopesticide strains were indistinguishable from other isolates in the applied tests. Thus, the use of these pesticides might indeed increase the risk for the consumers' health. Until complete information about the safety of the applied strains and formulations is available, consumers or manufacturers might benefit from the antimicrobial activity of EOs combined with thorough washing of the relevant foodstuffs to reduce the level of contamination.

Other

P8.4

Antimicrobial and antioxidant activities of side-stream lignins

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Lignin is a polyphenolic biomacromolecule found in woody parts of trees and other vascular plants, which is traditionally removed as an unwanted side stream from the biorefinery process. Its valorization from residual biomass into high value-added materials is of great interest within the circular bioeconomy framework, apart from its outstanding characteristics like antimicrobial, antioxidant, and UV-shielding properties, especially for packaging applications. The aim of this work was to compare two types of lignin, commercial (CL - Sigma Aldrich®) and Indulin AT (IND), regarding their total phenolic content (TPC), their antioxidant activity against the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, and their antimicrobial activity against Staphylococcus aureus ATCC 25923 and Salmonella Enteritidis ATCC 13076. The TPC was determined by the Folin-Ciocalteu method, using gallic acid as standard. The radical scavenging activity (%RSA) using 0.1 mmol L-1 DPPH radical solution was determined after 30 min of reaction of the lignins (both at 125 µg mL-1) at 517 nm. The minimum inhibitory concentration (MIC) was determined using the microbroth dilution technique at pH 5.5 and pH 7.4, and minimum bactericidal concentration (MBC) was also evaluated. The TPC values obtained were 281 ± 19 mg gallic acid equivalent (GAE) g-1 for CL and 327 ± 18 for IND. CL presented an RSA of 28.7 ± 0.6% against DPPH, while IND was able to inhibit 40.9 ± 0.5% of the radical. CL inhibited S. aureus growth showing a MIC of 2000 and 1000 µg mL-1 at pH 7.4 and 5.5, respectively, with bacteriostatic effect at the concentrations tested. IND showed a MIC of 1000 and 500 µg mL-1 at pH 7.4 and 5.5 with an MBC value of 2000 µg mL-1 at both pH for S. aureus. For S. Enteritidis, CL and IND showed a MIC of 2000 and 4000 µg mL-1 at both pH; however, no MBC was observed at the tested concentrations. Thus, both lignins denote potential sources of active compounds for the development of new food products or packaging materials.
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Other

P8.5 Towards the isolation of more robust next generation probiotics: the first aerotolerant Bifidobacterium bifidum strain?

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This work reports on the first described aerotolerant Bifidobacterium bifidum strain. One of the main challenges for exploiting the therapeutic potential of next generation probiotics is their inclusion into functional foods, which is hampered by extreme oxygen sensitivity. This is indeed an unmet technological problem of human gut bifidobacteria. Aerobic conditions are present during the processes of producing, handling, manufacturing and storing probiotics. B. bifidum species includes several probiotic strains of invaluable therapeutic potential, but they also exhibit one of the lowest resistance to oxygen among human bifidobacteria. In this work, we present strain Bifidobacterium bifidum IPLA60003, which has the ability to form colonies on the surface of agar plates under aerobic conditions, a weird phenotype that to our knowledge has never been observed in B. bifidum. The strain IPLA60003 was generated after random UV mutagenesis from an intestinal isolate. It incorporates 26 single nucleotide polymorphisms that activate the expression of native oxidative defense mechanisms such as the alkyl hydroxyperoxide reductase, the glycolytic pathway and several genes coding for enzymes involved in redox reactions. In the present work, we discuss the molecular mechanisms underlying the aerotolerance phenotype of B. bifidum IPLA60003, which will open new strategies for the selection and inclusion of probiotic gut strains and next generation probiotics into functional foods. Further, we addressed the technological application of IPLA60003 evaluating its survival and metabolic activity in milk for 28 days. Besides, we studied the survival of the strains in fresh cultures, after freezing at -80°C and after lyophilization by plate counts incubated in anaerobiosis and in the presence of O2. Globally, technological performance of strain IPLA60003 was better than other two non-aerotolerant B. bifidum strains.

P8.6 The ability of Streptococcus thermophilus BT01 to modulate urease activity in healthy subjects' fecal samples depends on biomass production process

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The probiotic market is increasing world-wide as well as the number of products marketed containing Streptococcus thermophilus strains at several dosages. However, the scientific evidence that should support the probiotic status of those S. thermophilus strains is often contradictory. Ability to survive the gastrointestinal transit is a key prerequisite in determination of probiotic effectiveness. The main aim of this study was to evaluate the recovery of viable S. thermophilus BT01 after GI transit in healthy adults when lyophilized using a simple sugar or a polysaccharide as cryoprotectants. To this aim a randomized-cross over study was carried out on 20 adult healthy subjects to evaluate viable, total loads, persistence of S. thermophilus BT01 and urease activity in stool samples. Strain-specific quantification combines culture-based method and molecular qPCR tool allowed to recover viable S. thermophilus BT01 strain in 90% of the subjects. S. thermophilus BT01 biomass, lyophilized using a simple sugar as cryoprotectant, showed significant increase of viable recovery compering to the same biomass lyophilized using a polysaccharide as cryoprotectant. S. thermophilus BT01 persisted in 50% of the subjects up to 3 days after the end of the biomass intake. Stool frequency, and consistency were improved during the treatments compering with baseline period irrespectively of the cryoprotectant used during the lyophilization process. Most importantly, S. thermophilus BT01 biomass, lyophilized using a simple sugar as cryoprotectant, was significantly effective to reduce urea content in fecal samples with a mechanism that can be linked directly to the urease activity harbored by S. thermophilus itself, and representing the first probiotic specific mechanisms described for this species.

Other

P8.7 Effect of high-intensity ultrasound on lactic acid bacteria during fermentation

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The High Intensity Ultrasound (HIUS) can improve lactic acid bacteria metabolism during fermentation. The HIUS cavitation can cause disturbance in cellular membrane. Thus, membrane sonoporation enhances mass transfer, enable enzymatic reactions and, remove cellular metabolism byproducts. Therefore, this study assessed the HIUS effect on lactic acid bacteria during fermentation. We evaluated the impact of different energy densities (1600 J/mL, 640 J/mL, and 200 J/mL) HIUS on fermentation time, microbial viability, and titratable acidity. The milk, for each experiment, was inoculated separately and in coculture with yogurt starter cultures (Lactobacillus delbrueckii subsp. bulgaricus e Streptococcus thermophilus) and Lactobacillus acidophilus La 5®. Microbial viability was estimated using MRS Agar, for milk fermented with yogurt starter cultures and selective MRS Agar supplemented with bile salts and cysteine in Lactobacillus acidophilus fermented milks. The plates were incubated at 37°C for 72 h under anaerobic conditions. The milk fermentation without HIUS treatment was used as a control. All experiments were carried out in triplicate. The results demonstrate that the HIUS treatment with reduces the fermentation time. The best condition for single yogurt starter culture was 640 J/mL energy density. The microbial population achieves 8.22 UFC.mL-1. The titratable acidity and pH were 0.8 g.100g-1 of product and 4.54 at the end of fermentation, respectively Also, the fermentation time was reduced, when compared with control was 345 min for control and 315 min for HUIS treatment. For Lactobacillus acidophilus, inoculated separately, the 200 J/mL energetic density had a higher effect on microbial viability. This treatment reduced from 480 min to 495. For HUIS sample final titratable acidity and pH were 0.78 g.100g-1 of product and 4.58 respectively. Nevertheless, the HUIS treatments do not influence microbial viability and physicochemical parameters for coculture experiments. The results evidence that HIUS treatment can optimize milk fermentation using single cultures.

P8.8 Insights on genomic, virulence and antibiotic resistance profiles of Escherichia coli O157:H7 from Brazilian beef

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Enterohemorrhagic Escherichia coli serotype O157:H7 is part of Shiga toxin-producing E. Coli (STEC) group, that poses a significant threat to human health due to the ability to cause gastroenteritis to hemorrhagic colitis and hemolytic uremic syndrome (HUS). In this study, we aimed to characterize thought a genomic approach the virulence and antibiotic resistance features of E. Coli O157:H7 isolated from a beef production chain. Enteropathogenic E. Coli was screened in a beef production chain in Brazil and characterized based on their pathotypes through PCR. Isolates identified as O157:H7 (n = 22) were subjected XbaI digestion and Pulsed-field Gel Electrophoresis (PFGE); based on the observed pulsotypes and original samples, isolates (n = 8, 2 from beef carcasses and 6 from feces) were selected and subjected to whole genome sequencing (Illumina). Genotypic analysis was conducted to identify serotype, virulence genes and antimicrobial resistance genes using the National Center for Biotechnology tools. Serotype identification performed with SerotypeFinder confirmed previous PCR results, O- antigen 157 and flagellin protein H7. Additional analysis allowed the identification of 23 virulence-related genes in all sequenced isolates: astA, chuA, eae, ehxA, espA, espB, espF, espJ, espP, etpD, gad, Iha, Iss, katP, nleA, nleB, nleC, ompT, tccP, terC, tir, toxB and traT; also, five isolates presented stx2A and stx2B. All isolates were included in ST11 according to MLST database. Antimicrobial gene screening did not identify major resistance genes among the sequenced isolates, although the presence of mdf(A), indicating that the isolates may carry a broad-spectrum specificity against several antimicrobial classes, including aminoglycosides, tetracycline, macrolides, ryfamicin and quinolones. Results showed a high number of virulence-related genes relevant for infection in all sequenced isolates obtained from different steps of beef production chain, and also antimicrobial features that must be taken into account as advantage under selective pressure. Acknowledgments: CNPq, CAPES, FUNARBE and FAPEMIG.

Other

P8.9 Effect of ultraviolet irradiation on Aspergillus flavus and Aflatoxin B1 in maize and peanut

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Aflatoxins and the producing fungi Aspergillus section Flavi are widely known as the most serious and dangerous issue in agricultural products. Present study aimed to investigate the effectiveness of the ultraviolet irradiation of the C region (UV–C) for the decontamination of A. flavus and Aflatoxin B1 (AFB1) on artificially inoculated maize and peanut using innovative decontamination equipment that supports vibrations to achieve semi-fluidization of the grain/kernel material getting equal irradiation of all surfaces of irradiated foods. Samples of maize and peanut were exposed to UV-C irradiation with a total dose in the range of 1080–8370 mJ/ cm2. Analysis by Tracker and ImageJ software confirmed the even distribution of irradiation on all surfaces during the entire duration of exposure. The highest reduction of A. flavus count was observed after ten days of incubation and irradiation treatment delivering a dose of 8370 mJ/cm2 achieving A. flavus count reduction of 4.4 log CFU/g in maize and 3.1 log CFU/g in peanut. Depending on the treatment, AFB1 reduction level in maize ranged from 17 to 43% and in peanut ranged from 14 to 51%. Sensory and physical testing of the peanut samples showed only minimal changes in the evaluated characteristics caused by different levels of the UV-C treatment. Presented results demonstrate a potential for the use of the presented approach as an effective reduction strategy for both A. flavus and AFB1 in maize and peanut.

P8.10 An expert knowledge ellicitation survey aimed to identify contamination spots in ready-to-eat meat and seafood processing facilities

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A real-time Delphi-type expert knowledge ellicitation (EKE) survey aimed at identifying the points in the production chain where contamination with Listeria monocytogenes would be most likely to occur as well as the sites where contamination niches harboring it was most likely to be found in food facilities was conducted. The survey focused on three major categories of ready-to-eat (RTE) food, that is, meat products, fish products, and food preparations containing meat or fish products. Also, each of the two former were divided into two sub-categories: cured food and heat-treated food. A total of 35 professionals from universities and research institutions, food production companies, food analysis and control service companies, and food safety inspection were involved.
Slicing of final product was considered by consensus as the operation with the highest risk of contamination in both cured and heat-treated meat products. Cutting and chopping were also identified as high-risk operations for the latter, whereas mincing, cutting and quartering of raw materials were for cured meat products. In cured and heat-treated seafood, cutting (as fillets, pieces or slices) of final product were agreed to have the highest risk of contamination. Equipment and tools used in cutting and filleting of raw seafood were also pointed as major responsible for the entry of L. monocytogenes into cured fish products. Lastly, cutting and handling of processed products were considered as the points in the prepared meals production chain with the highest probability of contamination.
Drainage systems and then areas or points of equipment and furniture of the processing line -and even installations- of difficult cleaning and disinfection were clearly identified as sites where contamination niches harboring L. monocytogenes were most likely to be found. In addition, slicing machinery (as a whole) was recognized as a high-risk site for both RTE seafood, heat-treated meat products and prepared meals. Mincers, cutting and filleting machines were also pointed out as hot-spots for heat-treated seafood and prepared meals. Table surfaces, conveyor belts, trolleys, etc. in the processing line in direct contact with raw materials and products were identified too in cured meat products processing facilities.

Other

P8.11 In vitro study of microbial interactions and metabolism of meat-related spoilage microorganisms under aerobic and anaerobic conditions (OLED_Lumin_FoodPack)

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Meat spoilage may be identified by the alteration of sensory characteristics related to microbial metabolism like off-odors. Knowledge about the fingerprint of volatile and non-volatile organic compounds may be useful for early detection of meat spoilage.
The aim of the study was the in vitro assessment of changes in significant metabolites produced by meat-related spoilage microorganisms co-cultured in a liquid model food under different storage conditions.
Pseudomonads, LAB, and B. thermosphacta were singly- or co-cultured in TSB (pH 5.8) and incubated at 4 and 10°C. The inoculation/gaseous headspace combinations tested were: (i) singly- and co-cultures of pseudomonads, LAB, and B. thermosphacta at equal starting level (3.5 log CFU/mL) under aerobic conditions and 100% N2 and (ii) co-cultures at different initial inoculum level, i.e., LAB and B. thermosphacta at 6.0 log CFU/mL and pseudomonads at 3.5 log CFU/mL, all stored under aerobic conditions, as well as pseudomonads and B. thermosphacta at 6.0 log CFU/mL and LAB at 3.5 log CFU/mL and stored under 100% N2. All microorganisms were enumerated on selective media, while organic acids and VOCs were quantified via HPLC-UV and SPME-GC/MS, respectively.
Mono- and co-cultures of pseudomonads and LAB at equal initial inoculation level revealed similar growth curves, while B. thermosphacta dominated in co-culture of all bacterial groups, at all assays except for 100% N2 at 10°C, where LAB dominated. When low inoculum of pseudomonads or LAB were co-inoculated with the other 2 bacterial groups at high initial inoculation level under aerobic and 100% N2, respectively, the results showed that pseudomonads and LAB were significantly inhibited by 1.0–3.0 logs. Among the tested organic acids, concentrations of acetic, oxalic, lactic, and propionic acid showed a clear monotonic increase, at all assays. A similar increasing trend was recorded in several VOCs such as 2-Methyl-1-butanol and 3-Methyl-1-butanol (aerobic and 100% N2) and 2,3-Butanediol (100% N2).
Knowledge of meat spoilage metabolites and their trend throughout storage under controlled in vitro conditions facilitates the correlation of microbiological with chemical spoilage indices, however, the type of meat is expected to affect the microbial status and metabolic fingerprint, necessitating the validation of the above on meat.

Other

P8.12 Microbial risk ranking is "a web-based decision support system" tool

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WHO estimates that more than 23 million people fall ill from eating contaminated food with bacteria, viruses, and parasitic protozoa in the European region every year, resulting in 4,654 deaths and more than 400,000 DALYs. Reducing these numbers has proven challenging because of the complex European food supply chain system and thousands of firms that provide consumers with hundreds of billions of euros worth of food each year. Moreover, this system is constantly in flux due to changing consumption patterns, the development of new products, and increasingly globalised food supply chains.
In a food safety system, decisions about food-borne risks from various products must be made consistently to minimise foodborne illness, reduce risks, and maximise benefits while also considering the cost of illness. The microbial risk ranking model, a web-based decision-support tool, will be developed to rank the most hazardous pathogen and food combinations to translate an academic understanding of the factors affecting the growth or inactivation of a pathogen in a specific food throughout the farm to fork scenario to estimate the final risk. It incorporates the disease-causing pathogen and properties of food, the influence of temperature change on pathogen growth and concentration at each stage of the supply chain, consumption patterns and the size and vulnerabilities of the populations affected, and economic factors to calculate the disease burden. In particular, the tool was intended to make the techniques of food safety risk assessment more accessible to non-experts in this field as an educational tool to assist in understanding the process of microbial food safety risk assessment. Furthermore, the tool helps teach the principles of risk assessment concerning food safety and highlights factors contributing to food safety risk. The tool also can be used by risk managers and others without extensive experience in microbial risk assessment or modelling and as a simple and quick means to develop the first estimate of relative risk. It can also be used as a training and risk communication aid to help determine data needs.

P8.13 Food safety: Knowledge and beliefs among the Portuguese population

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According to the World Health Organization, the reported cases of foodborne infections amount to 600 million a year and of these about 40% originate in the household. However, these figures may be even higher as most symptoms of foodborne infections disappear after only a day or two, and so often go unreported. Given that it is now accepted that a significant proportion of foodborne infections result from unsafe practices in food handling and preparation at home, it is important to understand what motivates these practices, what beliefs and convictions (myths) are widespread in the population and what socio-demographic factors contribute to their spread. An online questionnaire was conducted and answered anonymously by 486 consumers. The results were processed and statistically analysed using MS Excel® and Statistical Package for the Social Sciences (SPSS version 28®) software. The questionnaire contained 74 statements, some of which were food myths. Participants were asked to judge the truthfulness of each of these statements. Overall, a high percentage of respondents believed some safety-relevant myths (e.g., 37% and 27%; respectively, agree or have no opinion, that eggs that do not float in water can be eaten safely; 35% and 23%, respectively, agree or have no opinion, that raw meat should be washed before cooking; 31% and 19%, respectively, agree or have no opinion, that as long as it doesn't taste bad, food can be consumed after the expiry date). Age was the most important variable associated with belief in myths. This study demonstrates the need to invest in consumer education in order debunk widespread food safety myths.
This work has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 727580.

Other

P8.14 A review of milk kefir: Potential beneficial effects on human nutrition and health

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Fermented dairy products are formed during the acidification of milk through fermentation by suitable microorganisms; it contains different microorganisms in sufficient numbers and in an active state. A wide range of fermented milk products are produced and consumed around the world, including yogurt, kefir, koumiss, and yogurt beverages. There are various health benefits associated with the consumption of fermented dairy. Many studies reported that some fermented milk products have antimicrobial, antimutagenic, anticarcinogenic, and antihypertensive properties as well as provide benefits on mineral metabolism, reduce lactose intolerance symptoms and cholesterol levels. In addition to these effects, it has many other beneficial effects such as positive effects on type 2 diabetes and hypertension, antimutagen and antioxidant effects, and reduction of allergic symptoms. Dairy products including fermented milk are known to be the main carrier of probiotic microorganisms, and many clinical studies show the effects of probiotic strains on health. In this study, the effects of kefir, which is a popular milk beverage among widely produced fermented dairy products, on human health are mentioned.

P8.15 Bioactive compounds present in South African cannabis infused and highly hopped craft beers

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There is a worldwide trend towards cannabis legalisation for recreational use which has led to a bourgeoning industry of cannabis-infused edibles and beverages, including craft beer. Hops (Humulus lupulus), used as a bittering and flavouring component in brewing, is phylogenetically related to cannabis and although hops do not contain cannabinoids, terpenes are bioactive compounds that occur in both plants. Terpenes make up 98-99% of the total essential oil content of cannabis and hops and due to their low toxicity are used as biomedicine and food additives. Since the addition of THC to any alcoholic beverage remains illegal, cannabis-infused craft beers likely only contain cannabinoids and terpenes. South Africa has a select few commercially available cannabis-infused craft lagers and hoppy ales, but the bioactive components are not disclosed. The aim of this study was to analyse cannabis and hop bioactive compounds in commercially available cannabis-infused and hoppy craft beers. Cannabinoids (THC, CBC, CBD, CBN, CBG) and terpenes (20 most important based on abundance in both plants) were measured by HPLC and GC-MS analysis, respectively. Samples included cannabis/hemp seeds, CBD oil, terpene concentrate, hop varieties generally used in craft brewing (5), cannabis-infused beers (3) and hoppy beers (2). No cannabinoids or terpenes were detected in the hemp seeds used for producing Lager 1 (Durban Poison). The CBD oil used to infuse Lager 2 (Cann-O-Bliss) contained CBN, THC-V, Delta-9-THC and total potential THC, while Delta-3-Carene was the only terpene detected. Terpene concentrate added to the Pale ale (Girl Scout Cookies) after fermentation, showed CBD and terpenes α-humulene, α-pinene, β-caryophyllene, β-myrcene, β-pinene, limonene, linalool, ocimene and terpinolene (58.745mg/g). Hop varieties African Queen, Cascade, Centennial and Mosaic contained α-humulene, β-caryophyllene, β-myrcene and limonene. No cannabinoids were detected in any of the cannabis-infused beers and only one terpene was present, terpinolene at concentrations between 0.249 - 0.466 mg/g. The two highly hopped IPAs (Californicator and Jungle Paradise) also contained only terpinolene at concentrations of 0.431 and 0.497 mg/g, respectively. It is evident that terpinolene was the only terpene detected in the beers with no other bioactive compounds featuring. Its origin and persistent presence requires further investigation.

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