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FoodMicro

26th International ICFMH Conference

3–6 SEPTEMBER 2018

FREIE UNIVERSITÄT BERLIN

Conference Theme: “Biodiversity of Foodborne Microbes”

BOOK OF ABSTRACTS

Contents

Welcome Letters

Dear Delegate,

welcome to the FoodMicro 2018 conference in the Henry Ford Building of the Freie Universität Berlin, Germany! It is now 25 years ago that the FoodMicro conference was organized for the last time in Germany, and for us, this is a happy anniversary. The efforts to host the current FoodMicro 2018 conference in Berlin started approximately six years ago, and we are now very excited that this great scientific conference is taking place in Germany's capital.

The field of food microbiology is important for economics, medicine, veterinary medicine, food technology, nutrition, biotechnology, and other related areas. The importance of this field is underlined by the fact that over 430 delegates from more than 57 countries are participating in the conference. The FoodMicro conferences cover the most advanced research fields and therefore we have chosen "Biodiversity of Foodborne Microbes" as the common theme for the FoodMicro 2018 conference. We are looking forward to offering you an excellent programme, both in its scientific diversity and depth.

Organizing FoodMicro requires a multifaceted approach and is only possible with the help of numerous people and organizations: The ICFMH board members placed their trust in us to manage this important international scientific event and were valuable contact persons in all respects. The DGHM as our parent microbiological society gave us key support and paved the way to work with the congress organization MCI. The international experience and excellent management of the MCI staff was greatly appreciated. Moreover, the other German microbiological societies VAAM and DVG provided their support. Lastly, without the voluntary work of all scientists from the national and international scientific committees, this conference would not have been possible.

Besides offering a space to discuss science, we hope the FoodMicro 2018 conference will also be a platform to meet old colleagues and friends and to find new ones.

We hope that you enjoy FoodMicro 2018 and find it to be scientifically as well as personally rewarding!

Herbert Schmidt

Barbara Becker

Thomas Alter

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Invited Keynote Speakers | Monday 3rd September 2018

Lothar H. Wieler
Robert Koch Institute, Berlin, Germany

KN01

Food is central to one health

Food is of key importance when considering One Health research concerns. Food may be of animal, vegetable or fruit origin, but all food sources can be contaminated with pathogens over the full production cycle. Thus food safety is of utmost importance to reduce the burden of infectious diseases caused by the intake of contaminated food. Most infections caused by contaminated food are notifiable in Germany, but the numbers officially recorded in Germany are much lower than those truly appearing. The key issues in fighting outbreaks caused by contaminated food is the identification of the food incriminated and the epidemiological investigation tracing the route of food to prevent further spread of the pathogens.

The Robert Koch Institute is implementing the German electronic notification and information system (DEMIS) to foster fast information, spread valid information to the health authorities in due course and also lower technical barriers caused by the current notification system. Of particular interest is molecular epidemiology by whole genome sequence analysis of pathogens, as these data are internationally easily comparable, accessible and exchangeable. By two examples -outbreaks caused by *Listeria monocytogenes* and an EHEC O157:NM outbreak in recent years, this paper discusses the pros and cons of the implementation of molecular epidemiology as it is seen by the Robert Koch Institute.

Listeriosis is a serious, life-threatening infectious disease that mainly affects elderly and immunocompromised persons, pregnant women, and neonates. In Germany, listeriosis cases have been predominantly reported among nonpregnant women and men, and the source of most infections was unknown. The major risk factors identified for these cases were immunosuppressive therapy, immunocompromising diseases, gastric acid suppression, and frequently consumed ready-to-eat foods. *L. monocytogenes* is usually transmitted through food prone to contamination during manufacturing or postproduction processing before packing. In 2012, the number of *L. monocytogenes* cases in Germany started continuously increasing; 707 cases and a case-fatality rate of 7% were reported in 2016. Among the 6 most predominant enteric pathogens in Germany, *L. monocytogenes* has accounted for the highest number of years of potential life lost.

In February 2017, five cases of haemolytic-uremic syndrome (HUS) were notified in Germany with onset of illness in week 5, 2017, which constituted a marked increase compared with the mean in the same week of the previous 5 years (mean: 0.6; range: 0-2 cases;). In parallel, the consultant laboratory (CL) for HUS at the University Hospital of Münster detected Shiga toxin 2-producing (stx2) sorbitol-fermenting (SF) *Escherichia coli* (STEC) O157:NM isolates in four HUS patients with disease onset between December 2016 and February 2017.

Keywords: Genomic Surveillance, foodborne pathogens

Invited Keynote Speakers | Monday 3rd September 2018

Monika Ehling-Schulz
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KN02

Why to be serious about *Bacillus cereus?*

Bacillus cereus is involved in the industrialized world in foodborne disease as well as in systemic and local infections such as fatal bacteremia and a form of pneumonia remarkably similar to inhalation anthrax. Changing life styles, increased life expectancy and new eating habits have combined to increase the incidence of *B. cereus* infections and intoxications. The emetic type of the disease is attributed to the heat-stable depsipeptide cereulide, while different heat- labile enterotoxins have been linked to the diarrhoeal syndrome. The spectrum of potential *B. cereus* toxicity ranges from strains used as probiotics in animal feed to highly toxic strains already reported responsible for fatalities.

Due to high genomic plasticity, the population structure of *B. cereus* is quite dynamic allowing rapid adaptation of these volatile bacteria to changing environments. A link between virulence and the metabolism has been confirmed while persistence mechanisms are hitherto largely unexplored. To efficiently combat this microbe, an understanding of both, its virulence mechanisms as well as its persistence mechanisms, used for survival in diverse environments uncounted in foods and diverse hosts, is essential. Thus, this lecture will provide insights into the pathometabolism of *B. cereus* and will also discuss recent findings on potential survival and persistence strategies.

Keywords: Bacillus cereus, enterotoxin, cereulide, pathogen, intoxication

Invited Keynote Speakers | Tueaday 4th September 2018

Maria L. Marco
University of California, Davis, United States

KN03

Untangling *Lactobacillus* in foods from strain diversity to human health

Lactobacillus is an ecologically successful bacterial genus, evident by its widespread distribution in human, animal, insect, and plant microbiomes and high intra- and inter-specific genetic diversity. Lactobacilli are best understood for being essential together with other lactic acid bacteria for food and beverage fermentations which have been consumed since the origins of human civilization. Although *Lactobacillus* species commonly found in fresh fermented foods are also regarded to benefit health and well-being, the mechanistic basis for this and the importance of (fermented) food carriers and dietary backgrounds have remained elusive. Emerging evidence on the diet-dependent structure of gut microbiomes and *Lactobacillus* strain diversity and interactions with the mammalian intestine have resulted in new hypotheses on the value of these organisms for human health and facets that influence their efficacy. Our studies on *Lactobacillus* have shown that *Lactobacillus plantarum* has an expansive functional diversity and that host diets and food carriers can alter how these and other lactobacilli are received in the digestive tract. This work paves the way for the design of food fermentations that maximize species and strain-specific properties of lactobacilli.

Keywords: Lactobacillus

Invited Keynote Speakers | Wednesday 5th September 2018

Kostas Koutsoumanis

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KN04

Individual cell-based food microbiology: Insights into a “noisy” world

Bacterial cells within a clonal population can vary significantly in a number of phenotypic traits. The main source of this phenotypic heterogeneity is the stochastic variations associated with the genomic information flow including gene activation, transcription and translation. The fluctuations in the levels and activities of intracellular components, known as “molecular noise”, can lead to different behavior among genetically identical cells in a homogeneous environment. Heterogeneous behavior of individual cells is observed at the growth, survival and inactivation responses and should be taken into account in the context of Food Microbiology. Recent methodological advances can be employed for the study of single cell dynamics leading to a new generation of mechanistic models which can provide insight into the link between phenotype, gene-expression, protein and metabolic functional units at the single cell level. Such models however, need to deal with an enormous amount of interactions and processes that influence each other, forming an extremely complex system. In this review paper, we discuss the importance of noise and present the future challenges in predicting the “noisy” microbial responses in foods.

Keywords: Individual cell, stochastic models, growth, inactivation, survival

Invited Keynote Speakers | Thursday 6th September 2018

Una Mary Ryan
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KN05

Foodborne cryptosporidiosis and giardiasis – Current and future challenges

Cryptosporidium and *Giardia* are major causes of diarrhoeal disease in humans, worldwide. The burden of disease caused by food-borne transmission of these enteric protozoan parasites is under recognised, however notifications are increasing. In 2010 alone, both parasites were responsible for >36.8 million cases of foodborne diarrhoeal illness, 3,759 deaths (*Cryptosporidium* only) and 322,426 disability-adjusted life years (DALYs). The global nature of the food trade, increased international travel, an increase in the population of highly susceptible individuals, changes in consumer habits including eating outside of the home and consumption of more raw and undercooked foods, combined with improved diagnostic tools and communication are some factors associated with the increased diagnosis of these food-borne parasitic diseases worldwide. Existing and emerging global challenges and potential solutions will be discussed.

Keywords: Foodborne cryptosporidiosis and giardiasis

Keynote Abstract

Keynote Abstracts | Tuesday 4th September 2018

Topic A – Exploring biodiversity in microbial ecosystems along the food chain

K1

Insight into farmhouse PDO cheese primary production environment to reconcile sanitary control and microbial ecosystems

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Focusing on farms involved in the production of raw milk cheese, our objective is to analyze the possible links between farmers' practices and microbial composition, including micro-organisms of interest for cheese-making and pathogens, in various environments of the farms. Based on routine sanitary controls carried out on milk and cheeses in 200 farms, 14 farms belonging to two extreme classes (classes A and B, respectively characterized by low and high pathogens' prevalence) were selected. Seven different microbial niches (bedding area (BD), milking parlour ambient air (AI), feces (FE), teat surface (TS), milking machine inner surface (MM) and filter (FI), bulk milk (MI)) were sampled in triplicate from each farm at two seasons (indoor feeding vs. outdoor grazing). Pathogens were targeted using reference methods (*Listeria monocytogenes, Salmonella*, coagulase-positive staphylococci (CPS)) or PCR (Shiga-toxin producing *Escherichia coli* (STEC), *Coxiella burnetii)*. Microbiota were analyzed using high throughput sequencing (HTS) approaches targeting 16S rRNA genes. The results show the relevance of the strategy applied for farm sanitary classification. They confirm a higher prevalence of *L. monocytogenes* ($P< 0.001$ in BD) and CPS ($P< 0.05$ in MI and FI) in class B farms compared to class A. They suggest a key role of BD as a reservoir for *L. monocytogenes* and STEC. Across the total 546 samples analyzed by HTS, 3299 unique bacterial sequences spanning over 12 phyla and 492 genera were identified. Average Shannon diversity index ranged between 7 and 3, being the highest in FE, followed by BD, AI, FI, TS, MI and MM. Out of 642 unique bacterial sequences found across the 70 MI samples, 57% were shared with AI, followed by FI (55%), TS (46%), BD (42%), MM (25%) and FE (14%). The bacterial diversity profiles clustered first according to their environment of origin, second the season and third the pathogen prevalence class. Major shifts in bacterial taxa abundance were observed in BD with changing season. Twelve bacterial genera were differentially abundant ($P< 0.05$) depending on farms' pathogen prevalence class. Among them, *Cellulosilyticum* genus was more abundant in FE, BD, AI, FI and TS samples from class B farms, while *Kocuria* was more abundant in BD, FI, TS and MI samples from class A farms. Further statistical analyses are being carried out to infer potential interactions between microbial populations, pathogens' prevalence and farmers' practices.

Keywords: raw milk cheese, farm environment, microbial community assemblage, pathogens' control, metabarcoding

Keynote Abstracts | Tuesday 4th September 2018

Topic B – Ecology and interactions in food-associated microbial communities

K2

Inter- and intra-species interactions in wine fermentations

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Wine aroma and overall quality is greatly influenced by the alcoholic fermentation carried out by indigenous yeasts and/or intentionally added starter cultures. Yeast population dynamics at the onset and during the fermentation are the result of interactions of microorganisms with their continuously changing grape must environment but are also affected by microbial inter- and intra-species interactions. Such interactions not only are determinative for the kinetics of the different populations but have an effect on the chemical composition and analytical profile of the final wine.

In this study we sought to investigate both intra-*Saccharomyces cerevisiae* and *S. cerevisiae*-*Starmerella bacillaris* inter-species interactions during alcoholic fermentation of grape must. Fermentations were carried out both in flasks and in two-compartment bioreactors where cells of the different populations are kept separate. Viable counts and molecular analyses were performed in order to monitor the kinetics of the different populations while principal chemical components of grape must were also quantified during fermentations.

The performance of the yeasts in flasks was different than what was observed in the two-compartment bioreactor. More specifically, in the flasks we observed a competition effect that resulted in dominance of one of the two populations that were initially inoculated in the must. On the other hand, in the bioreactor both populations inoculated maintained high viability throughout the fermentation. This differential behavior was ascertained in both intra- and inter- species experiments and was further investigated by both molecular and culture methods to gain insights into the underlying mechanism governing dominance. RNA-seq pointed towards SO_2 expulsion pumps and cell aggregation as mechanisms of competitive advantage in *S. cerevisiae* intra-species interactions while in *S. cerevisiae*-*St. bacillaris* interactions, nutrient limitation and inhibitory compounds could not explain the dominance. The results obtained in this study underline the importance of the cell-to-cell contact in interactions between yeasts during alcoholic fermentation.

Keywords: population dynamics, microbial interactions, cell-to-cell contact

Keynote Abstracts | Wednesday 5th September 2018

Topic C – State of the Art techniques for the analysis of biodiversity and microbial interactions of foodborne microbes

K3

Extensive biodiversity of raw milk microbiota analyzed by amplicon sequencing and cultivation – Impact of methodology and library preparation

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Availability of Next-Generation sequencing based techniques has opened new horizons for analysing complex microbiota. However, sample preparation includes many different steps, which may have a larger or smaller impact on obtained results. In particular, samples having low bacterial counts are challenging, as only little DNA is available. Raw milk microbiota are complex communities with a significant impact on the hygienic, sensory and technological quality of resulting products. Fresh raw milk microbiota are notoriously difficult to investigate, as bacterial counts are usually very low and there is a high level of eukaryotic DNA originating from the cow's udder.

The aim of this work was to optimize a protocol for microbiome analysis of such difficult samples using high-throughput amplicon sequencing on an Illumina platform and to analyse the effect of different library preparation steps on the microbiome composition obtained. A DNA extraction protocol for raw milk with particular emphasis on the enrichment of bacterial cells and a reduction of eukaryotic DNA was developed. Then, the impact of different PCR conditions and DNA extraction kits on diversity estimates and reproducibility was tested to check whether and to which extent they introduce artefacts to the analysis. In addition, comparison of cultivation-dependent and amplicon-based approaches were performed in order to see, whether particular taxa are over- or underestimated.

Variation of different parameters in library preparation led to significant changes observed in the microbiota composition although single PCRs showed good reproducibility. Comparison of samples investigated by both cultivation-dependent and -independent approaches reveals systematic differences. Both approaches result in species not detected by the other technique, indicating that even NGS did not reveal the complete biodiversity. While the amplicon-based approach is by far superior in analyzing biodiversity in samples of high complexity, cultivation dependent techniques resulted in the detection of a surprisingly large number of novel genera and species that are lost using amplicon sequencing.

Keywords: raw milk microbiota, amplicon sequencing, library preparation

Keynote Abstracts | Wednesday 5th September 2018

Topic D – Impact of interventions during food production on microbial biodiversity

K4

Next generation bacteriophages: Novel tools for food microbiology

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The incredible host specificity and inherent antimicrobial activity of bacteriophages is the basis for many diagnostic and antimicrobial and therapeutic applications, in food safety and medical microbiology. Examples include the use of phage-encoded affinity proteins for specific labeling, immobilization and rapid diagnostics of target cells such as Listeria, Salmonella and Staphylococcus, and the specific targeting and killing of bacterial cells by both intact bacteriophage and phage encoded peptidoglycan hydrolases. Yet, application of bacteriophage may be restricted by several natural factors, such as narrow host ranges, transfer of potentially undesired genetic determinants, and development of resistance. To unleash the full potential of phage in therapy and biotechnology, genome editing is a promising way forward. Towards this aim, we used synthetic biology approaches to modify phages, employing cell wall-deficient bacterial L-form cells for rebooting of fully synthetic virus genomes. We also identified the first functional CRISPRcas system in *Listeria*, and developed it into a toolbox for rapid and efficient editing of very large, non-integrating phage genomes. These new approaches enable tailor-made design, conversion and arming of bacteriophages, and provide extremely useful tools for their application in control and detection of bacterial pathogens.

Keywords: Bacteriophage, detection, control, Listeria

Topic E – Microbiological spotlights

K5

Contamination of meat by *Escherichia coli* O157:H7: Characterisation of the bacterial cell interactions with the different skeletal muscle types, subtypes of myofibers and extracellular matrix components

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Escherichia coli O157:H7 is responsible for serious pediatric diseases following consumption of contaminated food products, especially beef meat in Europe. Meat contamination occurs at slaughtering, essentially at dehiding stage, where bacteria can be transferred from hides to carcasses. Despite being a key step in the ultimate human infection, the contamination of meat is poorly understood at molecular, cellular and tissue levels.

Bacterial adhesion to meat was investigated considering the different skeletal muscle types, constituent myofibers and components of the extracellular matrix (ECM) as well as the postmortem evolution of the meat matrice. Spatial distribution of bacterial cells in the food matrice was investigated following immunohistochemical analyses coupled to fluorescence microscopy as well as state-of-the-art synchrotron radiation beamline.

It first appeared that differential bacterial adhesion occurs at the surface of glycolytic and oxidative skeletal muscles. As revealed by the autofluorescences of deep UV excited muscle cells, the proportion of the four major types of myofibers greatly varies with the muscles types. While bacteria adhered similarly to these different constituent myofibres, spatial distribution analysis revealed that bacterial cells mainly adhere to the ECM. Environmental conditions greatly influenced bacterial adhesion to the ECM components, namely collagens I, III and IV, insoluble fibronectin, laminin-α2 and elastin. Specific bacterial adhesion to collagens I and III was influenced by the temperature and pH.

This investigation demonstrated the differential ability of *E. coli* O157:H7 to adhere to skeletal muscle tissue, especially at the ECM. This information is especially relevant to mitigate the contamination of meat, the food chain and ultimately human infection.

Keywords: extracellular matrix, meat contamination, bacterial adhesion, foodborne pathogens, STEC, EHEC

Topic E – Microbiological spotlights

K6

RAKIP: Resources for harmonized annotation and efficient exchange of risk assessment models

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The exchange of mathematical models generated in the areas of predictive microbiology (PM) and risk assessment (RA) is currently difficult and time consuming, mainly due to the lack of harmonized formats for model annotation and information exchange. This is true even though there are several modelling tools already available and frequently used, such as e.g. Combase, FDA-iRISK, MicroHibro, etc.
The Risk Assessment Modelling and Knowledge Integration Platform (RAKIP) Initiative is a joint ANSES, BfR and DTU Food effort aiming at creating open resources that support the efficient exchange of models and data between existing and future software tools in a transparent and consistent way. Among the developed resources, the Food Safety Knowledge Markup Language (FSK-ML) is a key element. This information exchange standard allows to annotate and exchange models in a harmonized way, even if the models were developed in different scripting languages, like R or Python. FSK-ML has been developed on the basis of similar data standards in Systems Biology (specifically SBML) and a generic metadata annotation schema for data and models that wherever possible and reasonable includes proper controlled vocabularies. Another resource developed for RAKIP is the open source software "FSK-Lab" that allows researchers and modellers to share their risk assessment models and associated data in a FSK-ML compliant way.
On the basis of these infrastructural resources, the RAKIP partners established the first web-based model repository for modellers, risk assessors and risk managers. Via this resource it is possible to search for models based on available metadata (e.g. hazard, population, food matrix) and even execute selected models online with own user-defined simulation settings (https://foodrisklabs.bfr.bund.de/rakip-web-portal/). Simulation results can be stored together with original model files and the applied simulation settings in the proposed FSK-ML information exchange file. In this way the RAKIP resources support full transparency of modelling work, covering model code as well as simulation settings and results.

Keywords: RAKIP; QMRA modelling; Information exchange format; Model annotation, Model repository

Conference Workshops

Conference Workshops | Monday 3rd September

3rd FMTGN symposium 'Food Microbiology Education in Practice'

S1

Present scenario and realistic constrains of food microbiology education in India

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The economic growth rate of India is very fast, but, one in every three malnourished children in the world lives in this country and this is due to lower per capita daily supply of calories, protein and fat, according to the Organisation for EconomicCo-operatio-nandDevelopment(OECD).The average Indian had access to 2,455 kcal per day and it is far lower than the at least 3,000 kcal per day availability for OECD nations.In this context, functional foods represent novel scientific paradigms that can surely improve the traditional nutritional deficiency.Apart from ex-situ food fortification techniques, fermented food is one of the age-old food culture of Indian people that enriched with diverse type neutraceuticals and improve the food digestibility.But due to civilization input, people are moving far away from this healthy traditional practice.The concept of probiotics, prebiotics, synbiotics is now emerging in the food industry.In these aspects, food microbiology education is in a very nascent stage.Only 23 educational institutes are now offering Food Science & Technology related Master degree level course.Microbiology in graduate and post-graduate level is offered by around 500 of college and university in this big and populated country.There are many institutes also offering Biotech, Nutrition, Dietetics, Bioprocess & engineering and other related courses at UG and PG level.It is noteworthy to mention that most of the course offered by the non-government organization, therefore, they support those courses which have market demand. Food microbiology is a minor part of these course curriculum by giving emphasis on lactic acid bacteria, food spoilage, probiotics, and relevant aspect.But most of the curricula are devoid of microbial food processing, value added food for human and veterinary animals, food virology, ethnic fermented foods, food hygiene, quality control norms, like contents.Besides very little emphasis has also given on hands-on study or skill development on this subject.No one institute is offering food microbiology as an independent course at any level.In related to this, very few microbial food products are also marketed except few dairy products and probiotic supplements for aquaculture and veterinary area.The poor market demand and industrial production are the major constraints behind the flourishing of food microbiology related courses in India.But, hope this darkness will be unlighted in recent future by depending upon effective education.

Keywords: India, food status, offered courses, food microbiology

3rd FMTGN symposium 'Food Microbiology Education in Practice'

S2

The training system in food and sanitary safety in the Senegalese institutions, Senegal

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In recent years, Senegal has sought to strengthen the governance capacity of higher education institutions in the area of food and sanitary safety systems (SSS). Indeed, food safety is a public health priority in Senegal, which justifies the actions carried out in the country. For example, international health regulations are well reflected in national policies and programs. In addition, there are efforts to support standards, codes of practice and guidelines issued by the Codex Alimentarius Commission (CAC). In sum, the country is implementing multiple initiatives to strengthen the SSS system and there are controls to ensure food safety and protect the health of consumers. In this context, higher education institutions have set up several training courses in this area of food, nutritional and health security. These are the Cheikh Anta Diop University of Dakar (UCAD), the Gaston Berger University of Saint-Louis, the Inter-State School of Sciences and Veterinary Medicine (EISMV).
At UCAD, several establishments carry training in food, nutritional and health security. These are the Polytechnic school (ESP), the Faculty of Science and Technology (FST) and the Faculty of Medicine and Ondtostomatology (FMPO). These courses cover several aspects including animal, plant and forest products; processing of agricultural products; food safety, quality in food; regulations, health and ethical standards; agricultural policies; entrepreneurship and management. These courses are of type: senior technician, license, master, engineer and doctorate.
All of these courses aim to provide learners with knowledge and skills for mastering the technologies and tools needed to ensure food, nutritional and health security.
In addition, a apply research and community service program accompanies the training component. Several laboratories and research teams are specializing in food, nutrition and health security. The analysis and testing laboratory plays an important role in the control of foodstuffs sold in Senegal. The Ministry of trade of Senegal through its laboratory deals with the inspection and control of imported food. The Food Technology Institute of Dakar is specializing in the control of aflatoxin in cereals.
The training system in food and sanitary safety in the Senegalese institutions is developed. Coordination of all training and research activities carried out in the different institutions is necessary.

Keywords: Training, research, food and sanitary safety, nutritional, Senegal

Conference Workshops | Monday 3rd September

3rd FMTGN symposium 'Food Microbiology Education in Practice'

S3

Teaching food microbiology at Moscow State University, Russia

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Food safety is a task that humanity has been trying to solve for a long time. In the IV century BC Hippocrates in the Diet wrote: "Our food should be a medicine, and the medicine must be food". However, cooking, preserving food with the help of microorganisms and damaging it are two sides of one process. In 1857, L.Pasteur opened a new era in food microbiology, scientifically proving that it is microorganisms that cause food spoilage and indirect infectious diseases of humans and animals. The Department of Microbiology of MSU was founded in 1953 by V.Shaposhnikov - the founder of technical microbiology, which includes all aspects of industrial use of microbes. Microbiology is a fundamental discipline in university programs aimed at training specialists - microbiologists of a wide profile. The main objective of any of these programs is to find an appropriate balance between the basic concepts of microbiology and the practical skills necessary for the work of specialists, including professionals in the food industry. The training also includes laboratory practice, the requirements for the organization of a laboratory for microbiological work with food, the control of the quality of food and raw materials in accordance with regulatory standards for sampling methods, the use of traditional and express methods of detection, identification and quantification of dangerous microbes, special attention is paid to methods for controlling the presence of antibiotic content in products which cause multidrug resistance of infectious agents, as indicators of food safety, ways of reducing microbiological hazard in food products. Students, masters, as well as professionals to improve their qualifications through the International Center for Biotechnology at the MSU take part in the training. Educational and methodical manuals on methods of microbiological work, detection of dangerous infections, colonizing products, permissible levels of their presence in the product, state quality standards have been published. Over the past decade, most of the microbiology courses in food have evolved to provide, in addition to basic knowledge of the microbiology of food, skills in critical thinking and solving problems in creating biopreservatives and food additives that enhance the biological, nutritional value and their health benefits human, perfection of quality control methods.

Keywords: methods for controlling, food safety, training of professionals.

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S4

Improving food safety management for small and medium enterprises in a developing country: Transferring knowledge and best practices through food microbiology education, Philippines

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A former Chancellor of the University of the Philippines (UP) once emphasized that "If UP is to fulfill its noble purpose as the national university then it must be the preeminent graduate and research university of the country as well as its leading public service university all at the same time." This statement therefore, has become the framework that we at the Laboratory of Food Microbiology and Hygiene (LFMH), use as guide in performing our mandate as a constituent of the only National University in the country. We understand that members of the faculty are tasked not only to transmit knowledge to students, but also to generate new knowledge through research and development activities; and to conduct community extension works that deliver information to sectors that do not have access to university education. The interfaces of this tripartite role of teaching, research and development, and public service are being explored to address the challenge of foodborne illnesses in the Philippines. The LFMH looks at this tripartite role as an opportunity to transfer food safety knowledge to micro-, small- and medium scale enterprises in a country where majority of food industry stakeholders are at these scales. In this presentation, we shall enumerate efforts of the LFMH in transferring knowledge to stakeholders from the food industry, academia, government units, and non-government organizations. We shall also share some of our experiences in merging teaching of university students and community extension activities to inculcate the values of service and grassroots empowerment. Learning and challenges we encountered in doing these activities shall also be discussed.

Keywords: Food Microbiology Education, Developing Country, Food Safety Management

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S5

From basic individual experiments to designing integrated laboratory analyses for a better understanding of microbial activity in practice, Turkey

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Microorganisms play an important role in changing the bio material (plant origin and animal origin) into simple or more complex products, so called fermentation process, when food is concerned. The characteristics of the end product is determined mainly by the type of microorganisms and the material. The intrinsic and extrinsic factors are also important for the microbal activity. During cheese production, for example, the raw material milk, looses its original characteristics and a new product cheese is manufactured at the end of the process. What we teach during a food microbiology course is the kind of microorganisms that can be used to produce cheese as well as which pathogens can be present in both milk and cheese and also the factors affecting the growth of the desired microorganisms. Students learn cheese manufacturing processes on the other hand, in technology lectures. They do not have the opportunity to combine the knowledge of microbiology and technology unless they have an advanced lecture in the programme. Food microbiology and food technology courses are mainly covered in the 5th and 6th semesters. They have design courses in the final semester. The content of the design courses is not enough for the students to understand the key points in food manufacturing. Since microbiology is the key difference between food engineering and other basic engineering diciplines such as civil, electric/electronic or mechanical engineering, an applied (theory and laboratory) course integrating these aspects is essential. The structure, content and the algorithm of such a course will be discussed in this presentation.

Keywords: microbiology, education, interdisciplinary, design, engineering

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S6

Molecular microbiology facilities: Impact on microbiology training in sub Saharan Africa countries, Nigeria

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Microbiology laboratories play an important role in food safety and food spoilage studies. Within microbiology laboratories, molecular microbiology techniques have revolutionized the identification and surveillance of pathogens and spoilage microorganisms. The combination of excellent sensitivity, specificity, low contamination levels and speed has made molecular techniques appealing methods for the diagnosis of many food borne and spoilage microorganisms.
In a well-equipped microbiology laboratory, the facility designated for molecular techniques remains indiscrete. However, in most Sub Saharan African countries, poor infrastructure and laboratory mismanagement have precipitated hazardous consequences. The establishment of a molecular microbiology facility within a microbiology laboratory remains fragmented.
A high-quality laboratory is expected to include both conventional microbiology methods and molecular microbiology techniques for exceptional performance. Furthermore, it should include appropriate laboratory administration, a well-designed facility, laboratory procedure standardization, a waste management system, a code of practice, equipment installation and laboratory personnel training.
This presentation highlights fundamental issues that need to be addressed when establishing a molecular microbiology laboratory in Sub Saharan African countries.

Keywords: Molecular Microbiology, Sub SaharaN Africa, Food Safety, Food Spoilage, Microorganisms

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S7

Newly introduced food microbiology module at University of Belgrade – Faculty of Agriculture

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Perceiving the need for well-trained professionals in the field of food microbiology, the Faculty of Agriculture (www.agrif.bg.ac.rs) has set up a whole new module "Food Microbiology" within basic academic studies (240 ECTS) integrated in the Department of Food Technology and Biochemistry. Within the module the following is studied: the basics of microbiology of food, industrial microorganisms in food of animal and plant origin, genetics of industrial microorganisms, microbiological food spoilage, food plant sanitation, probiotics and prebiotics, food infections and intoxications, microbiological methods of food analysis, etc. All lectures are followed by laboratory work. Most of the courses are present on e-learning platform moodle and accessible by students. Following good educational practices (GEP) we take care of regular up-date of the courses content and simultaneously promote and implement techniques of active learning. In order to achieve this goal, majority of lecturers have undergone various trainings to improve and develop new teaching skills. New accreditation of the Food Microbiology module have been opportunity to introduce some emerging topics, as e.g. foodborne viruses, increasing and serious concern in public health, or novelties in hygienic engineering and design, etc.

As a sequel, new module "Food and environmental microbiology" at the Master level of education (60 ECTS) was integrated in the curriculum of Food Technology. The new Master module consists of 3 compulsory subjects and 1 elective block from which 2 subjects are selected. Among others, the module includes subjects concerning introduction to scientific research, advanced methods in food microbiology, biotechnology in environmental protection, pathogens in food and environment, functional food, bioactive substances of microbiological origin, microbiological wastewater treatment, bioconversion of agro-industrial waste, etc. It is expected that well trained students - future specialists will be integrated in all part of the food chain production, contributing significantly to the safety of food products and consumers.

Keywords: food microbiology, curriculum, active learning

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S13

Biosafety challenges in students' dairy microbiology laboratories, Egypt

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Generally, the environment of any microbiological work must be in safe mode including laboratory coats, gloves, area of analyses and safety glasses which represents the minimal personal protective for students and their teachers. Also, Good microbiology techniques (GMTs) represent an important prerequisite for bio-safety of different dairy microbiology laboratories in research and teaching. In analyses of milk and its products by students or check the purity of microbial cultures, the analyses should be in bio-safety cabinet or using flame sterilization method for aseptic area. Teachers of food microbiology and their technical assistants should have enough knowledge and practice in using of different protective equipment and infection control measures in teaching laboratories in order to eliminate the spread of microbial hazards. The students' laboratories should provide with institutional bio-safety manual. Implementation of Good laboratory practice (GLP) could be a good solution for reducing the risk of microbial contamination especially by pathogens e.g. *Staphylococcus aureus* and *Listeria monocytogenes* from students' laboratories.

Keywords: Good microbiology technique, bio-safety, pathogens, microbial hazards, GLP

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S8

The update concepts in food microbiology education: An introduction of knowledge and skills for predictive microbiology and microbiological risk assessment in foods, Slovakia

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This contribution presents the concept and structure of education in food microbiology at Faculty of Chemical and Food Technology of the Slovak University of Technology in Bratislava, Slovakia. Since 2015 subject of Predictive microbiology and microbiological risk assessment including computational exercises is being lectured for last-year students of the Nutrition and Food Quality Assessment specialisations. Experiences from this period up until today will be shared as a means of encouragement to emphasize the role of science in the art of microbiological studies. This work was supported by the project of The Ministry of Education, Science, Research and Sport of the Slovak Republic KEGA 024STU-4/2018.
Contribution for the 3rd FMTGN symposium "Food Microbiology Education in Practice"

Keywords: Food Microbiology Education

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S9

Food microbiology training in Brazil – experiences with educational practices for undergraduate students from different majors, Brazil

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Food Microbiology is a topic included in the curriculum of many majors such as Food Engineering, Food Science and Technology, Nutrition, Veterinary Medicine, Gastronomy, Pharmacy and Biochemistry, among others. These curricula focus different aspects of the role of microorganisms in foods, according to the career. One of the main challenges is to present the appropriate content to such a heterogeneous group. Food safety and hygiene are taught in most of these careers, but other subjects are also taught, such as technological applications of microbes. In a Symposium during the Brazilian Congress of Microbiology in 2017, some professors have expressed difficulties in getting the content along, especially in those majors where food microbiology does not seem to be directly related to the profession. The use of personal devices such as cell phones and tablets during classes, which deviate the attention of the audience, requires new teaching strategies. For instance, some professors have incorporated extension work in the course in an attempt to immerse the students in the reality of many small businesses. In this experience, students were able to create new foods from wasted by-products and they have implemented good manufacturing plans in some industries. Other experiences include the development of research projects along the semester and the generation of articles that are often published in local journals. A few professors have tried active learning methodologies adapting ideas from methods such as team based and problem-based learning in the hopes to improve learning outcomes and increase students' interests by bringing real life problems to the classroom. In these settings, smartphones, tablets and laptop computers have been used successfully. Large outbreaks, news about recalls or national and international scandals, such as those related to meat products known in Brazil as "Weak Flesh Operation", have generated interesting discussions. We have noted an improved interest, participation, and critical thinking upon implementation of these methodologies. However, most professors throughout the country still use traditional teaching methods and their reluctance to change is related to lack of training and time. ilt is crucial that awareness concerning the basic curriculum considering different careers and the challenges and dilemmas facing education for the new generations be brought upon and dealt with by using creativity and effective teaching approaches.

Keywords: Education, food microbiology, teaching, smartphones

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S10

The role of the laboratory in undergraduate food microbiology education today, Australia

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There are many aspects of food microbiology that can be taught in an undergraduate food microbiology laboratory class. Laboratory classes can be designed for a range of important food microbiology topics including microbial fermentation, microbial spoilage, foodborne pathogens and rapid advanced analytical techniques. However, understanding how to identify spoilage microbes, determine the cause of foodborne disease, make a fermented product, or use the latest diagnostic instruments is complicated and requires advanced knowledge and technical expertise. Designing practical classes using problem solving to develop learning skills in the above situations can be difficult, especially as universities often have strict regulations around contact hours**.** Practical classes often have a large number of students, and staff may need training to prepare meaningful food samples for analysis. There may also be limitations on the use of the latest rapid advanced identification techniques. Instruments are often expensive and their use in associated research laboratories may preclude inexperienced students from using complicated sensitive instruments. In light of the above considerations, we should ask ourselves whether large traditional practical classes with limited time is still achievable for the study of practical food microbiology. What are the most important practical outcomes from an undergraduate food microbiology subject in a wider biotechnology or food science degree? A combination of the traditional and the virtual laboratory helps students obtain a broad and practical education in food microbiology. The use of a central consul linked to students' computer screens in large classes has assisted teachers at RMIT University to communicate and explain techniques, especially when combined with virtual laboratory demonstrations. This has enhanced the student experience and improved students' exposure to advanced techniques using online resources which can be integrated into traditional practical classes.

Keywords: Food microbiology, Undergraduate, Laboratory practicals, Virtual laboratories, Online resources

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S11

Good education practice: Use of web-based database for HACCP planning, Japan

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It is well known that the central objective of the FAO, WHO and EU policy and legislative framework related to food is always the provision of safe, nutritious, high quality and affordable food to the world's citizens and consumers. Thus regardless the challenges of the food system, such as climate change and resource scarcity, technological developments or the global economic and trade framework, the future education curriculum on Food Microbiology should be focused on food safety and nutrition. In order to be able to ensure the supply of safe and nutritious food to the consumer, we should prepare the *next generation students* **(ngs**) for food microbiology jobs (e.g., *food scientists and microbiologists in academia and government, food technologists and quality assurance specialists in the industry, food inspectors, food safety specialists and food safety lawyers, food processors, risk managers, food legislators, enforcement officials*) who are required to have solid knowledge and skills in microbiology *to solve problems that we don't even know they are problems yet, using approaches, technologies and/or developments that haven't been yet invented.*

Since we are living in exponential times, Food microbiology education, apart from the elementary subjects, such as (i) overview of food hazards, (ii) knowledge on microbiological and toxicological risk analysis, (iii) better interpretation of the information overload about hazards in food, (iv) why zero hazard does not exist, (v) ways to reduce hazards in food, should encompass additional aspects of food production, food stability (shelf life), food poisoning (infection and intoxication) and introduction of food waste or waste from food production processes (minimise environmental impact). Particularly, specific attention should be given to: (i) Next Generation Sequencing **(NGS)** that will allow students to have a greater depth of understanding on foodborne pathogens, (ii) access to technical information, as well as to new disciplines (big data, data analytics, computational biology) for better official inspections, i.e., explore the viability of technology-driven applications including nano-packaging, labelling, food-omics or molecular biology advances (e.g. DNA microarrays) and culture-independent techniques, or other rapid screening systems/sensors, on a large scale with reduced cost and easier mode of operation, adapted to small scale or home producers.

Keywords: education, future microbiology, safety

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S12

Teaching food microbiology in the non-university education, Poland

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We can assume that the learn of food hygiene and preparing meals should start already at the family level. However food microbiology has to be taught in different levels of formal education. Understanding the basic concepts of subject is critical in the context of water and food-related risk. This is a challenge to provide children or teenagers with basic knowledge in the field of food microbiology rising the awareness of the role of microorganisms in food and encouraging the exploration of this fascinating field of science. For many years Faculty of Food Technology participates many educational projects targeting primary and secondary school children. We have introduces diverse forms of lessons. The projects exist under common names Science Festival and WULS-SGGW Days and Open Labs. There are the food microbiology laboratory lessons organized for children with or without the supervision of their school teacher. Little biological knowledge, lack of skills in working with microscope and biological material are real challenges for an academic teacher. On the other side there is a lot of interest, dozens of questions and the desire of each of the children to independently perform the experiment. In an era of disciplinary specialisation and methodological focus the process of explanation the depth and breadth of microbiological research scientists to children is not always easy. The verbal interaction children-researchers are foundation of understanding. Teaching style influence the children and teenagers perception. The youngers see practical work as more enjoyable and olders as more more useful, clarifying or expanding knowledge. Practical examples answering some questions and trouble solutions will be presented and discussed.

Keywords: Education, Good practices, primary school, secondary school, laboratory lessons

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S14

Food microbiology education at university level in the future, Greece

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Information and/or data regarding microbial responses in foods are indispensable for constructing a plan for Hazard Analysis and Critical Control Point (HACCP). Graduate school of Agriculture, Hokkaido University opens a course for HACCP training. This course provides the skills of food safety management system, which identifies, evaluates and controls the hazards that are significant for food safety. This course is conducted as follows:

· make a team (group) with several students together and choose a food you want.

· consider and list up the possibilities of something dangerous happening (risk or hazard) and decide the seriousness of the hazard (critical point) during the food processing from raw materials to final products.

· propose how to control, manage and reduce the hazard during the food processing.

To facilitate planning a HACCP, web-based database and predictive tools such as ComBase (https://www.combase.cc) plays a key role. ComBase is a large database of microbial responses to food environments and has attracted the attention of many researchers and food processors. Although ComBase contains a vast amount of data, it is not easy to obtain desired information from the retrieved data. In the present study, we developed a new ComBase-derived database (Microbial Responses Viewer, MRV: http://mrviewer.info) consisting of microbial growth/no growth data. The growth/no growth data of nineteen different microorganisms were extracted from all the data in ComBase comprising 29 kinds of microorganism. The growth/no growth boundary was illustrated as a logistic regression model. The users easily enable to find out the critical limit for controlling targeted bacteria. Furthermore, the specific growth rate of each microorganism was modelled as a function of temperature, pH, and water activity (a_w) using a generalized linear model. The specific growth rate was illustrated using a two-dimensional contour plot with growth/no growth data. MRV provides information concerning growth/no growth boundary conditions and the specific growth rates of queried microorganisms. Using MRV, food processors and HACCP trainee easily find the appropriate food design and processing conditions. This database will contribute to the efficient and safe production and distribution of processed foods and also efficient training for HACCP planning.

Keywords: ComBase, Microbial Responses Viewer, HACCP, Critical limit

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PS.1

Food safety as poultry meat consumers understand it: Investigation at shopping points in Slovenia

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The importance of food safety culture has become important in the past few years, because many authors established that food safety behavior not always follow food safety knowledge. Griffith and co-workers (2010) argue that the greater the degree of safety attitude alignment between senior management and employee attitudes, the more likely they are to adopt positive behavioral attitudes, such as "handling food safely is good for the business". This study aimed to analyzed food safety culture in foodservice units (namely hotel restaurants) using questionnaire developed by Ungku Zainal Abidin (2013). With the statistical t-test for two independent samples and the analysis of the A-NOVA variance, we analyzed statistically significant differences between the individual groups by agreement for each category. A total of 185 useable survey responses were obtained and subjected to statistical analysis with six factors extracted: management and coworkers support, communication, self-commitment, environment support, work pressure, and risk judgment. Further analysis of the survey data showed employees´ perceptions on certain factors of food safety culture. Statistically significant differences were in question if they were attending food safety training (hygiene minimum) or not. The analysis shows that there are also statistically significant differences in categories associated with a set of questions about management, communication and work pressure. There were also statistically significant differences across time worked at current workplace in the category associated with set of questions about environment in the workplace. Significant differences in employees´ perceptions can guide development of interventions that support safe food handling practices in onsite foodservices. Further research is needed to confirm and validate the application of the food safety culture scale in other types of onsite foodservices.

Keywords: food safety culture, food handlers, foodservice units

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PS.2

A comparative study of free and immobilized brewing yeast fermentation performance based on kinetic parameters

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The study was focused on comparing two different immobilized brewing yeast, *Saccharomyces carlsbergensis* fermentation´s with traditional free cell fermentation in experimental scale. The immobilization techniques used were: entrapment and encapsulation method in alginate support. The objective was to choose the most suitable immobilization technique that protects the yeast cells providing a good fermentation performance compared to free yeast cell fermentation.
Kinetic parameters investigation of free and immobilized *Saccharomyces carlsbergensis* was done based on growth kinetics, ethanol productivity and substrate consumption (glucose) using computer simulation for different kinetic models. Entrapment and encapsulation immobilization techniques are applicable, effective and of economic benefit. These techniques protected the morphology of cells, and supported cells growth and budding. In normal fermentation conditions entrapment immobilization is similar with free yeast cell fermentation. In inhibitory condition both immobilized methods are more effective than free yeast cell traditional fermentation. Better results give encapsulation immobilization method. These results are supported also by kinetic parameter investigation.

Keywords: Yeast immobilization, entrapment, encapsulation, fermentation, kinetic parameters, modeling

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PS.3

The behaviour of some microorganisms isolated from medicinal plants to the action of artificial antimicrobials

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Albania has a varied and appropriate relief for the growth and development of traditional plants, depending on the climate and geographic localization. Also included are medicinal herbs, known and used too early for their positive influence on health and wellbeing. Medicinal plants are studied as origin of a considerable number of different microorganisms, that by their importance are generally classified in two groups: microorganisms of industrial importance and producers of biomolecules & harmful microorganisms that cause problems in the health and the environment. Referring to their presence in medicinal plants, isolated and purified microorganisms are both endemic and expanded microorganisms, such as microorganisms present in the plant due to microbial environmental contamination. It is not excluded the presence of poor pathogens and others come from air, soil, water, precipitations or even wastes and polluting materials. The study is based on a recent experience in the taxonomy of microorganisms isolated from plants, growing in areas with special characteristics. The experimental work was designed to identify the behavior of microorganisms present in two selected sources: Rosa canina of the Rosaceae family and the *common juniper*, Juniperus communis of the genius Juniperus, family Cupressaceae to antimicrobials as antibiotic drugs, that can be used in therapy along with the medical plants above. Isolated and identified species were grown in the presence of some inhibition drugs and was determined the level of resistance that microorganisms appeared under the action of the inhibitors used at the minimum effective levels of concentration (MIC). A slow growth, survival rate of species, reduced ability to produce biomolecules, as well as changes in reproduction and sporulation, were indicators of their sensitivity to the action of artificial inhibition and the effectiveness of combinations of selected plants with medicinal drugs.

Keywords: medicinal plants, Rosa canina, Juniperus communis, antimicrobials, MIC

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PS.4

Hygiene control a critical point to ensure microbiological stability in beer

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This paper aims to provide some solution of microbiological problems that affect beer quality during beer chain production in order to improve preventive measures and control methods. The origin of contaminant microorganisms came usually from wort, pitching yeast, and hygiene of equipments used. We have investigated the contamination rate during beer production in function of period of time, source of contamination, beer parameters etc. In order to ensure microbiological stability in beer it is very important to analyze inhibitor factors such as alcohol, hop compounds, carbon dioxide, oxygen, pH and also effectiveness of different processes such as filtration, storage at low temperatures, pasteurization etc.
Minimization the sources of contamination is the best way to control beer hygiene. The main measures undertaken consists on increasing the resistance of beer to microbial attack, optimization of CIP cleaning, optimization of processes that aim to reduce microbial load such as yeast filtration, pasteurization, and conditioning.

Keywords: beer, hygiene, microbiological stability, contamination, yeast

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PS.5

Consumption, perception and safety practices of Brazilian seafood consumers

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Seafood is source of proteins of biological value high and unsaturated fatty acids. The consumption of seafood is recommended by the World Health Organization (WHO), however, seafood is considered one of the main food categories associated with food borne disease outbreaks. Food-borne disease outbreaks linked with seafood consumption are mainly attributed to inappropriate handling practices and misjudged safety perceptions. Thus, the present work aimed to identify the profile of the Brazilian seafood consumer in order to determine the high-risk group, which is necessary to direct public education programs in seafood safety. Therefore, approximately 1,000 participants were invited to respond a survey composed by 29 questions, divided into three parts: demographic and socio-economic profiles; consumption habits; safety practices and risk perceptions. Multiple Correspondence Analysis was performed to group the consumers according to their profiles. The majority of the participants (89-90%) demonstrated to have knowledge about safe handling practices of seafood, reporting that they sanitize hands with soap, sanitize utensils, prepare dishes immediately after thawing and keeping raw foods separated from of ready-to-eat foods. However, it was not possible to infer whether such practices are really applied in the domestic environment and whether consumers understand their importance. Additionally, the survey revealed a low level of perceptions about seafood safety. A total of 64 to 71% of the participants demonstrated lack of knowledge of the main pathogens related to seafood disease outbreaks, and they also presented difficulties to identify consumption places that could result in higher microbiological risk. The consumers showed also be unaware of the responsible for ensuring seafood safety. Postgraduate participants presented positive correlations with the perception of microbiological risk in comparison with participants who have up to high school degree. Moreover, postgraduate consumers were positively correlated with adequate defrosting of seafood. The knowledge is an adequate tool for perception and judgment of microbiological risk, leading the consumer to action and adoption of safe practices. The education of seafood consumer about their safe, their handling, practices and perceptions is key for the success of farm to fork preventive measures aiming to protect public health.

Keywords: Survey, Seafood Handling, Safety, Microbiological Risk.

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PS.6

A bridge to interdisciplinary cooperation for graduate students through 'aziz sancar technocenter' at Istanbul Aydin University

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The rapid development in science and technology is a driving force to instruct new collaboration among disciplines. Biomedical, bioengineering, medical engineering are some of the examples that meet this need/gap, recently. However, collecting fundamental science lectures like mathematics, physics, biology/microbiology and engineering courses like fluid mechanics, thermodynamics, into a new hybrid discipline is not enough to give the intended viewpoint, that the target is a biological system showing behavior of living organisms. On the other hand, adopting a few biology related interdisciplinary selective courses into a graduate level classical engineering discipline program like mechanical, electric/electronics, civil engineering or keep the students together in some research group does not meet the expectations.

In many universities, different engineering disciplines study in separate buildings and come together only in social classes which account for the interdisciplinary work during their education. These courses are prepared at the intermediate level in terms of addressing everybody and are not considered as important within the main discipline. Therefore, it does not provide the expected benefits in interdisciplinary studies, and is, on the contrary, undervalued in the eyes of some disciplines. The intention of working together, however, is to bring together those who are competent and at the same time necessary to achieve a certain benefit in a particular context. The 'Technocenter' established in Istanbul Aydın University, laboratories belonging to related disciplines are gathered together. Thus, both students and teachers can see each other, talk about their problems, communicate and have the opportunity to work together. Within the context of this presentation it is aimed to present information about the new concept of IAU, which opens the horizons for innovation and entrepreneurship.

Keywords: new concept, technocenter, interdisciplinary, engineering, undergraduate education

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PS.7

International mobility – Opportunity for collaborative research and student centered project-oriented learning and training

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The research group of the authors has a long-lasting international cooperation in pedagogical and research work with several institutions abroad (Raspor and Smole Možina, 2012). The activities connect bilateral research projects and university networks (Erasmus+, CEEPUS), resulting in improve quality of educational and research work. Cases of teachers' and students' mobilities, where academic staff and students actively participate before, during and after mobility at a guest and host institution will be presented. Team work with doctoral students (researchers) contributes to the overall research objective and optimal transfer of knowledge, skills and attitudes. Doctoral students gain teaching experience. By individualized approach, they get immediate feedback on the process, communication and guidance of the student through the research work quickly improves. Learning and training tailored to individual students' needs improves quality of education, makes it flexible and contributes to acquisition of knowledge and overall mobility experience of the students on exchange. They gain experience in research, develop laboratory skills and critical thinking, independence, tolerance and adaptability, learn to process obtained data, report results and how to write scientific papers. All involved in mobility are testing the international environment, practice foreign language, learn about new culture but at the same time also represent their own. These increases the potential for successful international cooperation and contribute to the development of inclusive society.

Keywords: international mobility, cooperation, tailored training

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PS.8

Outcomes of the project innovirology: The network of European teachers/trainers of virology

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The European Union had funded through Erasmus+ programme an initiative for innovation in teaching and training of Virology as well as for improving dissemination. In collaboration of seven institutions from different European countries, the INNOVIROLOGY project has dealt successfully with the promotion of technologies of information and communication in teaching and learning, supporting learning and access to open educational resources in the field of education and training, and has contributed to the modernization of the European systems of higher education.
Multiple outcomes had been achieved and could be accessed at the webpage of the Project http://www.innovirology.com
A European laboratory training manual for teachers and trainers: a total of 55 documents had been gathered, including protocols, laboratory practices, syllabi, and lectures. All these materials may be used for theoretical lectures, practical work with students, or as a protocol.
Online Virology Courses: four online courses have been developed in the format of videos - Diagnosis in Virology, Animal Virology, Plant Virology and Bacteriophages. Subtitles and/or transcripts are available in the six different languages. These courses are of exceptional quality, actual, informative, and fun to follow. Two other online courses have been developed as slide shows: Basic Virology and Food Virology. All the courses provide modern pedagogical material and will help students to better understand the world of viruses. (http://www.innovirology.com/resources/online_courses/)
An educational Smartphone app: a Smartphone application game on viruses has been developed. This app has the advantage that it may also be played in computers and tablets. It consists of a total of more than 200 multiple choice questions, on average quite easy and funny, to motivate the player to get introduced into the world of Virology. Each game comprises 20 questions, and points are earned depending on the speed in which each question is answered. All questions are offered in the six languages.
Virology webinars: A collection of recorded lectures is offered in the webpage.
A collaborative textbook for training in Virology: Virology - An interactive guide (http://www.innovirology.com/resources/virology_-_an_interactive_guide/)
The audience to be approached are high school students or undergraduate students and an attractive, updated, interactive book had been written and prepared.

Keywords: virology, innovation, teaching, textbook, on-line courses

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PS.9

The basic platform for higher education in food microbiology

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One of the essential targets of ICFMH is to prepare a proposal for a harmonized curriculum for education of food microbiologists at university level. This activity is discussed more or less regularly last 15 years at FOOMICRO congress. The inquiry in many countries has shown that, even in the EU member states, education and training in food microbiology are extremely unstructured and differ widely from country to country and from type of university or school conducting the program. The authors see as *minimum requirement* for a complete curriculum in food microbiology in the following structure - 3 years of basic studies, 2 years of advanced studies with half a year for preparing a master thesis, which is in total 5 years. Basic studies can be in the area of agriculture biology, food science, and technology, veterinary, hygiene an closely related programs as these programs deliver essential basic knowledge for the students wishing to continue their education in food microbiology as field of their practical expertise for employment within food supply chain or in relevant research or advisory or inspection institutions. The paper tries to cluster relevant knowledge and skills on the way to make it relevant and transparent. On top of this basic structure they add assortment of activities carried by food microbiologists in industry, in the service of the government, in research and at universities, to illustrate and to complement the knowledge, skills and attitudes needed for highly professional food microbiologist. The present paper has ambition to provide a basis for discussions with relevant authorities, national bodies for food microbiology and those involved in academia as well. Harmonizing the curricula in food microbiology will create a scientifically sound basis for the efficient protection of consumer health, and will make a significant contribution to increasing efficiency of agro-food industry also with reduction of food losses through food supply chain.

Keywords: Food Microbiology Curriculum, Harmonization, Education, Training, Knowledge, Skills, Attitude

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PS.10

Problems of food microbiology and advanced methods of teaching microbiology of food products in Ukraine

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Food Microbial education for students in Ukraine, in particular, in the Odessa National Academy of Food Technologies (ONAFT) and Sumy State University (SSU) is given out by lecturing, laboratory classes, non-academic scientific work with students. Educational books and manuals were written by department teachers for studying microbiological disciplines at the modern level, involving new foreign and own scientific and methodological developments. These are "Technical Microbiology" (2017), "Microbiology of food production" (2016), "Microbiology of milk and milk products" (2015) a number of methodological developments for laboratory research. To prepare scientific works, students also use monographs written by teachers, for example, "Preservation of food products. Microbiology, energy, control", "Microbiology, packing and control of the canning production" etc.
Students actively participate in conferences, congresses and publish scientific articles in journals independently or together with teachers. The teachers and students of the academy carry out their scientific investigations in a scientific laboratory equipped with modern devices. Students also participate in state-funded programs, for example, on the topic: "Scientific basis for improving the sanitary control of safety of food raw materials and processed products".
Such results are possible with active scientific work of teachers, who developed 6 priority methods for controlling microbiological safety of food raw materials and processed products only in 2015-2017.
Thus, the main directions of teaching microbiological disciplines in ONAFT are (1) creating manuals containing up-to-date information on useful microorganisms and spoilers and foodborne disease agents and methods of their detection; (2) participation of students in the scientific works and publications; (3) introduction into the educational process of own and foreign scientific developments on the improvement of methods for controlling the safety of food raw materials and products of their processing.

Keywords: teaching microbiology of food products in Ukraine

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PS.11

Methodology of accelerated monitoring and assurance of sanitary quality and food safety in Ukraine

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The purpose of the work is to develop the scientific principles of accelerated determination of sanitary quality and safety of food raw materials and products of its processing by differentiated molecular-biological diagnostics of microorganisms-contaminants (*Bacillales spp., Cronobacter spp. (E. sakazakii), Shiga toxin-producing strains of Escherichia coli (STEC), methicillin-resistant Staphylococcus aureus*) according to their genetic determinants, as well as scientific substantiation of the research methodology with taking into account some modern conditions and principles of HACCP.
The applied morphological, physiological-biochemical and other classical microbiological, molecular-biological and molecular-genetic methods of identification of microorganisms and received new actual results regarding the composition of microbial contaminants of food, zoned and industrially processed in Ukraine, - quantitative and species composition of microorganisms in the raw material, at the stages of the technological cycle and products of its processing. Investigation of the methodology and methods of control of regulated microorganisms showed the inadequacy and inaccuracy of their phenotypic diagnostics due to the similarity of morphotinic properties within individual groups, the inconsistency of a number of biochemical features, the appearance of new metabolic features such as the synthesis of the toxicity gene with species traditionally considered to be non-pathogenic. Monitoring of the ability of isolated strains to synthesize toxic genes was conducted and new methods of preparation of samples for the determination of regulated contaminants were proposed. Proposed methods are tested on various types of food products. Molecular genetic diagnosis showed the specificity of the bacillary contaminants in Ukraine: the presence of the *nhe* gene was detected in 100% of *B.cereus* strains, *hbl* in 60% and *cyt K* about 40% of the strains studied. Resistant to penicillin and oxacillin isolates of *S. aureus* were typing by mec A gene in PCR with two primers (MecA147-F and MecA147-R). The results show that 66,7% of *these* isolates yielded a *mecA* product. The stx1 gene was the predominant gene detected in all STEC positive samples. The *eae* gene was found in one of examined isolates from beef carcass. Three isolates from swabs of beef carcass carried both *stx1* and *stx2* genes, one isolate showed association between *stx1* and *eae* genes, one isolate was positive for *stx1* gene only. Also, our results indicate that the dairy farm environments are a potential source of *Cronobacter* spp in raw milk. It was found that detection of these bacteria from raw cow´s milk on average is 19.4 %.
The priority genotype diagnostics of toxigenicity of microorganisms with the use of molecular genetic methods, in contrast to the phenotype, allows to carry out accelerated microbiological control of food safety taking into account the peculiarities of their composition and properties, ensures the accuracy of identification, the possibility of monitoring and prediction of microbiological risk, is a reliable method of their sanitary control.

Keywords: assurance of sanitary quality and food safety in Ukraine

Oral Abstract

Oral Abstracts | Tuesday 4th September 2018

Exploring biodiversity in microbial ecosystems along the food chain

O1.1.

"House" microbiota in a cold smoked salmon processing environment: A potential spoilage source

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Cold smoked salmon (CSS) is a lightly preserved fish consumed as a Ready to Eat Product (RTE) without heat treatment. Bacteriostatic treatments (salting and smoking) are used to prevent from foodborne and spoilage hazards. CSS contamination depends on raw materials, human activities, air flow and processing environment. Microorganisms can persist in processing plants due to growth at low temperatures, biofilms formation and tolerance to biocides. The relationship between a product and its processing environment is a major source of contamination. A house microbiota may affect the food quality.

In man-made building like food factories, air, cleaning, temperature and humidity are mainly used to drive microbial communities dynamic and structure. With the growing field of NGS in food environmental microbiology, microorganism's behavior can be evaluated by metabarcoding. The aim of this study is to monitor bacterial communities in a CSS processing environment using 16S metabarcoding. Microbial ecology knowledge in this complex ecosystem could be useful to characterize microbial reservoir, improve targeted hygiene procedures and lead to shelf life and products quality optimization.

Surfaces samples from CSS processing environment and products were analyzed by 16S metabarcoding. DNA was extracted from samples, used to PCR amplify the V3-V4 region of 16S rDNA then sequenced on Illumina MiSeq. Taxonomic classifications were obtained using FROGS pipeline, Silva 16S reference database and RDP classifier.

Several OTUs have been identified. The processing environment is mainly composed of *Acinetobacter, Pseudomonas, Aeromonas* and *Psychrobacter. Photobacterium*, *Enterobacteriaceae*, *Brochothrix,* Lactic Acid Bacteria (LAB) are dominant on salmon products.

Beta diversity revealed a core microbiota between the processing environment and products. This core community is mainly composed of Gram positive spoilage bacteria: LAB, *Brochothrix* and Gram negative: *Enterobacteriaceae*, *Psychrobacter*, *Pseudomonas, Aeromonas* and *Shewanella*.

These findings allowed identify residential bacteria within the processing environment. This persistent flora could have an impact on products quality. Spoilage contamination hotspots were detected. A better understanding of microbial dynamics within processing environment could help to reduce contamination and spoilage. Thus, improve cleaning and disinfection procedures to reduce food wastage and enhance products quality.

Keywords: house microbiota metabarcoding spoilage food wastage processing environment smoked salmon

Exploring biodiversity in microbial ecosystems along the food chain

O1.2.

Diversity in microbiota of chicken skin through raw poultry processing and refrigerated storage

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Richness and abundance of microbiota present in raw meat products play an important role in the shelf life of the products, their microbial safety, and therefore the consumer health. Sources of contamination in raw meat are animal and environment microbiota, and depend on the farming and slaughtering process. Poultry meat can host very diverse microbial communities including spoilage bacteria affecting the shelf life.
We used 16S sequencing to describe microbiota of skin from chicken legs and neck across evisceration and after the chilling tunnel in a poultry processing plant. Changes in bacterial communities of MA-packed cuts (wing, breast, leg), freshly produced and products stored until expiry date were included. At each sampling site, 3-5 samples were collected. Relative abundance of bacterial species was determined by 16S sequencing on Illumina MiSeq. Microbiota samples were collected, total DNA was extracted, and an amplicon of approximately 460 bp of the V3-V4 fragment of 16S rRNA genes were amplified and sequenced. The sequence data were processed on BION software version 16.12 (Danish Genome Institute) as well as NCBI and EzTaxon databases.
Significant shifts in the bacterial communities was observed between sampling sites across the evisceration processes and during the shelf life of 11 days. *Aeromonas veronii* was the dominating species on chicken leg skin and neck skin before evisceration and on chicken neck skin at end of evisceration process. During the evisceration processes, the microbiota on chicken leg skin shifted towards dominance of *Acinetobacter johnsonii* and similar changes was observed for the chicken neck skin after the chilling tunnel. Bacterial spoilers, *Brochothrix thermosphacta*, *Pseudomonas* spp., and *Carnobacterium* spp., as well as *Acinetobacter iwoffii* were dominant on skin of MA-packed raw chicken meat cuts (legs, breast, wings) after cold storage for 11 days. All the genera and species observed in chicken skin microbiota have been described as food spoilage bacteria and some as animal pathogens. This diversity of species is a reflection of the chicken rearing environment and handling during slaughter. Slaughter process equipment, e.g. feather picker and mechanical evisceration, could explain the presence of bacteria associated to the chicken gut microbiota. The presence of several psychrotolerant species in the chicken skin sample microbiota is a possible consequence of cold storage affecting the microbial communities.

Keywords: chicken skin, microbiota, 16S sequencing, poultry processing, shelf life

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Exploring biodiversity in microbial ecosystems along the food chain

O1.3.

DaQu fermentation selects for heat resistant *Kosakonia cowanii* and bacilli

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DaQu is a spontaneous solid state cereal fermentation used in Chinese vinegar and liquor production, which is the first fermentation stage aiming to generate saccharolytic enzymes. Evolution of microbiota in the spontaneous fermentation is mainly controlled by the control of the temperature profile. DaQu fermentation reaches a temperature of 50~65 °C for several days. Despite this high temperature, mesophilic *Enterobacteriaceae* and bacilli are present throughout the DaQu fermentation. This study aimed to determine whether DaQu spontaneous solid state fermentation selects for the heat resistant variants of these organisms. Replicate samples were obtained from a medium-temperature DaQu fermentation with a peak temperature of 58.00 ± 1.38 °C after 12 days. Heat resistance in *Enterobacteriaceae* is mediated by the locus of heat resistance (LHR). Screening of 14 *Enterobacteriaceae* isolated form DaQu identified one LHR-positive strain of *Kosakonia cowanii*. This strain also exhibited higher heat resistance than the closely related LHR-negative *C. sakazakii*. Heat resistance in *Bacillus* endospores is mediated by the *spoVA*^{2mob} operon. The copy number of the *spoVA*^{2mob} operon in genomes of DaQu isolates was determined by qPCR. Out of ten isolates of the species *Bacillus licheniformis*, *Brevibacillus parabrevis*, *Bacillus subtilis*, *Bacillus amyloliquefaciens* and *Bacillus velezensis*, 5 did not contain the *spoVA*^{2mob} operon, 3 contained one copy and 2 contained two copies. The proportion of *spoVA*^{2mob}-positive isolates was higher for samples taken at the end of the fermentation process. The presence and copy number of the *spoVA*^{2mob} operon increased the resistance of spores to treatment 110 °C. Thermal death time curves were modeled with the Weibull model; both the shape parameter *p* and the inactivation rate *k* of the Weibull model were highly correlated to the copy number of the *spoVA*^{2mob} operon. To confirm selection of LHR- and *spoVA*^{2mob}- positive strains during DaQu, the copy numbers of these genetic elements in DaQu samples were quantified by qPCR. The abundance of LHR and *spoVA*^{2mob} operon in community DNA relative to the abundance of total bacterial 16S rRNA genes increased threefold and sevenfold, respectively, during fermentation. In conclusion, culture-dependent and culture-independent analyses demonstrate that DaQu fermentation selects for heat resistant *Enterobacteriaceae* and bacilli.

Keywords: Heat resistance, DaQu, locus of heat resistance, spoVA2mob operon, selective pressure

Exploring biodiversity in microbial ecosystems along the food chain

O1.4.

Screening of *Staphylococcus carnosus* strains as starter cultures in raw ham fermentation

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Northern European raw hams are fermented for several months to ensure their organoleptic properties. The application of starter cultures might improve and accelerate this process. As the properties of raw ham starter cultures differ from those of strains applied in raw sausage fermentation, this study aimed at identifying strains that are safe for application, resistant to the conditions applied in the industrial process and show the desired metabolic traits. Thus, 39 different *Staphylococcus carnosus* strains were assessed for virulence and pathogenicity determinants as well as fermentation properties with regard to their application as starter cultures.

The Qualified Presumption of Safety concept of the European Food Safety Authority was used as a guideline to estimate the potential health risk for consumers, with emphasis on antibiotic resistance, presence of toxin genes and production of biogenic amines. Among the 17 antibiotics tested with the agar disc diffusion test according to the Clinical and Laboratory Standards Institute, ten strains were resistant or intermediate resistant against cefotaxime, chloramphenicol, oxacillin or trimethoprim/sulfamethoxazole. None of the strains was PCR-positive with primers targeting the staphylococcal enterotoxin genes (sea-see, seh), the exfoliative toxin gene (eta) or the toxic shock syndrome toxin gene (tst-1). Two strains showed β-haemolysis on human blood agar plates. The remaining 22 antibiotic-sensitive and non-toxigenic *S. carnosus* strains were analysed for the production of biogenic amines by HPLC-analysis. None of the strains produced cadaverine, putrescine and histamine under the experimental conditions used, but 12 strains produced phenethylamine in concentrations ranging between 2.6 and 15.0 µg/mL.

The proteolytic and lipolytic properties, the nitrate reductase activity as well as tolerance to sodium chloride and nitrite concentration applied in industrial settings were assessed *in vitro*. As expected, strain specific profiles were determined. Five strains yielded results favourable for raw ham fermentation.

The results of this study indicate that safety risks, such as antibiotic resistance and biogenic amine production, are quite common among strains of *S. carnosus*, and that fermentation properties are strain specific. Consequently, this study highlights that each strain should be analysed individually before its application as starter culture in fermented meat products.

Keywords: Staphylococcus carnosus; raw ham fermentation; starter culture

Exploring biodiversity in microbial ecosystems along the food chain

O1.5.

Dietary interventions modify the gut microbiota during pregnancy in patients with gestational diabetes mellitus (GDM)

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Gestational diabetes mellitus (GDM) is one of the most common pregnancy complications, associated with an increased risk of maternal and perinatal outcomes. It has been hypothesized that interventions on life style can have beneficial effects due to the modulation of the maternal gut microbiota during pregnancy.

Microbiota is remodeled at several body sites during pregnancy and dietary habits and quality of micronutrients may affect gut microbial composition. Increased knowledge of how change in foods and nutrients can modulate gut composition in pregnant women is thus of importance, not only for the mother but also for the unborn child.

We performed a prospective observational study evaluating the microbiota of 41 patients with GDM from the second to the third trimester of pregnancy after following a diet in line with given guidelines. The fecal microbiota was assessed by 16S amplicon based sequencing and daily dietary information as well as blood metabolites were analyzed. Overall we found a higher bacterial richness as pregnancy progressed in our GDM patients with strong correlation between pro-inflammatory taxa associated with GDM and metabolic and inflammatory variables. After the dietary counselling, 34.1% of the participants showed to be adherent to the given dietary recommendations. Adherent patients showed significantly reduced intakes of simple sugars and increased consumption of fiber, oligosaccharides and polyunsaturated fatty acids (PUFA) if compared with non-adherents. C-reactive Protein (CRP) values, blood glucose, fasting insulin and Homeostasis Model Assessment Insulin Resistance (HOMA-IR) score were significantly lower in adherents. This reduction in inflammatory variables was strictly correlated with the increase in *Faecalibacterium*, *Blautia* and R-*Ruminococcus*. In addition adherent showed significant reduction of *Bacteroides, Veillonella* and *Rikenellaceae.* We then observed a negative correlation between *Blautia* and total cholesterol, CRP and HOMA-IR. At sub-genus level, we observed a higher number of *Blautia* oligotypes and several oligotypes were associated with PUFA or negatively correlated with cholesterol. That evidence suggests an effect of the dietary intake at sub-genus level highlighting a possible different strain-dependent effect on gut.

Patients with adherence to the given dietary recommendations showed a significant reduction in inflammatory variables and pro-inflammatory microbiota with respect to non-adherents.

Keywords: Gut Microbiota; Gestational Diabetes Mellitus (GDM); Dietary Interventions; Metabolic Syndrome; Diet

Exploring biodiversity in microbial ecosystems along the food chain

O1.6.

Functional lactic acid bacteria from traditional Korean fermented food for combating obesity

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Kimchi, a Korean fermented food, has been mentioned as one of the healthiest foods in the world. In addition to the nutritional properties of kimchi, its major beneficial feature may be based on the lactic acid bacteria (LAB) associated with its fermentation. Scientific evidence supports anti-obesity functions of LAB in rodents fed with a high fat diet. Yet, there is only sparse information on the mechanism of this effect. The objective of this study was to investigate the effect of eight different LAB strains, isolated from Korean fermented food, on the weight of a high-fat diet (HFD) induced obesity mouse model.
Eight different LAB strains were isolated from the traditional Korean fermented foods, chili, doenjang and kimchi. The strains were identified as *Enterococcus faecium*, *Lactobacillus sakei*, *Lactobacillus alimentarius* and *Lactobacillus plantarum*. To evaluate their functionality for alleviating obesity, the strains were freeze-dried and mixed with a HFD. Male C57BL/6 mice were fed with the mix for 10 weeks and water was provided *ad libitum*. The mice were housed at 23 ± 1 °C and 55 ± 10% humidity, in a 12 hours light/dark cycle. Body weight and feed consumption were measured once a week. The animals were sacrificed, and serum, adipose tissues and feces were collected. The *E. faecium, L. plantarum* and *L. sakei* strains significantly ($p < 0.05$) reduced visceral fat mass in mice compared to the control group; however, *Lactobacillus alimentarius* did not show any effect. The main reduction was detected for epididymal fat. Traditional Korean fermented foods are considered to be a rich source of beneficial microorganisms. Their functionality appears to be related, at last in part, to associated LAB strains, thereby suggesting a possible approach for treatment of obesity.

Keywords: Lactic acid bacteria, fermented food, obesity

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Exploring biodiversity in microbial ecosystems along the food chain

O1.7.

Internalisation of *Escherichia* coli O104:H4 into the roots of lettuce plants grown in different soil types

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In 2011, a large foodborne outbreak of diarrhoea and the haemolytic-uremic syndrome (HUS) was recorded in Germany, which was caused by consumption of fenugreek sprouts contaminated with a Shiga-toxin producing enteroaggregative *E. coli* O104:H4 strain. Within the last years an increasing number of food-associated outbreaks caused by enteropathogenic *E. coli* were traced back to the consumption of contaminated fresh produce. The ability of pathogenic *E. coli* to colonise plants, including roots, is an important issue as bacterial contamination of fresh produce may occur directly on the field *via* manure, faecal contamination, irrigation or surface water. The aim of this study was to analyse the overall ability of *E. coli* O104:H4 strain C227/11ϕcu to adhere to and to internalise into the roots of lettuce plants under environmental conditions present in a green house. Moreover, the influence of the soil and lettuce type on adherence and internalisation was investigated.

Lettuce and lamb's lettuce were grown in non-sterile diluvial sand and alluvial loam. The plants were contaminated with fluorescently labelled *E. coli* O104:H4 strain C227/11ϕcu *via* irrigation water. Three biological and three technical replicates were performed for each condition. After four days of incubation in a biosafety level 3 greenhouse, plants were excavated. Roots were washed, homogenised and spread plated to determine the number of adherent bacteria. Internalised bacteria were analysed by homogenisation and plating of washed and surface disinfected roots.

Quantitative analysis showed that the extent of adherence was significantly influenced by the soil type used for plant growth whereas the extent of internalisation was mainly affected by the host plant. Significantly more internalised bacteria were found in the roots of lettuce compared to lamb's lettuce. Furthermore, depending on the host, the used soil type could influence the extent of bacterial internalisation.

This study demonstrated the ability of *E. coli* O104:H4 strain C227/11ϕcu to colonise plant roots in a lettuce and soil type dependent manner. Therefore, cultured plants should be taken into account as reservoirs of enteropathogenic *E. coli* strains.

Keywords: E. coli O104:H4, BSL3 greenhouse, roots, lettuce, soil type, internalisation, adherence

Exploring biodiversity in microbial ecosystems along the food chain

O1.8.

Genotyping, virulence and antimicrobial resistance of methicillin-resistance *Staphylococcus aureus* isolates from two municipal abattoirs in Nigeria

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Methicillin resistant *Staphylococcus aureus* (MRSA) colonization has gained interest in recent years in veterinary medicine due to its zoonotic potential. Currently, there are no information about the genotypic and virulence characteristics of MRSA isolates detected in Nigerian abattoirs.

In order to gain a deeper understanding into the epidemiology of MRSA associated with the abattoir food chain environment in Nigeria, a total of 18 isolates - previously *spa* typed from samples obtained from Ibadan and Ilorin abattoirs - were recovered and characterized by *Staphylococcus* cassette chromosome *mec* (SCC*mec*) typing,multi-locus sequence typing (MLST), antimicrobial resistance, and DNA micro-array analysis.

The SCC*mec* typing of MRSA strains revealed two distinct types (IVa and V). MRSA with new *spa* type t16571 (n = 10) belonged to SCC*mec* type IVa. Other MRSA *spa* types (t091, t1931, t816, and t4235) have the mobile genetic element of the SCC*mec* V type. MLST of the MRSA t16571 strains revealed that 7 of the 10 isolates belonged to ST 88 while 3 other strains all from the Ilorin abattoir (slaughterhouse wall of cattle section: 2 and nasal sample of an abattoir worker: 1), were found to belong to a novel ST 3614. A 100% resistant to β-lactams antibiotics (Cefoxitin and Penicillin) was observed among isolates. These strains were also resistant to Tetracycline (72.2%), Trimethoprim (72.2%), Ciprofloxacin (22.2%), Gentamicin (5.6%), Kanamycin (5.6%) and Tiamulin (5.6%) demonstrating six different resistance patterns. Isolates of same SCC*mec* type and colonality demonstrated similar resistance profile. Results from DNA microarray showed a high homogeneity among the MRSA strains. Resistance (*mec*A, *blaZ, dfrS1, tet(k)*) and virulence (*sea*, *sec*, *seh* and *sel, edin*B and *pvl*) genes including haemolysins, proteases, superantigens, and biofilm-associated genes were typed in the isolates from the various abattoir sections and workers.

Measures should be implemented at the abattoir food chain to prevent spread of MRSA among abattoir workers, food animals and the environment.

Keywords: MRSA, Abattoir, genotypic characteristics, virulence, Antimicrobial susceptibility, Nigeria

Exploring biodiversity in microbial ecosystems along the food chain

O1.9.

Variation of bacterial load on pacific oyster *(Crassostrea gigas):* Influence of seasons and differences between body compartments and harvesting wate

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Oysters are lamellibranch molluscs consumed worldwide and with rising commercial value. As they usually are consumed raw, they are frequently implicated in foodborne outbreaks. The aim of this study was to evaluate the influence of bacterial load from the harvesting area on the bacterial content of different body compartments of this filter-feeding shellfish.
Oyster and water column samples were collected between July 2016 and May 2017 from a bivalve production area located in Aveiro, Portugal, classified as a class B production area. Following international standard methods, total aerobic bacteria, Enterobacteriaceae, *Escherichia coli*, coagulase-positive staphylococci, *Clostridium perfringens*, *Salmonella* spp. and *Listeria monocytogenes* were quantified/detected. Aerobic bacteria and faecal indicators were also evaluated on superficial biofilm, intra-valvular fluid and hemolymph samples. Quantification of *Vibrio* spp. was performed by fluorescent in-situ hybridization (FISH) for superficial biofilm, hemolymph and intra-valvular fluid samples. DNA from oyster flesh was extracted and subjected to next-generation sequencing (NGS) for studying its microbiome.
Salmonella spp. were detected on water samples collected during spring and winter, whereas the highest levels of *E.coli* and *Enterococcus* spp. were recorded on autumn samples. Water contamination was not reflected on oyster bacterial contamination. *E.coli* and *Enterococcus* spp. levels were very low or undetectable in all oyster samples analysed. All flesh and intra-valvular liquid oyster samples presented *E.coli* contamination levels compatible with a class A production area. None of the samples presented unsatisfactory results for the pathogenic bacteria evaluated.
V.aestuarianus was the specie of *Vibrio* most abundant on the samples analysed by NGS. Autumn was the season with highest abundance of *Vibrio* spp. (74.2%). The highest counts of *Vibrio* spp. by FISH were obtained for superficial biofilm and intra-valvular fluid sampled during summer, while for hemolymph sample levels of *Vibrio* spp. were similarly high in spring, summer, and autumn seasons. A drastic reduction on *Vibrio* spp. was observed in all winter samples. These results suggest that although oysters can concentrate bacteria from the surrounding waters by their filter feeding ability, they are also able to eliminate bacteria functioning as filtering and depurating animals.

Keywords: Pacific oyster, production area, faecal contamination, bacterial indicators, microbiome,

Exploring biodiversity in microbial ecosystems along the food chain

O1.10.

Stress tolerant biofouling communities on RO membranes used for treatment of whey water in a dairy industry

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Whey has changed from being a side stream to being a valuable source of dairy ingredients. After recovering the valuable fractions, the remaining liquid may also be used after treatment for various in-factory purposes in order to decrease both intake of drinking water and factory water discharge. Reverse Osmosis (RO) filtration is extensively used in the food industry for treatment and water recovery. The surfaces of the membranes will eventually experience biofouling. In this study, RO membranes used for after-treatment of ultra-filtrated whey water in dairy industry were sampled after Cleaning-In-Place (CIP) application to investigate the ecology of the biofilm communities on both the retentate and permeate side. Light and Confocal Laser Scanning Microscopy (CLSM) were used to visualize the biofilm on direct samples, while 16S, 26S and ITS rRNA sequencing were used for identification. A dense multipecies biofilm consisting of filamentous yeast, budding yeast and gram negative bacteria was observed on the RO membranes from the same water recovery process line. To our surprise, the filamentous yeast was also found on the permeate side of the RO elements. Microscopically, these yeast isolates seemed - due to the large surface coverage of the filamentous cells - to be the primary, physical component of the biofilm. They were found to be tolerant towards typical CIP cleaning and also displayed considerable heat resistance. Their hyphae may initiate attachment and spreading on membrane surface, being a harbor for budding yeast and bacteria. Since neither cfu numbers nor DNA based methods reflect the physical importance of these filamentous yeasts, their role in biofilm formation and membrane fouling could be underestimated. In order to elucidate their role, it is suggested that further research on membrane microbial communities include methods for filamentous yeast detection.

Keywords: filamentous yeast; reverse osmosis membrane; biofilm; water re use; dairy

Ecology and interactions in food-associated microbial communities

O2.1.

Intraspecific interference competition and genetic diversity in *Carnobacterium maltaromaticum*

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Carnobacterium maltaromaticum is a lactic acid bacterium of great interest in the field of biopreservation. Several strains belonging to this species are indeed capable of inhibiting the food-borne pathogen *Listeria monocytogenes* by interference competition, *via* the production of bacteriocins. While the impact of inhibitory properties on food-borne pathogens is being widely documented, few studies have focused on the ecological impact of interference competition on food bacteria. The aim of our study was to assess the extent of intraspecific interference competition in the species *C. maltaromaticum* and the link with population diversity. In this respect, a high-throughput interaction assay was performed to screen for pairwise antagonistic interactions between 76 *C. maltaromaticum* strains. This led us to investigate 5776 pairwise interactions where each strain of the tested collection underwent two situational simulations: that of the sender and that of the receiver strain. The results showed that intraspecific competition is major in *C. maltaromaticum* species, with approximately 60% of the sender strains inhibiting the growth of at least one receiver strain. In addition, the screened antagonistic interactions presented a high diversity in terms of width of activity spectra, width of sensitivity spectra, and target specificity. This suggests a high diversity of the produced antagonistic substances and of the resistance mechanisms. Besides, the population structure was established by MultiLocus Sequence Typing (MLST) and exhibited a high level of diversity. All these results reveal that interference competition plays a major ecological role in the species *C. maltaromaticum* and are in agreement with models predicting that competition is compatible with genetic diversity.

Keywords: Carnobacterium maltaromaticum, biopreservation, interference competition, population structure

Ecology and interactions in food-associated microbial communities

O2.2.

Interaction of *Aspergillus flavus* and *A. parasiticus* with *Salmonella* spp. isolated from peanut

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Although *A. flavus* and *A. parasiticus* are the main microorganisms of concern in peanuts, several *Salmonella* outbreaks in this product have been reported in the last ten decades. Thus, it is important to establish a strategy to control these microorganisms in order to understand their interaction in the peanut supply chain. The purpose of this study was to evaluate the co-cultivation of *Aspergillus* section *Flavi* and *Salmonella* isolated from peanuts. Three strains of *Aspergillus* section *Flavi*: *A. flavus* producing aflatoxin B; *A. flavus* non producing aflatoxin and *A. parasiticus* producing aflatoxin B and G were co-cultivated with seven serotypes of *Salmonella* (*S.* Typhimurium ATCC 14028, *S.* Muenster, *S.* Miami, *S.* Glostrup, *S.* Javiana, *S.* Oranienburg and *S.* Yoruba). With the exception of *S.* Typhimurium, all the strains were isolated from peanut samples. Each *Salmonella* strain was inoculated separately by using pour plate technique (*ca.* 5 log cfu /mL) in PDA (potato dextrose agar). After agar solidification, each pre-cultured fungus was inoculated in the center of a 90 mm petri dish. The plates were incubated at 30° C and the fungal colony diameter was measured once a day for 7 days. In addition, each *Aspergillus* strain was cultivated in the absence of *Salmonella* culture as a control. After 7 days, the aflatoxin production was determined by using the agar plug technique on thin layer chromatography (TLC). All three strains of *Aspergillus* reached the maximum growth as pure cultures, being significantly different ($p < 0.05$) from the co-cultures. The minimum fungal growth was observed in co-cultivation with *S.* Oranienburg 7 days later. The colony diameter was 69 mm for *A. flavus* aflatoxin B producer; 70 mm for *A. flavus* non producer; and 66 mm for *A. parasiticus*. However, no significant difference ($p < 0.05$) was observed among the other *Salmonella* serotypes. For the aflatoxin production test, both strains of *A. flavus* preserved their characteristics of toxin production after co-cultivation with *Salmonella*. On the other hand, *A. parasititicus* did not produce aflatoxin G in co-cultivation with *Salmonella*. These results suggest that the interaction of *Salmonella* and *A. parasititicus* interferes with the aflatoxin production.

Keywords: co-cultivation, Aspergillus section Flavi, Salmonella

Ecology and interactions in food-associated microbial communities

O2.3.

Study of the inhibition mechanism of the foodborne pathogen *Listeria monocytogenes* by *Lactococcus piscium* using proteomic and transcriptomic approaches

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Lactococcus piscium CNCM I-4031 is a psychotrophic lactic acid bacterium which presents an interesting potential as bio-preservative in seafood products owing to its inhibition property against foodborne pathogen *Listeria monocytogenes*. The mechanism of growth inhibition of *L. monocytogenes* by *L. piscium* remains to be established. However a previous study demonstrated that cellular contact is required to obtain the antagonistic activity of *L. piscium* against *Listeria*. In this setting, we performed label-free quantitative proteomics and RNA-seq based transcriptomic analysis of *L. piscium* CNCM I-4031, in monoculture and in mixed culture with *L. monocytogenes*. Both culture conditions were performed in chemically defined medium (MSMA) based on shrimp composition and reproducing the inhibition observed in shrimp. The combination of both approaches allowed us to determine the up regulation of a set of genes involved in bacterial late competence and peptidoglycan hydrolysis. These genes that stand out from the rest of the proteome and transcriptome could probably be involved in the contact dependent growth inhibition of *L. monocytogensis* by *L.piscium.* But further investigations are needed.

Keywords: Lactococcus piscium, Listeria monocytogenes, inhibition mechanism , proteomic, transcriptomic

Ecology and interactions in food-associated microbial communities

O2.4.

Ecology of *Salmonella* and antimicrobial resistance in a pig slaughterhouse

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Salmonella is a bacterial pathogen responsible for a large number of food associated infections with an estimate of 666 cases per million of inhabitants in France per year. In order to guarantee food safety, a better understanding of *Salmonella* ecology and adaptation strategies in the food production chain constitutes a prerequisite. In a *One Health* perspective, data on *Salmonella* antibiotic resistance in food environments are also crucial to decipher transmission routes of resistant foodborne pathogens as well as resistance genetic determinants involved, and the role of process and selection pressures (as cleaning and disinfection) in bacterial adaptation and antimicrobial resistance emergence.

Using a pig slaughterhouse as a model food environment, occurrence of *Salmonella* was investigated at six different areas along the slaughter chain and through 4 sampling campaign, each time before and after cleaning and disinfection procedures (a total of 48 surface samples). Characterization of isolated *Salmonella* strains using serotyping and PFGE typing enabled to identify and trace persistent strains in the slaughterhouse. Minimal inhibitory concentrations (MIC) were also determined for various relevant antibiotics and for biocides used in the slaughterhouse. In addition, associated indigenous bacterial communities were characterized using 16S rRNA amplicon sequencing.

Results revealed the presence of *Salmonella* at all sampling areawith five serotypes: S.4,5,12:i:- (50%), Rissen (16%), Typhimurium (16%), Infantis (10%) and Derby (8%). Approximately 70% of isolated *Salmonella* strains exhibited resistance to ampicillin and sulfamethoxazole, 80% to tetracycline and 10% to chloramphenicol. Bacterial diversity analyses showed that populations in slaughterhouse were highly dominated by ɣ-proteobacteria and especially by the Moraxellaceae family (genus *Psychrobacter*, *Moraxella*, *Enhydrobacter* and *Acinetobacter*) at the different sampling areas. Population compositions were overall stable in time at a given sampling area suggesting that the surface populations are resident populations within the slaughterhouse, rather than populations introduced weekly by the new swine bands to be slaughtered.

Together, such data participate to the construction of a comprehensive view of *Salmonella* ecology in food environments integrating associated microbial flora and the distribution of antimicrobial resistance in relation to processing conditions.

Keywords: Salmonella, antibioresistance, cleaning and disinfection, microbial ecology, pig slaughterhouse

Ecology and interactions in food-associated microbial communities

O2.5.

Deterministic heterogeneity drives differential inactivation dynamics within clonal populations of foodborne pathogens

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Even under homogeneous environmental conditions isogenic cells within a clonal bacterial population tend to display intercellular differences that can result in differential behavior. An important example of such phenotypic heterogeneity in the context of food preservation and safety is the typical observation that not all cells within a clonal population are equally sensitive to a stressful encounter, with the underlying reason or mechanism often being elusive. However, using time-lapse fluorescence microscopy to scrutinize individual cellular fates within stressed populations of *Escherichia coli* and *Salmonella* Typhimurium, we found several cellular markers whose distribution within the population correlates well with individual cellular survival chances. Further study of these markers reveals that the seemingly stochastic variability in survival among (isogenic) cells of stressed (clonal) populations of foodborne pathogens can often be deterministically explained and predicted by mechanistically tractable intercellular differentiation processes.

Keywords: Salmonella Typhimurium, population heterogeneity, stress response, survival

Ecology and interactions in food-associated microbial communities

O2.6.

Increased content of non-fatty acid lipids modulates membrane fluidity in food pathogens and food spoilage microorganisms at low temperatures

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For different bacterial isolates from food we found an increased amount of lipophilic compounds in their membranes at low temperatures (10/6°C) in comparison to mesophilic temperatures (37/30°C). Based on this observation we suspected a fatty acid-independent membrane modification mechanism at low temperatures. We tested the impact of increased isoprenoid quinone content in 10 *Listeria monocytogenes* strains and two *Paenibacillus glucanolyticus* strains at low temperatures. Elevated carotenoid content was examined for two *Staphylococcus xylosus* as well as two *Micrococcus luteus* strains.
Quantitative and qualitative analyses of isoprenoid quinones and carotenoids revealed a clear increase of these lipophilic compounds under low temperature growth conditions. In the *L. monocytogenes* strains, we were able to show a negative correlation between the extent of their fatty acid adaptation and their menaquinone content at 6°C. The supporting function of menaquinones and carotenoids in membrane fluidization for the species mentioned above was confirmed by *in vivo* membrane fluidity analyses by measuring generalized polarization and anisotropy with the fluorescent dyes laurdan and TMA-DPH, respectively.
Strains with increased quinone or carotenoid concentration showed an expanded membrane transition phase which resulted in more fluid membranes at low growth temperatures and more stabilized membranes at higher temperatures. Additionally for *L. monocytogenes* strains, the correlation between menaquinone concentration and membrane transition phase expansion was confirmed by suppression of the quinone synthesis. This finding revealed an additional mechanism improving the adaptation to temperature shifts for *L. monocytogenes* strains.
This finding could improve the understanding of food spoilage microorganisms and pathogens and their membrane adaptation in chilled food. Fluid membranes pose a key function in bacterial survival at low temperatures and the tested lipophilic compounds could support their ability to maintain membrane fluidity by lipid composition adjustment. For example *Listeria monocytogenes* is well known as a food pathogen capable of growing at a broad temperature range, from 50°C down to -2°C. This outstanding temperature-range for growth could probably be enabled by the increased menaquinone content.

Keywords: Low temperature adaptation, isoprenoid quinones, carotenoids, membrane fluidity, food-borne pathogen

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O2.7.

Natural encapsulation of beneficial probiotic bacteria in extracellular matrix from biofilm-forming *Bacillus subtilis*

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Probiotics, which are live microbial supplements, are often incorporated into foods and beverages to provide putative health benefits. To ensure their beneficial effects, these organisms must survive processing and storage of food, its passage through the upper gastrointestinal tract (GIT), and subsequent chemical ingestion processes until they reach their target organ. However, there is considerable loss in viability of probiotic bacteria in the acidic conditions of the stomach and the high bile concentration in the small intestine. *Bacillus subtilis*, a spore-forming non-pathogenic bacterium, recently has gained interest in its probiotic properties; it effectively can maintain a favorable balance of microflora in the GIT. In addition, *B. subtilis* produces an extracellular matrix that protects it from stressful environments. We suggest that the extracellular matrix produced by *B. subtilis* could protect other probiotic bacteria and therefore potentially could be used as a vehicle for delivering viable probiotic cells to humans. Therefore, we have developed a novel cultivation system that enables co-culturing of *B. subtilis* along with probiotic lactic acid bacteria (LAB) by increasing production of the extracellular matrix by *B. subtilis* cells. Moreover, we showed that *B. subtilis* improved survivability of LAB during food preparation, storage and ingestion. Consequently, we believe that the results of our study will provide a novel technique of using a natural system for preservation and delivery of probiotics to humans.

Keywords: Beneficial bacteria, B. subtilis, biofilm formation, probiotics, extracellular matrix

Ecology and interactions in food-associated microbial communities

O2.8.

Improving bacterial spoilage prediction by multi-omic analyses and statistical modeling of metabolic activities among synthetic communities

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In Europe, about 20% of the meat produced is wasted, mainly because of early spoilage or wrong estimation of spoilage time. The lack of good models to predict meat spoilage is due to the versatile diversity of the bacterial communities growing on meat and secondly, because of the intricate metabolic interactions causing organoleptic defects (texture, color, odor, aspect). The goal of this work, which is part of the REDLOSSES project, funded by the French National Research Agency, was to establish a strategy combining multi-omics analysis and *de novo* construction of synthetic spoilage bacterial communities based on comparative genomics of sequenced strains. We also carried out the construction of a simplified raw pork sausage matrix on which we inoculated our synthetic communities and formulated various highly controlled environmental conditions of the meat process (lactate concentration and type of packaging). To construct the synthetic bacterial community, we first analyzed by 16S rRNA and *gyrB* amplicon sequencing the diversity and dynamics of bacterial communities among a wide range of raw pork sausages samples at spoilage time. We then selected 12 species from the core communities and built up a simplified community from selected strains with known genome sequence. Based on comparative genomic analysis on putative spoilage promoting metabolic pathways, these 12 species were separated into 3 different groups of spoilers, whether they were involved in the potential production of biogenic amines, organic acids or volatiles spoilage compounds. The community was inoculated on sterile synthetic meat matrix and incubated for several days at 8°C. To model the influence of each factor (i.e. packaging type and lactate concentration), a composite central design was carried out and used to estimate the role of each sub-population in the production of spoilage and how abiotic parameters influences some bacterial species more than others. The expression of eighty genes implied in metabolic functions associated with spoilage was profiled by qRT-PCR. A metabolomic study by UHPLC-HRMS was conducted to monitor quantitatively more than fifty metabolites. First results revealed that both factors greatly influenced metabolic profiles associated to meat spoilage. Major population shifts were also observed among *Firmicutes* and *Proteobacteria* phyla. Correlation could be made between these ecological changes, genes expression and metabolites production.

Keywords: Meat spoilage, qRT-PCR, metabolomics, composite central design, bacterial function modeling

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Ecology and interactions in food-associated microbial communities

O2.9.

The surface smear microbiome of red-smear cheese and its role in the development of defective smear after vacuum film pre-packaging

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Surface-ripened cheese is characterized by the development of an orange-red pigmented smear layer representing a complex microbial ecosystem. Today cheese is increasingly sold as vacuum film pre-packed cheese portions. However, vacuum film pre-packaging of surface-ripened cheese is often accompanied by undesired changes of olfactory characteristics and consistency of the cheese surface smear. Due to the lack of scientific knowledge, the development of defective smear during storage of pre-packed cheese is not predictable and no preventive measures are available.

The aim of this study was to analyze and compare the composition of the surface smear microbiome of vacuum film pre-packaged cheeses with good quality smear and defective smear. For this purpose, a 16S rDNA and rRNA-based next-generation sequencing analysis was performed and the results were compared with data from a previously conducted culture-based study. The 16S rDNA-based approach revealed a highly diverse microbiome composed of 132 bacterial genera representing typical smear bacteria, as well as bacterial taxa so far not associated with the cheese ecosystem. The numbers for typical smear bacteria such as *Staphylococcus*, *Brevibacterium*, *Brachybacterium* and *Agrococcus* revealed a decline after vacuum pre-packaging, whereas non-enteric Gram-negative bacteria turned out to be increased in the surface smear of pre-packaged cheese. This distinct shift in bacterial populations was in particular pronounced in samples from pre-packaged cheeses showing defective smear. The alterations observed for defective smear from pre-packaged cheeses were further supported by the results from the rRNA-based transcriptome analysis, which indicated to a reduced metabolic activity for *Brevibacterium*, *Brachybacterium* and *Agrococcus.* The increase in reads from *Halomonas* and *Psychrobacter* pointed to a rise in their metabolic activity, which was again pronounced in defective smear from pre-packaged cheese. As some *Halomonas* spp. are known to represent strong exopolysaccharide producers and *Psychrobacter* spp. are known to generate volatile sulfur compounds, bacteria from these genera may have the potential to contribute to the observed smear defects. Thus, the further investigation of their role in the smear of surface-ripened cheese and their potential contribution to the development of defective smear will be the subject of future research.

Keywords: surface-ripened cheese, defective smear, transcriptome analysis, next-generation sequencing,

Ecology and interactions in food-associated microbial communities

O2.10.

Viability and physiological responses of yeasts exposed to stress conditions of West African fermented cereal doughs

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This study investigated the viability and physiological responses of twelve yeast strains including three *Saccharomyces cerevisiae* strains (Sc1, Sc2 and Sc3), three *Candida glabrata* strains (Cg1, Cg2 and Cg3), three *Kluyveromyces marxianus* strains (Km1, Km2 and Km3) and three *Pichia kudriavzevii* strains (Pk1, Pk2 and Pk3) all isolated from fermented cereal doughs in Benin. The yeast strains were exposed to individual stress factors of cereal doughs [low pH 3.4, ethanol concentration 3% (EtOH), total lactic acid concentration 285 mM (LA) and total acetic acid concentration 150 mM (AA)] and to the combinations of these stress factors (LA+AA and LA+AA+EtOH); all stress conditions were adjusted to pH 3.4 using hydrochloric acid, 2N. Single cells were studied for cell viability, intracellular pH (pH_i) and micro-colony development by flow cytometry combined with fluorescence microscopy using propidium iodine, SYTO13 and carboxyfluorescein diacetate succinimidyl ester. The combined approach provided information regarding population heterogeneity when exposed to the stress conditions. Additionally, the fluorescence microscopy provided information about the pH_i recovery and the time required for stressed single cells to resume proliferation when transferred in optimal growth condition (MYGP, pH 5.6). The individual effects of low pH (3.4) and ethanol (3%) seemed not significant on cell viability. For all strains, lactic acid (285 mM) induced prolonged lag time. However, acetic acid (150 mM) and the combinations of the stress factors were so inhibitory that no growth was observed over 24 h periods. *S. cerevisiae* strains were more resistant followed by *P. kudriavzevii* strains, whereas *C. glabrata* and *K. marxianus* were more sensitive. When the most resistant *S. cerevisiae* strain (Sc2) and the most sensitive *K. marxianus* strain (Km1) were stressed in the combined stress factors (LA+AA+EtOH) for 6 h and further transferred to optimal growth condition, 45.2 % of single cells of the most resistant strain Sc2 was able to recover their pH_i to near physiological range and therefore, was able to resume proliferation after 3-24 h of incubation. However, the stressed single cells of the most sensitive strain Km1 were killed and could not recover the pH_i or repair their membrane when transferred to optimal growth condition. This study demonstrated that *S. cerevisiae* strain Sc2 is a relevant candidate in development of starter cultures for fermented cereal doughs production.

Keywords: Yeasts, intracellular pH, flow cytometry, fluorescence microscopy

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State of the Art techniques for the analysis of biodiversity and microbial interactions of foodborne microbes

O3.1.

Limits of detection of next-generation sequencing in food microbiology

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Next generation sequencing (NGS) is increasingly being applied to food microbiology due to its untargeted nature and ability to probe non-culturable organisms, as well as its potential to give genomic information about the organisms found. Recent studies report a breadth of microbes associated with fresh produce, but very few have utilised NGS to detect human pathogenic organisms.

The purpose of this study was to explore the limits of detection of a number of NGS technologies for the foodborne bacterial pathogen *Salmonella* and phage MS2, which is a surrogate for the viral pathogen norovirus, in a background of lettuce homogenate. Methods examined were RNA-seq, using both the Illumina ScriptSeq and NEBNext kits, and 16S amplicon sequencing, using the Illumina MiSeq platform. The Scriptseq methodology had the most sensitive limit of detection (LoD), being able to detect *Salmonella* at 10^4 CFU and MS2 at 10^5 PFU. The NEBNext, although a similar methodology, had the least sensitive LoD, detecting both *Salmonella* and MS2 at 10^6 CFU / PFU respectively. 16S amplicon-sequencing gave an LoD of 10^5 CFU of *Salmonella*, with similar numbers of sequences, at 10^5, to those seen with the Scriptseq methodology. Due to the targeted nature of the methodology, 16S amplicon-sequencing cannot detect MS2. All methodologies had a high level of lettuce co-sequenced.

The detection limits found are less sensitive than currently used methodologies and the concentrations of pathogens usually reported for fresh produce, and therefore cannot be used as a screening tool for human pathogens. It is anticipated that this work will inform future experiments on the potential uses of NGS within food microbiology and the issues surrounding the detection of species at low level concentrations within a complex, microbiologically diverse, matrix.

Keywords: Next-Generation sequencing, Food microbiology, microbiome, lettuce, Salmonella, MS2 phage

State of the Art techniques for the analysis of biodiversity and microbial interactions of foodborne microbes

O3.2.

Quick genome analysis using TORMES

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Microbial Genomics is increasing widely by the use of High-Throughput Sequencing (HTS) technologies and bioinformatics, adapting to the different software available already for the users that in many cases requires great computing resources and skills, making it challenging for non-bioinformaticians researchers.
We present TORMES, a free, user-friendly software for conducting quick genome analysis without the need of advanced computing resources or skills. TORMES is a pipeline that wraps several software and processes: quality filtering of raw reads, assembly, species identification, MLST, antibiotic resistance and virulence genes search, genome ordering and annotation and pangenome-comparative analysis of samples. Results are then used in R environment to render an interactive web-like report. Special analysis for specific genera are available by enabling the --genera option: "Salmonella" (serotyping, point mutations that confers resistance to antibiotics and plasmid search) and "Escherichia" (serotyping, fimH-typing, point mutations that confers resistance to antibiotics and plasmid).
TORMES was used to analyze the genome of 10 Salmonella isolated from several food samples confiscated from passengers arriving on flights from non-European countries at the International Airport of Bilbao (Spain). The quick identification of bacteria that could harbor antibiotic resistant or virulence genes in mobile genetic elements is critical to control the potential source of foodborne pathogenic agents that are introduced to Europe.
TORMES analysis of the 10 isolates was performed and lasted 1 hour and 20 minutes using a 124GB RAM 32 cores computer. Samples were found to be *Salmonella enterica* subspecie enterica and belonged to 6 different ST (4, 11, 23, 45, 64 and 279) and 6 serotypes (Anatum, Enteritidis, Montevideo, Newport, Oranienburg and I 4,[5],12:d:-). All isolates had genes involved in the multidrug and metal efflux pump complex MdsABC (*mdA*, *mdsB*, *mdsC* and *golS*). 3 samples had de *aac(6')*-Iaa that is involved in aminoglycoside resistance, whereas 2 also harbored *qnrB5*, involved in quinolone resistance. The other 7 harbored *aac(6')*-Iy involved in aminoglycoside resistance. 3 samples harbored *cdtB* that encodes the cytolethal distending toxin B that is involved in chromatin disruption and cell death.
TORMES can be efficiently applied to perform quick genome analysis without the use of web servers or high computing resources.

Keywords: bioinformatics, bacterial genome analysis, DNA sequening, pipeline, software

State of the Art techniques for the analysis of biodiversity and microbial interactions of foodborne microbes

O3.3.

An information technology based platform for food safety and quality management systems

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Currently, consumers demand constant reassurance of the origin, quality and compliance to label of the food products they purchase. Therefore, food industries, retailers and authorities have to develop advanced, effective and relatively low-cost solutions for quality and safety assurance as well as fraudulent practices. In this context, various approaches focus on: (a) metabolomics, (b) the application of advanced data analysis and machine learning strategies, and (c) Information technologies.
In recent years, web-based applications and online data repositories have played a major role in advancing food-related research, by providing scientists and researchers with means of knowledge sharing, communication and data exchange, regardless of their dispersed geographical locations. The Symbiosis Online Research Framework-Machine Learning (SorfML) was developed to provide the food research community with an interactive web application for data management, exchange, visualization and analysis. SorfML supports a wide variety of experimental platforms thanks to its generic entity-attribute-value (EAV) database backend. The generic nature of the EAV allows the framework to manage heterogeneous data types without the need to provide separate storage modules for every experimental platform applied. The SorfML makes data management straightforward despite the complex relationships typically found in food research conducted on a systems-level, where information is collected on a multiple knowledge level.
The SorfML front end, uses the latest web technologies to allow users to upload, query, browse, and visualize data through a series of data views, exploratory data analysis tools and relational visualizations. The application is currently being used as a central data repository for various EU (eg PhasmaFOOD), national (NovelEYE, QAPP) projects, aiming to provide novel methodologies for assessing freshness of animal and plant origin products. Indeed SorfML is tested on beef fillets, minced beef, rocket salad, stored under aerobic and MAP, analyzed with diverse instruments such as e-nose, HPLC, FT-IR, GC-MS, and Multispectral imaging. Populations of TVC, LAB, pseudomonads, Enterobacteriaceae & *B. thermosphacta* were predicted. As a result, recommendations were obtained for the suitability of each analytical instrument and machine learning approach to predict the counts of each bacterial group.
This work has been supported by the project “PhasmaFOOD

Keywords: Machine learning, Infomration technologies, omics, management system

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O3.4.

Rethinking the *Bacillus cereus* group in the age of whole-genome sequencing: Novel insights into phylogenetic topology, taxonomy, and virulence potential

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The *Bacillus cereus* group is populated by numerous closely-related species, including foodborne pathogen *B. cereus sensu stricto*, bioterrorism agent *B. anthracis*, and biopesticide *B. thuringiensis*. While efforts to characterize the group using whole-genome sequencing (WGS) have only recently been undertaken, the amount of publicly-available data for these organisms has increased dramatically in recent months; for example, between April 2017 and March 2018, the number of *B. cereus* group genome assemblies in the National Center for Biotechnology Information Reference Sequence database has more than tripled. Furthermore, the number of published *B. cereus* group species has doubled since 2016. With the rapid and very recent increase in WGS data for the group, understanding of the group's taxonomy, virulence potential, and phylogenetic topology have lagged behind. Here, we present the most up-to-date, genomics-based portrayal of the *B. cereus* group. We applied BTyper, a tool for rapidly characterizing *B. cereus* group genomes using a combination of virulence- and phylogenetic-based markers, to over 2,000 *B. cereus* group genomes. All genomes underwent *in silico* (i) virulence gene detection, (ii) multi-locus sequence typing (MLST), (iii) *panC* clade assignment, (iv) *rpoB* allelic typing, (v) 16S rDNA gene identification, and (vi) antimicrobial resistance (AMR) gene detection. In addition to providing updated distributions of virulence and AMR genes present in the group, we produced a phylogeny that revealed numerous genomes that fell well outside of the 7-clade scheme that is currently used for characterizing the group. 16S rDNA gene sequences for these outgroup genomes were more than 97% identical to previously published *B. cereus* group species type strains, indicating that, while they are still members of the *B. cereus* group, they may be novel species. To identify potential new *B. cereus* group species, FastANI's implementation of the average nucleotide identity (ANI) metric was used to compare selected genomes to the type strains of all 18 published *B. cereus* group species, as well as genomes from three proposed *B. cereus* group species. Using the accepted threshold of 95% ANI as a species cutoff, 20 potential new *B. cereus* group species were identified. These results indicate that the 7-clade *B. cereus* group as it is currently portrayed may need to be revised to accommodate more diversity as WGS becomes a routine method for characterizing these organisms.

Keywords: Bacillus cereus group, whole-genome sequencing, virulence, antimicrobial resistance, taxonomy

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O3.5.

Towards the discrimination of closely related species from *Bacillus cereus* group using MALDI-TOF MS proteomic profiles – Or how to find a needle in a haystack!

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Bacillus cereus sensu lato, also known as the *B. cereus* group, includes closely related Gram-positive, spore-forming and aerobic bacilli, widely distributed in the environment and food matrices. Besides characteristic colonies on Mossel agar, these species exhibit highly divergent properties and their distinction remains challenging. Presently their classification rely mainly on distinctive phenotypic traits, such as pathogenic potential to mammals (*B. anthracis*, *B.cytotoxicus*, emetic/diarrheic strains of *B. cereus*) and insects (*B. thuringiensis*), enzymatic ability causing food spoilage (*B. weihenstephanesis*, *B. wiedmannii*), thermotypes, as well as colony morphology (*B. (pseudo)mycoides*). Recently, *Bacillus toyonensis, Bacillus manliponensis*, *Bacillus gaemokensis* and *Bacillus bingmayongensis* have been recognized as plausible members of this group.

The aim of this study is to investigate the use of MALDI-TOF Mass Spectrometry to characterize and distinguish *B. cereus* contaminations.

Because available databases do not cover such wide diversity, a collection of 120 strains representative of *B. cereus* group diversity were characterized by the presence of toxin encoding genes and presence of parasporal crystalline inclusions, the phylogenetic classification, as well as by the acquisition of molecular fingerprints and proteomic profiles. More specifically, overnight grown isolates were analysed by MALDI biotyper using the standard direct transfer sample preparation procedure. Biostatistical analyses were performed using different clustering tools.

Creation of the main spectra profiles were made according to manufacturer quality controls and procedure for our strain collection. Calculated composite correlation index matrices show clear match with Guinebretiere phylogenetic groups and sub-groups. Identification of relevant peaks is under process for tentative biomarker assignment. Furthermore, additional strains of B. cereus will be tested to validate our database.

Preliminary data are promising and the use of MALDI-TOF MS seems interesting as a reliable, easy to use and fast laboratory-based analysis tool for the distinction between strains of *B. thuringiensis* subspecies approved in Europe for crop protection, spoilage *B. weihenstephanensis* or highly pathogenic *B. cytotoxicus* in less than 30 min.

Acknowledgement to BtID CASDAR project and consortium aiming at distinction and traceability of Bt contamination from farm to fork.

Keywords: MALDI, proteomic profiles, B. cereus, B. cytotoxicus, B. thuringiensis, biodiversity

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O3.6.

Molecular typing of Listeria *monocytogenes* in isolates from foodstuffs and food-processing plants in Germany

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Listeriosis, caused by the bacterium *Listeria monocytogenes (Lm)*, has the highest proportion of hospitalised cases of all foodborne zoonoses under EU surveillance. Human infections mainly occur in elderly, pregnant and immunocompromised people after consumption of contaminated foodstuffs. Its high case fatality rate renders listeriosis a major public health concern. For effective surveillance and disease control, comprehensive molecular typing of *Lm* isolated from food, food-processing plants and humans is indispensable. Still, the analytical basis for that purpose needs to be improved.

In the MolTypList[1] project, *Lm*-isolates from ready-to-eat food products and isolates from food-processing plants sent to the German National Reference Laboratory (NRL) for *Lm* in 2016 were analysed by pulsed-field gel electrophoresis (PFGE) and by whole genome sequencing (WGS). The current gold standard, PFGE, and WGS were compared as typing tools and representative typing data were generated. Both methods revealed diverse *Lm*-strains in Germany and clusters based on epidemiologically linked isolates. However, resolution varied markedly and was clearly in favour of WGS-based typing.

Within various WGS-based clusters, a very close genetic relationship between *Lm*-isolates from food products sampled on different stages of the product's life span (manufacturer, distributor) or between isolates from food product and food-processing plant could be proven. This reflects the way of *Lm*-contamination in food production and retail systems. Furthermore, genetic relatedness of isolates sampled at different time points possibly gives a hint to persistence of specific genotypes in food-processing plants, thus highlighting the need for improved hygiene measures. Main differences appeared between *Lm*-isolates of the food categories fish products and meat products. While *Lm*-isolates from fish products supra-regionally showed a close genetic relationship, isolates from meat products appeared to be more diverse.

Our WGS-database did not only shed light on the population structure of *Lm*-isolates from foodstuffs and food-processing plants in Germany, it also provided the key to tracing back more than 70 human cases in a protracted listeriosis outbreak to a contaminated food product and its manufacturer and thus outbreak clarification.

[1]This project is supported by a grant of the Federal Ministry of Health (GE20160326) within the framework of the German Research Platform for Zoonoses.

Keywords: Listeria monocytogenes, molecular typing, whole genome sequencing, population structure, trace-back

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O3.7.

Deep metatranscriptomic sequencing indicates stable microbial community across seasons and suppliers for protein meal factory ingredient

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High-throughput sequencing of food samples can simultaneously reveal, in an untargeted way, multiple important details about the microbial community and food ingredients in the sample. For example, observing the presence of toxin producing genes or allergens is critical for food safety and consumer health. Metatranscriptome deep sequencing of food samples is a novel application area aiming to reveal the active microorganisms and predict their functions in the sample. We investigated 31 poultry meal metatranscriptomes with total RNA high-throughput sequencing. The samples were collected as part of regular monitoring of a pet food factory raw ingredient to establish a baseline and describe the typical microbiome of these samples.

Starting from input of hundreds of millions of sequenced reads per sample, we observe between 460 and 751 microbial genera per sample as well as the food ingredient host composition. The core poultry meal metatranscriptome appears remarkably stable across batches sampled from different vendors during a one year period, with *Bacteroides, Clostridium, Lactococcus, Aeromonas,* and *Citrobacter* being the most abundant. When characterizing the baseline, we observe three samples with additional non-poultry meal food matrix content. This unexpected and noticeably different content would have remained undetected without shotgun sequencing. These samples also differ from the baseline in their microbial community composition and cluster separately from the other samples using distance-based metrics.

Several challenges exist in studying metatranscriptomes— from molecular depletion of host content in order to amplify the faint microbial signal, to developing robust bioinformatics pipelines for accurately quantifying the microbial community. Our ongoing research in this space include automated outlier detection, robust sample comparisons and visualizations, and differential microbial abundance estimation. Within the Consortium for Sequencing the Food Supply Chain, we have built an automated pipeline to process raw sequencing read files to understandable microbial and food matrix profiling results. This enables understanding of food safety hazards and quality issues that may arise in the supply chain by detecting deviations from the normal baseline microbial profile. By surveilling the microbiome of food ingredients, we can develop methods and best practices that can be used to improve food testing standards and security of the food supply chain.

Keywords: Metatranscriptomics, Food microbial community, Food authentication, Bioinformatics pipeline

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O3.8.

Microbial diversity and functional diversity in lowtemperature Daqu microbial community revealed by metagenomics

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Although traditional starter cultures are used for the production of fermented food for centuries, the fermentation mechanisms of microbial communities within traditional starter cultures still remain unexplored. Low-temperature *Daqu* is a typical traditional fermentation starter for the production of light flavor Chinese liquor. Complex community structure contributes to diverse functions within the community including saccharification, fermentation, and production of flavor substances. In this study, we investigated both microbial diversity and functional diversity of low-temperature *Daqu* to determine the functional species in the community.
We collected smashed ready-to-use low-temperature *Daqu* for amplicon sequencing and shotgun metagenomic sequencing. Taxonomy analysis was conducted with the Silva 16S rRNA database and NCBI nr database. KEGG and CAZyme databases were used for functional annotation.
In low-temperature *Daqu* community, 43 bacterial genera and 13 fungal genera were presented by amplicon sequencing. A total of 1847 species of 781 genera were detected by shotgun metagenomics. *Lichtheimia*, *Lactobacillus*, *Rhizopus*, *Leuconostoc* were extremely abundant with the abundance of 23%, 18%, 17% and 16% respectively. Based on the KEGG and CAZyme annotation, 10 genera were revealed as functional microorganisms related to the production of amylase, protease, and esterase. *Lichtheimia*, *Lactobacillus*, *Rhizopus,* and *Bacillus* were presented with the abilities of starch degrading, protein degrading and aroma producing in low-temperature *Daqu*. *Leuconostoc*, *Pichia,* and *Streptomyces* had the potential to produce protease, flavor components and precursors. *Lactobacillus plantarum*, *Lactobacillus paralimentarius*, *Lichtheimia ramose*, *Lichtheimia corymbifera*, *Rhizopus delemar*, and *Bacillus licheniformis* were the key functional species.
Although low-temperature *Daqu* was a complex community with 781 genera, the total abundance of 11 dominant genus was 93.05%, which indicates that low-temperature *Daqu* is a diverse but relatively concentrated microbial community. Based on the functional analysis, fungi plays an important role in the community. The results of six functional species can be the foundation for recreating the community and industrial production of *Daqu.* Further investigation of possible microbial interactions among functional species would reveal more insights for in vitro community reconstruction.

Keywords: Daqu, Shotgun metagenomics, Microbial diversity, Functional diversity

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O3.9.

Characterization of new food species and potential symbiotic interactions by metagenomics

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Traditional fermented food products contain a large variety of microorganisms, whose origin although not completely defined, stems from the raw material itself, the recipient/tools used to collect and process the raw materials, and from backslopping. Initially, their microbial composition was determined by culture dependent methods giving an approximate idea of present species, although often biased by lack of appropriate culture media. At present, Metabarcoding, a method based on the amplification of markers such as 16S for bacteria and ITS for fungi is widely used. It provides taxonomic information at genus and in certain cases species level. However, metagenomics is a more powerful method. We used it to explore microbial diversity of number of food products using HiSeq sequencing (~15 M paired reads) processed by an in-house INRA developed bioinformatics tool, Food-Microbiomes. This tool combined with metagenomics read assembly allows analysis at the strain level and genome reconstruction of dominant strains. Interestingly, although European dairy products were extensively studied, this approach allowed evidencing new food species or frequent occurrence of poorly described species.
In the present communication, we will present studies focused on traditional African fermented products, which were still poorly explored. The first one is sour wort, which is the first step of Tchepalo processing (sorghum based African beer) but also a non-alcoholic beverage with high nutritional value for woman and children. While classical microbiology indicated a lactic fermentation dominated by *L. fermentum*, metagenomics revealed a poorly culturable dominant Lactobacillus belonging to a new subspecies of *L. delbrueckii* that was only occasionally isolated. The second product is Suusac, an East African fermented camel milk, in which classical microbiology indicated *Streptococcus infantarius* sp *infantarius* as the dominant species. Our analysis showed that indeed, this species shares the lead with a Strepcococcus from the salivarius group belonging to an undescribed species yet. In both cases, it was then possible to isolate routinely these species by adapting culture media or developing probes.
These results showed that in both cases, lactic fermentation is driven by an association of 2 species that are stably established and may have developed symbiosis relationship in the same manner than *S. thermophilus* and *L. bulgaricus* in yogurt.

Keywords: metagenomics, Tchepalo, Suusac, Lactobacillus,L. fermentum, L.delbrueckii, streptococcus infantarius

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O3.10.

Halophilic and halotolerant bacteria: From environment to cheese

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Halophilic bacteria are usually reported as living in environments such as see, salted lake and soil. However, they can also be found in food, such as cheese, salting and see products. In cheese, species belonging to the genera *Psychrobacter*, *Halomonas* and *Pseudoalteromonas* were occasionally isolated. Previous studies reported that presence of Psychrobacter species is linked to rapid aging of some cheeses. Likewise the presence of *Halomonas* species in several cheese factories has been considered as indicator of hygienic problems. Nevertheless, it has been also suggested that *Psychrobacter* and *Pseudoalteromonas* could play major roles on cheese ripening. Nevertheless, recent studies based on non-culturable methods showed that these bacteria could be the dominant microbiota on the surface of cheese of excellent quality, suggesting that they are indeed underestimated technological bacteria.

In view of the lack of studies related to these bacteria in cheese, we decided to develop several approaches to assess their origin and role in this context. For this purpose, we used metagenomic analysis of 54 cheese rinds to determine the prevalence of these bacterial groups. Moreover, we used an improved isolation protocol based on marine broth medium supplemented with different salt concentrations and incubated under three different temperatures 20, 25 and 28°C). Phenotypic screening of species was preliminary performed by using gram staining and optical microscopy, and followed by molecular identification through 16S rRNA gene amplification by using universal primers.

To date, we isolated from 12 different cheeses 30 independent clones belonging to 4 *Halomonas* species, 2 *Pseudoalteromonas* species, and 5 *Psychrobacter* species. Metagenomic profiling has revealed a high abundance of the 3 genera in several cheeses (up to 23.17% of the total reads number for *Halomonas*, ≤37.05% for *Pseudoalteromonas* and ≤32.70% for *Psychrobacter*.

The high abundance of these species suggests an important impact on the cheese properties. Therefore we are currently developing functional genomic approach on cheese rind models to determine the main functions involved in cheese adaptation and interaction with other cheese bacteria.

Keywords: Cheese, metagenomics, Psychrobacter, Pseudoalteromonas, Halomonas

Impact of interventions during food production on microbial biodiversity

O4.1.

The synergistic effect of combining low and high radio frequency electric fields on microbial inactivation of *Escherichia coli* in saline water and fruit juices

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The application of high intensity electric fields including radio frequency electric fields (RFEF) and pulsed electric fields (PEF) has been introduced as an alternative non-thermal food processing technology to ensure food microbial safety as well as preserving its quality and nutritional attributes. The mechanism of inactivation in both RFEF and PEF is due to a phenomenon called irreversible electroporation, which can be affected by key factors such as temperature, electric field strength, and treatment time.
In PEF, a synergistic effect was shown when a combination of nanosecond and microsecond pulse durations was applied, which eradicated a higher level of microbial population compared to when nanosecond or microsecond pulses were applied separately. It was assumed that microsecond pulses affect cell membrane integrity, while nanosecond pulses affect internal organization. Nanosecond and microsecond pulses in PEF are equivalent to high and low frequencies in RFEF, respectively.
A set of experiments was conducted to investigate the possible synergistic effect of combining high and low frequency electric fields on *Escherichia coli* inactivation in saline water and fruit juices. The experiments focus on comparison study between different protocols of RFEF treatment including combination of high and low frequency, high frequency and low frequency electric fields separately as well as the effect of order of treatments on microbial inactivation. In order to distinguish the difference between the high and low frequency electric fields in microbial inactivation, mechanistic studies were conducted by using electron microscopy techniques and polymerase chain reaction (PCR) test. Finally, an energy efficiency study was carried out to determine the advantage of combining high and low frequency electric fields treatments.

Keywords: intervention strategies, food processing, RFEF, non-thermal processing, E.coli, food microbiology

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O4.2.

Identification of novel genes involved in high hydrostatic pressure resistance of *Escherichia coli*

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High hydrostatic pressure (HHP) is an interesting hurdle in minimal food processing approaches that aim to synergistically combine different stresses to improve microbiological safety and stability while maximally preserving food quality. In order to come to a proper design of HHP-based hurdle technology, it will be important to understand why HHP interacts synergistically with some other stresses, and thus to establish the cellular impact of each stress on foodborne pathogens. The impact of HHP on cellular physiology has to a large extent been coined from the effect of HHP on biomolecules and from the genetic analysis of bacterial mutants that were selected to become HHP resistant. Nevertheless, the mechanism leading to HHP-mediated cell death is still unclear. To further zoom in on this mechanism, we decided to screen for loss-of-function mutations in *E. coli* able to affect HHP sensitivity. More specifically, ca. 5,000 random Tn*10*dCm transposon mutants of *E. coli* MG1655 were individually exposed to HHP, after which the phenotype of interesting loss-of-function mutations was confirmed. Interestingly, our results reveal that alterations in the tricarboxylic acid cycle can profoundly affect HHP resistance of *E. coli*, although they hardly affect the susceptibility to heat.

Keywords: E. coli, high hydrostatic pressure resistance, heat resistance, TCA cycle

Impact of interventions during food production on microbial biodiversity

O4.3.

How to design heat treatment regimes in a variable world: Is the average microbial resistance, the 95th percentile, or the 99th percentile the best target?

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For food safety we want to be absolutely sure ! But absolute does not exist; food products, microorganisms and humans are biological entities showing large variability. And on top of that also technical process parameters (temperature, time) show variability. In order to validate processes in our variable world these variabilities need to be taken into account. Data sources to support design of heat treatment regimes are literature data, databases, historical data, experimental data, storage tests, challenge tests, predictive models, basic knowledge and logic. All these data sources have their weak and their strong points, and therefore it is useful to make use of all kind of sources of data. This makes it on the one side more diffuse and difficult to judge due to large variability, but on the other hand also more realistic, since in reality variability does exist.
Nonetheless, a difficult decision remains how safe you should be. A scientific answer cannot be given to such a question, yet the impact of various variability sources can be compared to prioritize them and to evaluate the impact on microbial inactivation efficacy. We will illustrate this by selecting several percentiles in the variability distribution of most relevant parameters (D-value, heat temperature and time), and to discuss the consequences on the outcome of heat treatment regimes. Targeting the 99th percentile in microbial resistance (i.e. the most heat resistant strain out of 100) can be shown to be in many cases clearly not sufficient.
The approach presented can give insight in effects of biological heterogeneity on the ultimate variability. This can help to identify the most relevant parameters for variability in the outcome and can help to direct how far in certain parameters one should go to reach a targeted reduction.

Keywords: Validation, thermal processing, heterogeneity, variability, extremes, distribution tails

Impact of interventions during food production on microbial biodiversity

O4.4.

Microbiota analysis to reveal temperature abuse of fresh pork

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Temperature control is important to minimize microbial growth along the fresh meat processing chain. Violations of temperature regulations (temperature abuse) may therefore negatively affect meat safety and quality. Few methods are available to reliably distinguish between old meat and meat that has been subjected to temperature abuse. Specific temperature exposures may, however, lead to systematic changes in the diverse bacterial communities associated with the meat due to different *in situ* growth characteristics. We investigated whether temperature-driven patterns in the community composition on fresh meat surfaces reflect the temperature-history (combination of time and temperature). Sterile pieces of pork were inoculated with a carcass swab homogenate, to which *Salmonella* was added. Changes in the meat microbiota were monitored during aerobic chill-storage (4 °C and 7 °C) and temperature abuse (12 °C and 16 °C) for 96 hours, using culture-based methods and 16S rRNA gene sequencing. Bacterial genera that dominated during prolonged temperature abuse were *Acinetobacter*, *Serratia* and *Pseudomonas,* whereas chill-stored meat was dominated by *Pseudomonas* only. By comparing meat inoculated with the carcass microbiota to meat inoculated with porcine feces as a reference, we showed that the composition of the initial community affects subsequent changes during storage. The presented results suggest that principal coordinate analysis of beta diversity could be a useful tool to reveal temperature abused meat. Analysis of both sequence data and culturing revealed a strong positive correlation between *Escherichia coli* and *Salmonella*, which suggests that *Escherichia coli* may be used as a microbial indicator for growth of *Salmonella* on the surface of fresh pork. In conclusion, the present work showed that 16S rRNA gene sequencing methods are practical and efficient tools that expand our understanding of microbial community dynamics and diversity in foods. Such methods can further facilitate easy screening and identification of safety indicators disclosing pathogen growth.

Keywords: 16S rRNA gene sequencing, growth indicator, Salmonella, carcass microbiota, feces, community dynamic

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O4.5.

Effect of pluck set removal techniques during slaughter on pig carcass contamination with hygiene indicator bacteria, ESBL/AmpC-producing *E. coli, Salmonella* and *Yersinia enterocolitica*

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Pigs are asymptomatic carriers of pathogenic and antibiotic resistant bacteria, which may contaminate pig carcasses during slaughter. Especially, opening the oral cavity during the pluck set (i.e. lungs, heart, liver, and tongue) removal is a potential risk for spreading bacteria over the carcass. Therefore, the aim of this research was to compare carcass contamination between pigs of which the pluck set was removed following standard procedures and pigs of which the pluck set was alternatively removed (leaving tongue and highly contaminated tonsils inside the unopened oral cavity).

From each of 12 pig batches, 20 carcasses (ten slaughtered normally and ten slaughtered alternatively) were sampled after pluck set removal by swabbing the elbow, throat and sternum (100 cm^2 each) in two Belgian slaughterhouses. Samples were analyzed using direct plating to quantify total aerobic bacteria, *Enterobacteriaceae* and *E. coli,* as well as investigated for the presence of ESBL/AmpC-producing *E. coli*, *Salmonella* and *Yersinia enterocolitica*.

Average total aerobic counts for throat samples ranged between batches from 2.1 to 3.8 $\log_{10}$ CFU/cm^2 with mean reductions up to 0.6 $\log_{10}$ CFU/cm^2 when using the alternative method. Median throat *Enterobacteriaceae* and *E. coli* numbers varied between batches from 0.6 to 2.8 $\log_{10}$ CFU/cm^2 and 0.4 to 2.3 $\log_{10}$ CFU/cm^2, respectively, with maximal mean reductions of 1.0 $\log_{10}$ when applying alternative slaughter. The proportions of *Salmonella* and *Y. enterocolitica* positive throat samples were equal for both slaughtering methods and pathogens (1.7%). The presence of ESBL/AmpC-producing *E. coli* on throat samples diverged from 5% (normally slaughtered) to 14% (alternatively slaughtered). Similar results were seen for other carcass areas. Consequently, the alternative pluck set removal method, requiring only minimal adaptations in the slaughterhouse, may contribute to improve the microbial quality of pig carcasses.

Keywords: Pig; Slaughter; Salmonella; Yersinia; ESBL; Escherichia coli; Pluck Set; Hygiene; Contamination

Impact of interventions during food production on microbial biodiversity

O4.6

Cultivation-independent quantification of thermotolerant *Campylobacter*

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Campylobacter is the major food borne bacterial pathogen in European Union, causing 246,307 reported campylobacteriosis cases in 2016. Human infections are most frequently associated with handling, preparation and consumption of broiler meat. According to risk assessment studies the number of the cases could be reduced by decreasing numbers of *Campylobacter* on the meat. Quantification of *Campylobacter* on broiler meat is currently performed by cultivation method. This is however biased, since bacteria might lose cultivability due to cold or oxygen stress during storage at retail. As alternative, a real-time PCR assay combined with pre-treatment of the samples with the DNA intercalating and crosslinkable dye propidium monoazide (PMA) quantifies bacteria with an intact membrane, which are putatively infectious. Reliable results however depend on sufficient signal reduction from dead cells, e. g. influenced by incubation temperature, organic load of the sample and efficiency of crosslinking of the dye.
The aim of the study was to develop an internal sample process control (ISPC), consisting of a distinct number of peroxide-killed *C. sputorum* cells. The ISPC shall monitor reduction of the signal from dead cells and putative DNA losses from live cells during sample processing.
We designed the ISPC, i.e. a species-specific fragment of the 16S rRNA gene of *C. sputorum* DSM 5363, having similar length and copy number per chromosome compared to the thermotolerant *Campylobacter* target. We proved the suitability of this ISPC in exclusivity tests. Further, using chicken rinse samples we proved an accordance of the ISPC signal reduction with the thermotolerant *Campylobacter* target in the presence of PMA and upon loss of DNA during extraction.
In practice the ISPC shall be added to chicken rinse samples for monitoring live/dead discriminatory quantification of thermotolerant *Campylobacter*. Our study provides a crucial step towards implementation of cultivation-independent quantification for improved food safety of fastidious bacteria. Our further work will focus on conservation of the standard and characterization of its membrane permeability properties for distribution of the ISPC to various laboratories. In the following the method will be validated in an international ring trial, organized by the Bavarian Health and Food Safety Authority.

Keywords: viable Campylobacter, real-time PCR, propidium monoazide, internal sample process control

Impact of interventions during food production on microbial biodiversity

O4.7.

Clostridium botulinum – New extensive cardinal parameter growth and growth boundry model for saltreduced lightly preserved seafood

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Salt is important to manage growth and toxin formation by non-proteolytic *Clostridium botulinum* in chilled seafood. *C. botulinum* can grow and produce toxin at temperatures as low as 3.5 °C but this risk is managed by 3.5% water phase salt (WPS) in chilled products. Reduced salt intake is important for health reasons and included in recent dietary recommendations. This creates a demand to replace salt with other environmental factors in order to control *C. botulinum* in seafood. Some mathematical models are available to predict growth of the pathogen. Those models contain the effect of five environmental parameters or less including temperature, CO_2, salt, pH, lactate and nitrite (Food Cont. 29, 2013, 309-317). However, available models lack the effect of several important antimicrobials and therefore are not well suited to predict how the inhibiting effect of salt can be replaced by changes of other environmental factors. Therefore, the objective was to develop an extensive predictive growth and growth-boundary model for non-proteolytic *C. botulinum* in seafood with a range of different environmental factors.
A cocktail of four non-toxin producing *C. botulinum* group II isolates were studied and cardinal parameter values for the effect on growth of temperature, pH, salt/$a_{w,}$ nitrite and organic acids (acetic, benzoic, citric, lactic and sorbic) were determined in broth. A cardinal parameter model for the nine environmental factors and their interactive effect was developed. 39 growth curves in seafood and data from the literature were used for product calibration and evaluation of model performance.
Estimated cardinal parameter values were 1.64 °C (T_{min}), 5.14 (pH_{min}), 3.75% WPS/0.977 ($a_{w\,min}$), 20.0 ppm (nitrite) and 11.4, 0.19, 0.05, 2.8 and 1.2 mM, respectively, for undissociated acetic, benzoic, citric, lactic and sorbic acids. Model predictions corresponded to guidelines for management of *C. botulinum* including absence of growth (i) at 3.5 °C, (ii) with pH 5.0 at 10 °C and (iii) with 3.5% WPS at 5 °C. Evaluation of the model suggests it can be used to facilitate product development for a wide range of seafood as shown here for salt reduced brined shrimps at 5 °C, pH 6.15, 2500 ppm acetic, 860 ppm benzoic and 430 ppm sorbic acids. When the %WPS is reduced from 3.5% to 1.0%, the ψ-value changes from 2.3 to 0.8 and growth was predicted. However, by increasing the benzoic acid to 1600 ppm growth of non-proteolytic *C. botulinum* was prevented.

Keywords: Clostridium botulinum, predictive modelling, predictive microbiology

Impact of interventions during food production on microbial biodiversity

O4.8.

Population genetic structure of *Listeria monocytogenes* strains isolated from the pig and pork meat production chain in France

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Listeria monocytogenes is an ubiquitous pathogenic bacterium, transmissible to humans through the consumption of contaminated food. The pork production sector has been hit hard by a series of *L. monocytogenes*-related food poisoning outbreaks in France. An overview of the diversity of strains circulating at all levels of the pork production chain, from pig farming to finished food products, is needed to identify the contamination routes and improve food safety. Until now, no typing data has been available on strains isolated across the entire pig and pork production chain.

Here, we analyzed the population genetic structure of 687 *L. monocytogenes* strains isolated over the last 20 years in virtually all the French *départements* from three compartments of this production sector: pig farming (PF), the food processing environment (FPE) and finished food products (FFP). The genetic structure was described based on MLST clonal complexes (CCs). The CCs were obtained by mapping the PFGE profiles of the strains. The distribution of CCs was compared firstly between the three compartments and then with CCs obtained from 1106 strains isolated from other food production sectors in France.

The predominant CCs of pig and pork strains were not equally distributed among the three compartments: the CC37, CC59 and CC77 strains, rarely found in FPE and FFP, were prevalent in PF. The two most prevalent CCs in the FPE and FFP compartments, CC9 and CC121, were rarely or never detected in PF. No CC was exclusively associated with the pork sector. Three CCs (CC5, CC6, CC2) were considered ubiquitous, because they were observed in comparable proportions in all food production sectors. The two most prevalent CCs in all sectors were CC9 and CC121, but their distribution was disparate. CC9 was associated with meat products and food products combining several food categories, whereas CC121 was not associated with any given sector. Based on these results, CC121 is likely able to colonize a larger diversity of food products than CC9. Both CCs being associated with the food production suggests, that certain processing steps, such as slaughtering or stabilization treatments, favor their settlement and the recontamination of the food produced.

Keywords: Listeria monocytogenes, pig, pork, PFGE, MLST, population structure, genetic diversity

Impact of interventions during food production on microbial biodiversity

O4.9.

Lactic acid bacteria producing anti-fungal compounds: From plant protection to cereal product

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Fungal food spoilage plays a pivotal role in the deterioration of food and feed systems and some of them are also able to produce toxic compounds for humans and animals. The mycotoxins produced by fungi can cause serious health hazards, including cancerogenic, immunotoxic, teratogenic, neurotoxic, nephrotoxic and hepatotoxic effects and Kashin-Beck disease. In addition to this, fungal spoilage/pathogens are causing losses of marketable quality and hygiene of foodstuffs, resulting in major economic problem throughout the world. Nowadays, food spoilage can be prevented using physical and chemical methods, but no efficient strategy has been proposed so far to reduce the microbial growth ensuring public health. Therefore, lactic acid bacteria (LAB) can play an important role as natural preservatives. The protection of food products using LAB is mainly due to the production of anti-fungal compounds such as carboxylic acids, fatty acids, ethanol, carbon dioxide, hydrogen peroxide and bacteriocins. In addition to this, LAB can also positively contribute to the flavour, texture and nutritional value of food products. This presentation focuses on the use of LAB for food preservation given their extensive industrial application in a wide range of foods and feeds.

Keywords: lactic acid bacteria, bioprotection, antifungal compounds

Impact of interventions during food production on microbial biodiversity

O4.10.

Harnessing environmental biocontrol lactic acid bacteria for fresh produce safety

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Foodborne outbreaks from the consumption of fresh minimally processed vegetables and fruits occur a regular intervals globally. Prevention of initial pathogen contamination is key to reducing illness, however approaches to prevent pathogen growth following infrequent low level contamination events would also provide an additional safety hurdle. In this work a large collection of lactic acid bacteria (LAB) isolates from fresh produce was obtained and screened for growth inhibitory activity against *Salmonella* and *Listeria monocytogenes* to identify biocontrol isolates. A wide range of vegetables, fruits and herbs were sampled to obtain a large LAB collection (n=897). An agar spot method was used to identify pathogen inhibiting isolates which were then identified using 16S rDNA sequencing. Antimicrobial isolates were tested in a cut iceberg lettuce model against the pathogen over 7 days of storage at 12°C. Sixty-nine LAB isolates had activity against *Salmonella* and/or *L. monocytogenes*. These LAB were identified as members of the 'generally recognized as safe' *Leuconostoc*, *Lactococcus*, *Carnobacterium* and *Weissella* genera. Testing of a subset of these LAB revealed strong *Salmonella* and *L. monocytogenes* inhibition in a cut lettuce model assay with 3-4 log CFU/g lower level of pathogen present after 7 days, compared to the no LAB control. LAB from fresh foods and the environment have potential as pathogen growth inhibitors which can improve the safety of minimally processed vegetables and fruits. These isolates could be applied as protective additives directly on fresh vegetables and fruits pre- or post-harvest, or may in the future be selected for using farming practices, providing a natural protective fresh produce microbiome.

Keywords: biocontrol, safety, lactic acid bacteria, fresh prodcue

Microbiological spotlights

O5.1.

Cold adaptation of *Bacillus cereus:* What happens during the lag phase?

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The spore-forming bacterium *Bacillus cereus* is a major cause of foodborne outbreaks in Europe. Some *B. cereus* strains can grow at low temperatures and consequently during the storage of refrigerated foods. Bacterial cold adaptation is characterized by a lag phase in which the bacteria do not multiply and modify their physiology to cope with the constraints of low temperatures. During the lag phase, diverse events take place and these are poorly understood. From a practical point of view, the risk of *B. cereus* multiplication in refrigerated foods is consequently difficult to predict.

Our aim is to determine a sequence of molecular and physiological events during the lag phase at low temperature of the mesophilic *B. cereus* ATCC 10876 and the psychrotrophic MM3 strains. The expression of genes essential for cold adaptation (*cshA* coding for a helicase RNA and *desA* encoding a membrane fatty acid desaturase) or involved in the transition between lag and exponential phase of growth (*abrB* coding for a transition regulator) was studied by transcriptional reporter systems with the fluorescent proteins GFP and mCherry. The kinetics of the promoter activity was measured by quantification of the fluorescence at the population level during the lag phase at low temperature.

An increased expression of the *cshA* promoter was observed at 12°C at the beginning of the lag phase without any increase in the number of cells, suggesting *cshA* activity during adaptation. The expression of *abrB* at 12°C occurred at different times, during lag phase for the ATCC 10876 strain and during the exponential growth phase for the MM3 strain. The expression of these genes is currently studied at the single cell level by epifluorescence microscopy time-lapse. In contrast to *cshA* and *abrB*, *desA* seemed to be expressed later in the growth for both studied strains. The fatty acid desaturase encoded by *desA* has been considered responsible for the increase in unsaturated fatty acids, that restore membrane fluidity at low temperature. We observed that this increase in unsaturated fatty acids, as well as the increase in the anteiso/iso fatty acids ratio, began after lag phase, during the exponential growth phase. The major change in fatty acids profiles during lag was an increase in the ratio of iso-C15: 0/iso-C13: 0 fatty acids.

Keywords: Bacillus cereus, Cold adaptation, Lag phase, Fluorescence, Fatty acids

Microbiological spotlights

O5.2.

Differential diagnostics of enteropathogenic *Bacillus cereus* based on marker proteins

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Bacillus cereus is one of the most common pathogens linked to food poisoning, which can become manifest as an emetic or diarrheal type. Different exotoxins, namely the non-hemolytic enterotoxin complex Nhe, the hemolytic enterotoxin complex HBL and the single-protein cytotoxin K (CytK), have been linked to the diarrheal type of the disease, which resembles *Clostridium perfringens* infections. However, neither the sole presence of the toxins nor the amount of the secreted enterotoxins directly reflects the actual toxigenic potential of a strain. Due to the high prevalence of *B. cereus* in various natural habitats, contaminations of food production and processing chains cannot totally be avoided. Thus, it is important to establish a system to differentiate between strains with high- and low enteropathogenic potential to prevent loss of precious foods due to recalls of food charges contaminated with low-or non-pathogenic *B. cereus.*

Subcellular proteomics was used to identify toxicospecific marker proteins that are secreted by the bacterial cells or are expressed on the surface. Different methods, such as biotinylation, trypsin shaving and 2D DIGE, were applied to identify virulence factors located on the cellular surface and differential 2D gel electrophoresis was used for analysis of the secretome. By using this polyphasic approach, we could successfully identify marker proteins, which are expressed by highly toxigenic but not by low toxigenic *B. cereus* strains. An antibody-based system was successfully established that allows the quantitative detection of the marker proteins.

The detection of toxicospecific marker proteins, combined with the information of bacterial total cell count, will provide the basis to establish a diagnostic risk assessment tool, in order to evaluate the risk potential of *B. cereus* contaminated food charges. It is expected that such risk orientated diagnostic tools could help industry in decision making and contribute to the prevention of precious foods loss.

Keywords: Bacillus cereus, enterotoxins, food poisoning, marker proteins

Microbiological spotlights

O5.3.

Phenotypic microarray analysis reveals variations in carbon source utilization and stress resistance profiles among selected clinical and food related *Listeria monocytogenes* strains

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Listeria monocytogenes is an important food-borne pathogen that accounts for serious public health problems as well as significant food safety challenges and economic losses to the food industry. Infections of susceptible individuals results in various life-threatening conditions often associated with high mortality rates. Although most cases are sporadic in nature, occasionally large-scale outbreaks linked to common ready to eat food sources occur. To survive in rapidly changing harsh environmental conditions, *L. monocytogenes* has evolved various adaptive response mechanisms making its control in food a great challenge. This in turn has generated populations that are phenotypically heterogeneous in natural situations. Different approaches including the OmniLog phenotype microarray (PM) assay and whole genome analysis were applied to selected clinical and food related *L. monocytogenes* strains to uncover genotype and phenotype associations that are linked to stress resilience and host pathogenicity amongst the selected strains.

Growth performance of the strains was assessed for carbon sources utilization, pH stress resistance and osmo-tolerance. Several food-relevant phenotypic differences with respect to carbon source utilization, resistance to acid and osmotic stress conditions were uncovered amongst the strains on phenotypic microarrays. Targeted phenotypic analyses were applied that confirmed selected PM array phenotypes as well as uncover phenotypic variation in virulence among the examined strains. Despite high phenotypic variability the strains showed, high genomic and key metabolic pathway conservation suggesting that gene expression and regulation could be responsible for the observed differences. These nutrient and stress resistance profiles obtained from a diverse set of *L. monocytogenes* strains provides a potential basis for the future design of *L. monocytogenes* specific media to enhance routine detection of this pathogen and nutrient utilization pathway inhibitors or protocols that can improve food safety.

Keywords: Listeria monocytogenes, Phenotypic Microarray, Whole genome analysis

Microbiological spotlights

O6.1.

Tracing back multidrug-resistant bacteria in fresh herb production: From chive to source through the irrigation water chain

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The emerging threat of antibiotic resistance (AR) may herald a new era of infectious disease and understanding the development and spread of resistance is therefore crucial. Little is known about the transfer of resistance from the environment to humans, which occurs most likely via the food chain. Currently it remains unclear which AR bacteria (ARB) and AR genes are transferred onto food plants and if the resistance persists on the plants. An experimental site used primarily for salad growing was chosen for a field study in Switzerland. The site was optimal due to the irrigation water for the plants being pumped from a nearby river, where an upstream wastewater treatment plant (WWTP) releases its effluents into the river. Lettuce heads were planted under two different conditions: i) plain soil or ii) manure-amended soil and were treated with either i) WWTP downstream effluent or ii) UV-treated irrigation water. Sampling took place for 7 time-points (week 0 - week 6) and samples from each treatment and time-point were screened for culturable extended spectrum beta-lactamase (ESBL)- and carbapenemase producing *E. coli* and *K. pneumoniae*. Colonies were morphologically characterized and checked by MALDI-TOF biotyping.Agar plates selecting for ESBL- and carbapenemase-producing *E. coli* and *K. pneumoniae* on lettuce showed an increase in bacterial counts from around 10^4 CFUs in week one to 10^5 CFUs in week 6 in all treatments, however, no effect between treatments was observed. Bacterial counts in UV-treated irrigation water were 10^2 CFUs lower than in WWTP-effluent irrigation water. However, bacterial soil counts did not show a difference in treatments and time-points and remained the same at approximately 10^5 CFUs. Gamma-proteobacteria and genera from the taxonomic classes Bacilli and Acinetobacteria were identified from ESBL selecting agar plates. While alpha- and beta-proteobacteria were largely identified in all three sample types from carbapenemase selecting agar. *E. coli* or *K. pneumoniae* were not identified by MALDI-TOF biotyping, which may be due to overgrowth of other species. This issue will be addressed by sequencing the 16S rRNA of the microbial community in the different treatments and time-points and will provide an in-depth characterization of the microbiome. Our results so far show ARB in different environmental reservoirs and will help to understand the microbial communities involved in the transfer of AR from the environment into the food chain.

Keywords: antibiotic resistance, cultivation, microbiome analysis, amplicon sequencing, MALDI-TOF biotyping

Microbiological spotlights

O6.2.

Prevalence of mycotoxins in selected Nigerian fermented foods

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In Africa, fermented foods and beverages play a significant role in contributing to food security and their production is dominated by informal processing sectors (cottage and rural small-scale processors) that make use of different traditional processing methods thereby, bringing about variation in substrates used, processing conditions (time, temperature, moisture etc.), packaging materials, handling, and storage practices. These factors determine the quality and safety of the final products. This study evaluated the safety quality in terms of mycotoxin prevalence of some fermented foods obtained from Nigerian markets. Fermented food samples (*n* = 191) including maize gruel (*ogi*), sorghum gruel (*ogi baba)*, melon seed (*ogiri*), locust bean (*iru*) and African oil bean seed (*ugba*) from Southwest Nigeria were quantified for 23 mycotoxins, including aflatoxin B_1 (AFB_1), fumonisin B_1 (FB_1), and sterigmatocystin (STE) using liquid chromatography-tandem mass spectrometry. Data obtained revealed that 82% of the samples had mycotoxins occurring singly or in combination. FB_1 was present in 83% of *ogi baba* samples, whereas 20% of *ugba* samples contained AFB_1 (range: 3 to 36 µg/kg) and STE was present in 29% of the *ogi* samples. Ochratoxin A was found in *ogi baba*, *iru*, and *ugba*, at mean levels of 6, 6, and 9 µg/kg, respectively, whereas, roquefortine C was only detected in *iru* and at a low incidence rate (range: 10-14 µg/kg). HT-2 was the most frequently occurring trichothecenes in the samples and the level of deoxynivalenol in all the positive samples (*n* = 18) reached a maximum of 118 µg/kg. In terms of multi-mycotoxin contamination, FB_1 + FB_2 + FB_3 + STE + AFB_1 + alternariol + HT-2 co-occurred within one sample. This is the first study that assessed the presence of mycotoxins in *ugba* and *ogi baba* and the first to report a wide range of previously unreported mycotoxins in *iru, ogiri* and *ogi* consumed in Nigeria. The extent to which the analysed mycotoxins contaminated these food commodities justifies the need to enact fungal and mycotoxin mitigation strategies along the food chain.

Keywords: Mycotoxins, Fermented foods, Processing conditions

Microbiological spotlights

O6.3.

Bacteriophage-encoded tail fibers for improved detection of pathogenic bacteria

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Rapid and sensitive detection of pathogenic bacteria is critical to the global food industry. Demand has driven the development of rapid detection systems for foodborne pathogens. A critical component of these systems is choosing an affinity molecule that retains specificity and sensitivity for a target pathogen over a wide range of *in situ* applications. Antibodies are the most extensively used affinity molecules for enrichment (e.g., immunomagnetic separation) and bacterial detection (e.g., ELISA and biosensors). Unfortunately, antibodies are not always ideal for bacterial detection in food; common problems include cross-reactivity with background flora and fluctuating binding sensitivity.

Bacteriophages (phages) are viruses that infect bacteria. The first stage of any phage infection is adsorption to a host cell, mediated for many phages by their long tail fibers (LTFs). We engineered the LTF from a broad-spectrum *Salmonella* phage S16 into an affinity molecule (S16 LTF) for specific detection of *Salmonella*. S16 LTF conjugated to paramagnetic beads (LTF-MBs) could capture and enrich various *Salmonella* strains from food pre-enrichments or solution with no cross-reactivity. We developed LTF-MB enrichment into an ELISA-like detection test using S16 LTF conjugated to horseradish peroxidase (HRP-LTF) as a detection probe. The novel enzyme-linked LTF assay (ELLTA) uses LTF-MBs to enrich over 95% of *Salmonella* from solution. Subsequently, HRP-LTF binds to bead-bound *Salmonella*, which then catalyses the conversion of a chromogenic substrate (TMB) into a visible blue product. ELLTA is a quick and sensitive assay, detecting down to 10^2 cfu/ml in two hours.

To explore the extraordinary binding ability of the S16 LTF we also solved the structure of its binding tip using X-ray crystallography. We reveal how receptor-binding specificity is determined by a single - and structurally unique - protein (gp38) attached to the end of the LTF. The distal domain of gp38 represents a feat of protein folding, containing rare polyglycine helices folded into an unusual lattice structure, we named “polyglycine sandwich”. The distal end of the sandwich features the receptor-binding sites that determine host specificity. Overall, tail fibers (and other phage binding proteins) have enormous potential for development into affinity molecules to improve bacterial detection in near limitless applications for agriculture, environmental control, and food production.

Keywords: bacteriophage, tail fibers, X-ray crystallography, Salmonella, pathogen detection

Poster Abstract

Exploring biodiversity in microbial ecosystems along the food chain

P1.1

Genetic diversity and population structure of *Lactobacillus plantarum* – The predominant core microbiota of traditional spontaneously fermented bamboo shoot

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Lactobacillus plantarum is an industrially important lactic acid bacterium with diverse ecological niche adaptability and high genetic diversity. Its technological and functional properties are generally strain-specific rather than species-specific. Our earlier high-throughput amplicon sequencing study revealed *L. plantarum* as the predominant core microbiota of traditional spontaneously fermented bamboo shoots. The present study examined the genetic diversity and population structure of these wild *L. plantarum* strains using pulse-field gel electrophoresis (PFGE)-based chromosomal restriction analysis and multi-locus sequence typing (MLST). SfiI-PFGE analysis of 85 *L. plantarum* strains from four different types of fermented bamboo shoots of North East India evidenced the presence of at least one dominant clone unique to each food type indicating food niche specific clonal selection. The *L. plantarum* population was further analysed by MLST based on five conserved housekeeping genes in comparison with the corresponding sequences from 20 publicly available reference genomes of *L. plantarum* from various sources of different geographical origin. The analysis produced 26 unique sequence types (STs), the majority of which were assigned into two clonal complexes (eBG-16 and eBG-24). Only fermented food isolates formed the founder genotype of the clonal complexes. The wild strains of fermented bamboo shoot origin were assigned into 15 STs, of which six STs clustered within eBG-24 clonal complex and nine STs were singletons with two strains forming genetically distant separation from the founder genotype. This finding reaffirmed the high genetic diversity of *L. plantarum* in the fermented bamboo shoot ecosystem. Although the geographical distribution of the strains amongst the clonal complexes was uneven, all the human intestinal isolates were disseminated within eBG-16 clonal complex, supporting the reported hypothesis (Seizen *et al*. 2010) that human-related *L. plantarum* strains originate from the food consumed by the individuals. Excess of low frequency allelic variation without recombination indicated a recent selection sweep during *L. plantarum* evolution. Our study demonstrated a genetically heterogeneous population structure of *L. plantarum*, which is largely different from previously reported findings.

Keywords: Lactobacillus plantarum, Fermented bamboo shoot, Genetic diversity, Population structure, MLST, PFGE

Exploring biodiversity in microbial ecosystems along the food chain

P1.2

Inactivation of human pathogenic bacteria by food materials extracted from cultivated and natural plant flora collected from Palestine

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Summary: This research was conducted to study the antibacterial activity of some Palestinian plants against seven human pathogenic bacteria using the agar disk-diffusion method. Evaluation of the antibacterial activities of plant saps based on the width of the bacterial inhibition revealed that *Eucalyptus camaldulensis* (0.3 cm), *Allium sativum* (0.2 cm), *Ceratonia siliqua* (0.15 cm) and *Amygdalus communis*(0.15 cm) have the best antimicrobial activities against the bacterial mixture compared with the other fourteen tested plants. Furthermore, *E. camaldulensis* showed the strongest antimicrobial activity among the four plants. Also, *A. sativum* have the maximum anti-microbial action against all types of the tested bacteria. In addition, saps of *E. camaldulensis* and the mixture of *E. camaldulensis* and *A. sativum* have a strong ability to kill all types of the tested bacteria followed by the mixture of *C. siliqua* and *A. sativum*, the mixture of *C. siliqua*, *A. sativum* and *E. camaldulensis* and the mixture of *A. communis, A. sativum* and *E. camaldulensis* that have significant results as anti-microbial agents against most types of the tested bacteria. The results showed that *A. sativum* and the mixture of *A. sativum* and *C. siliqua* have the maximum antimicrobial affectivity against *Staphylococcus aureus,* whereas, *Micrococcus luteus* was strongly inhibited by *E. camaldulensis, A. sativum,* the mixture of *E. camaldulensis* and *C. siliqua,* the mixture of *E. camaldulensis* and *A. sativum,* and the mixture of *E. camaldulensis, A. sativum* and *C. siliqua. Escherichi. coli* was efficiently inhibited by *A. communis, A. sativum,* and *E. camaldulensis* and also by the mixture of *A. sativum* and *E. camaldulensis. Pseudomonas aeruginosa* was inhibited in a significant amount by *E. camaldulensis* and *A. sativum*, whereas, *Proteus vulgaris* was strongly inhibited by the *A. sativum*. *Bacillus subtilis* was strongly inhibited by *A. sativum*, while, for the *Klebsiella pneumoniae*, most saps revealed an intermediate inhibition except the *A. communis,* which showed the lowest inhibition value.

Therefore, the current study elucidated that *E. camaldulensis*, *A. sativum*, *C. siliqua* and *A. communis* are the best tested Palestinian plants containing the antibacterial agents against the tested bacterial types.

Keywords: Antibacterial agents, food material, self-medication, medicinal plants

Exploring biodiversity in microbial ecosystems along the food chain

P1.3

Influence of Afzelia *africana* ('Akparata') and *Mucuna flagellipes* ('Ukpo') on the quality of set yoghurt

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Set yoghurt was processed using local stabilizers- 'akparata' (*Afzelia africana*) and 'ukpo' (*Mucuna flagellipes*) flours. The seeds of *Afzelia africana* were cleaned, roasted for 20 minutes, de-hulled, winnowed and milled into flour. The seeds of *Mucuna flagellipes* were cleaned, de-hulled, soaked in water for 24 hours, boiled for 60 minutes, oven dried at 70 ºC and milled into flour. Plain set yoghurt samples were produced with 'akparata' flour and 'ukpo' flour at increasing concentrations 0.1 - 0.4%. The yoghurt sample without the stabilizers served as then control. The samples were subjected to physicochemical, microbial and sensory analyses using standard methods. Results show that the fat ranged from 1.01 % for sample Y + C (control) to 2.14% for sample Y+A (containing 0.4 % stabilizer). The protein content ranged from 2.79% for sample Y+C to 3.83 % for sample Y+A (0.4% stabilizer) while moisture decreased from 90.68 % for the control to 81.19% for sample Y + A (0.4 % stabilizer) and carbohydrate which ranged from 4.98% for sample Y+C to 11.46% for sample Y+A (0.4% stabilizer). The calcium content was 16.55 mg/ 100 g for sample Y+A (0.1 % stabilizer) to 32.46 mg/ 100 g for sample Y + C while phosphorus content ranged from 129.19 mg/100g for the control to 134.53 mg/ 100 g for sample Y+U (containing 0.4% stabilizer). The vitamin A content ranged from 25.13 mg/ 100 g for sample Y + U (0.4% stabilizer) to 35.92mg/100g for sample Y+C while vitamin C ranged from 20.43 mg/ 100 g for sample Y+U (0.4 % stabilizer) to 38.93 mg/ 100 g for the control. The total titratable acid increased with decrease in pH of samples while viscosity increased with increase in concentration of the stabilizers. The total viable count of the samples ranged from 1.37×10^5 cfu/ml for sample Y+U (0.1 % stabilizer) to 2.98×10^5 cfu/ ml for sample (0.3 % stabilizer). The lactic acid bacteria count ranged from 4.4×10^4 cfu/ml for sample Y+U (containing 0.4% stabilizer) to 2.9×10^5 cfu/ml for sample Y+ U (0.3 % stabilizer). The mould count ranged from 1×10^1 cfu/ ml for sample Y + U (0.1 % stabilizer) to 5×10^1 cfu/ ml for sample Y+A (0.3 % stabilizer). Sensory scores obtained showed that sample Y+C (control) was the most preferred for colour, aroma, taste, consistency, mouthfeel and overall acceptability.

Keywords: Afzelia africana, Local stabilizers, Mucuna flagellipes, Set yoghurt

Exploring biodiversity in microbial ecosystems along the food chain

P1.4

Probiotic characteristics of LAB isolated from fish products

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Objective: The objectives of this study were to isolate LAB from fish products in UAE and investigate their probiotic characteristics. This study aimed also to characterize the isolated LABs which possessed a bio-preservative (bacteriocin) properties.

Method: Physiological properties, cell surface properties (hydrophobicity, autoaggregation, co-aggregation), acid and bile tolerance, bile salt hydrolysis, cholesterol removing, exopolysaccharide (EPS) production, haemolytic, resistance toward lysozyme and six antibiotics were examined. The rRNA sequencing was carried out to identify the LAB isolates and to acquire Genbank accession numbers. The antimicrobial activity of the neutralized cell-free supernatant (NCFS) of 39 LAB isolates was tested according to referred method. Selected LABs were employed to product fermented fish sausages (FFS). The health-promoting benefits (antioxidant, antihypertensive and cytotoxicity) of the FFS were examined.

Results: A 39 LABs were isolated and identified as Enterococci, Lactobacilli and Streptococci. Virulence genes for Enterococci were tested and found safe. In general, all identified LAB (Lactococcus lactis KX881768, Lactobacillus plantarum KX881772, Lactococcus lactis KX881782 and Lactobacillus plantarum) showed auto-aggregation ability, high cholesterol removal ability, high co-aggregation, strong antimicrobial activity and EPS production. Among the isolates, Lactococcus lactis KX881768, Lactobacillus plantarum KX881772, Lactococcus lactis KX881782 and Lactobacillus plantarum KX881779 exhibited remarkable cholesterol removal abilities. Similarly, Lactobacillus plantarum KX881779, and Lactococcus lactis KX881782 showed very promising fermentation profiles. Enterococcus spp showed good probiotic activities and remarkable antimicrobial properties. The health-promoting benefits of FFS fermented by Enterococcus species were greater than Lactobacillus ones.

Conclusions: Selected isolates from camel milk exhibited outstanding probiotic characteristics. Results of this study showed that LABs isolated from traditional fish products in UAE had great potential to be used in food industry. Further studies are required to explore the health benefit of these isolates of fermented foods made by these isolates.

Keywords: Bio-preservative, probiotics, Fish, Fermented Sausage

Exploring biodiversity in microbial ecosystems along the food chain

P1.5

Tracking bacterial contamination from animal reservoirs to meat during pork production by 16S rRNA gene sequencing

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Contamination of meat during production is unavoidable. Yet, there is a general lack of knowledge concerning source attribution of meat contamination and microbial community dynamics during processing. Here, we compared the microbiota on pork surfaces at slaughter and cutting to bacterial communities in animal reservoirs, i.e. faeces, tonsil tissue, and carcass skin. These animal reservoirs are presumed to contribute to meat contamination. Sampled meat surfaces included the carcass opening along the midline at slaughter as well as sub-primal cuts of pork neck and loin at cutting. We sampled random animals and meat surfaces along the pork processing chain to investigate whether general patterns in microbiota compositions in animal reservoirs and on meat surfaces could be observed. A total of 92 samples were analysed by both 16S rRNA gene sequencing of the V3-V4 region and culture-dependent methods. Diversity and population structures were analysed and compared and indicator value (IndVal) indexes were calculated to investigate the potential of certain taxa to be indicative of a specific contamination source. Results showed that animal reservoirs varied significantly in their microbiota and that each animal reservoir contained characteristic groups of genera that may be suitable to identify source specific attribution of meat contamination. Data analysis revealed that bacteria on meat were partly derived from faeces, tonsils, and skin; however, other sources may also contribute to meat contamination. Data further indicated that the meat microbiota varied on the different primal cuts and that processing practices, such as scalding and chilling, potentially induce shifts in the microbiota. Lastly, we showed that consistent microbial patterns were observed at population level, i.e. when random animal reservoirs and meat surfaces were sampled. Overall, the present work showed that 16S rRNA gene sequencing methods are practical and efficient tools that expand our understanding of microbial behaviour and diversity along food production chains. Moreover, we demonstrated that 16S rRNA gene sequencing facilitates easy screening and identification of indicator organisms for source attribution of contamination.

Keywords: Food microbiota, microbial diversity and ecology, indicator organisms, microbial source attribution

Exploring biodiversity in microbial ecosystems along the food chain

P1.6

Effect of meat plant environmental microbiota on biofilm formation and sanitizer sensitivity of *Salmonella* Typhimurium

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Biofilms containing foodborne pathogens are a serious concern for the food industry, the formation of which is dependent on the environment and microorganisms present. The objective of this study was to examine the influence of meat plant commensal bacteria on the formation of biofilms harbouring Salmonella Typhimurium. Biofilm formation for 50 bacterial isolates recovered from beef packing plants was tested in mono-cultures or in co-cultures with S. Typhimurium. The isolates included, 18 Gram negative aerobic bacteria (GNA), 8 Gram positive aerobic bacteria (GPA), 5 lactic acid bacteria (LAB), 9 *Enterobacteriaceae* (EB), and 10 generic Escherichia coli (GEC). Biofilms developed at 15°C for up to 6 days were quantified using Crystal Violet (CV) staining at days 2, 4 and 6. Selected cultures in planktonic form or in biofilms were also tested for sanitizer susceptibility. In mono-cultures, ≥61, 13, 20, 89 and 80% of GNA, GPA, LAB, EB and GEC developed measurable biofilms after 6 days. However, all co-culture combinations did so on all three sampling days. The interaction between commensal bacteria and S. Typhimurium was species/strain dependent, varying from no impact to synergistic or antagonistic effects. The majority of GPA (7/8), and 5/10 GEC strains had synergistic or antagonistic effect, respectively, on the biofilm formation of S. Typhimurium. No effect was observed for the remaining members of each respective group. When planktonic cells (mono-cultures or co-cultured with S. Typhimurium) of Acinetobacter sp., Janthinobacterium sp., Paenibacillus sp., Pseudoclavibacter sp., and Serratia sp., were treated with a quaternary ammonia or a peroxyacetic acid-based sanitizer, the complete inactivation of cells (up to 6 log CFU) was attained. However, when in biofilms, viable cell numbers for both mono- and co-cultures were only slightly reduced or unaffected by the sanitization treatments. The effect of sanitization on the numbers of viable Salmonella in biofilms was affected ($P < 0.05$) by the companion co-culture species as well as the type of sanitizers used. The results of this study clearly demonstrate that the potential impact of commensal bacteria must be taken into account when designing strategies to combat pathogen biofilms. In addition, the antagonistic effects of commensal bacteria against pathogen biofilms should be further explored to assess their potential as novel bio-control strategies.

Keywords: biofilm, commensal microbiota, Salmonella, meat plant environment

Exploring biodiversity in microbial ecosystems along the food chain

P1.7

Selection of lactic acid bacteria as starters for the fermentation of tiger nut *(Cyperus esculentus)* milk for yoghurt production

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Introduction: Tigernut is rich in dietary protein but it is underutilized because it is highly perishable. This study isolated strains of Lactic Acid Bacteria (LAB) from sorghum and commercial yoghurt. Their technological properties were studied to select the best strain(s) as starter culture for production of tiger nut yoghurt with a view to obtaining a relatively less expensive and nutritionally improved quality yoghurt.

Materials and methods: Strains of LABwere isolated and identified from Ogi prepared under strict hygienic conditions and commercially sold yoghurt samples purchased in Ile-Ife. Standard biochemical, physicochemical and technological properties were used for the selection of best starters for yoghurt production. Production of antimicrobials by the organisms, bile salt tolerance and carbon assimilation tests, Exo-polysaccharide and enzymes production, production of biogenic amines and antagonistic activities of the starters against selected pathogens were some of the parameters used to screen the best starters.

Results: A total of forty seven LAB were isolated from the samples and identified as L.plantarum, L. acidophilus,L. fermentum, L. casei, L. pentosus, L. lactis and Streptococcus thermophilus. The technological properties showed L. plantarum,L. fermentum and L. lactis as the best starters showing high exopolysaccharide production. Diacetyl, hydrogen peroxide and lactic acidproduction ranged from 0.53gL-18.1g/L respectively. The optimum temperature and pH for starter selection were 30°C and 5.5. There was no production of biogenic amines.They also demonstrated antagonistic activities against known pathogens.

Conclusion: LAB isolated from fermented sorghum could be used as starters for improved tiger nut yoghurt production in milk and dairy industries.

Keywords: Ogi, Tiger nut, Yoghurt, Lactic acid Bacteria, Nutritional improvement.

Exploring biodiversity in microbial ecosystems along the food chain

P1.8

Assessment of the nutritional quality of lactic acid bacteria fermentation of tiger nut (*Cyperus esculentus)* milk for yoghurt production

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Background: Protein deficiency and malnutrition is on the rise especially among children of weaning age. This is because protein sources from plant or animals are usually expensive and beyond the reach of low income earners. Regular supply of food crops is also being threatened by possible crop failure, drastic climate change and lack of basic method of extending the shelf life of perishable goods which makes them susceptible to microbial attack and lack of accessibility, availability and sustainability throughout the year round.

Objectives of the study: This study focused on isolation and identification of strains of Lactic Acid Bacteria (LAB) from fermented cereal sorghum gruel.. The technological properties of the organisms were studied,and selected as starter for production of tiger nut yoghurt with a view to extending the shelf life of the highly perishable food product and improve the availability and nutritional quality of the starter produced yoghurt that can be used as weaning food.

Method: Strains of LAB were isolated from fermented cereal gruel Ogi prepared under strict hygienic conditions. They were identified using classical and molecular methods. Technological properties of the organismsinclude production of exo-polysaccarides, antimicrobial agents, enzymes, absence of biogenic amines were monitored using standard procedures. Yellow variety of tiger nut was obtained from Sabo market, in Ile-Ife, Nigeria. The nuts were sorted, cleaned, washed and surface sterilized and milled, sieved with 45um mesh size to obtain the milk. The milk was pasteurized at 85°C for 15minutes and fermented in three different batches..It was packaged as improved tiger nut yoghurt as weaning foods. The raw tiger nut and produced yoghurt were analyzed for their physicochemical, nutritional, anti-nutritional and organoleptic properties. The shelf life of the starter -mediated yoghurt was also studied.

Results: There were significant improvements in constituents of the tiger nut yoghurt compared with the raw samples. There was increase in nutritional composition and physicochemical parameters and a reduction in the anti-nutritional content of the yoghurt. The shelf life of the yoghurt was extended by the bio-preservative activity of LAB.

Conclusion: The starter-culture mediated yoghurt can solve the problem of malnutrition and lactose intolerance in weaning infants and therefore the research advances the field of Food and Nutrition Security measurement .

Keywords: Protein deficiency ; Tiger nut ; Nutritional improvement; Weaning food; Food Security

Exploring biodiversity in microbial ecosystems along the food chain

P1.9

Viability of intestinal *Lactobacillus* casei strain in traditional Labneh

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Labneh (concentrated yoghurt), as a transmission medium, was classified into four treatments; the first one was contained 2% free cells of *Lactobacillus (Lb.) casei* FEGY as control. Second, third and fourth treatments were included with 2% of encapsulated cells of *Lb. casei* with different capsule materials including sodium alginate, alginate- milk and κ-carrageenan served as T_1,T_2 and T_3 respectively. Results revealed that viability of both yoghurt starter cultures and *Lb. casei* were increased in the first week of cold storage (5±1 °C) and slightly decreased at 15 days. Moreover, the data showed that encapsulated *Lb. casei* by alginate- milk was more resistant to simulated gastric conditions compared to other treatments when exposure for 2h. Also, we observed significant ($P< 0.05$) differences in chemical properties during storage between different treatments whereas treatments that contained different encapsulated materials had a higher moisture content more than control. Moreover, the concentration of both acetaldehyde and diacetyl as flavor compounds was higher in Labneh contained microencapsulated *Lb. casei* than control.

Keywords: Intestinal lactobacilli, Labnah, Diet, Microencapsulation, Sustainability

Exploring biodiversity in microbial ecosystems along the food chain

P1.10

Development of a rapid PCR-based method to discriminate the flavour-forming *Macrococcus caseolyticus* species from related *Staphylococcus* species

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Macrococcus caseolyticus has been identified as a member of the secondary microflora of certain dairy products and proposed as a driver of flavour development due to its highly proteolytic nature. A PCR-based method for the rapid detection and discrimination of *M. caseolyticus* from related *Staphylococcus* species was developed based on a primer set designed to amplify part of the cytochrome c oxidase subunit II (*ctaC*) gene. The *ctaC* gene encodes one of the core subunits of the multi-subunit cytochrome c oxidase complex, the terminal enzyme complex in the electron transport chain. The majority of *Staphylococcus* species have an alternative terminal electron complex, and this difference between *M. caseolyticus* and *Staphylococcus* species can be exploited for discrimination purposes. Following PCR optimisation with a number of type strains of *M. caseolyticus* and other staphylococcal species, complex environmental sources including raw milk and bovine tongue were screened for the presence of *M. caseolyticus* using the primer set. Each *ctaC*-positive isolate was subsequently subjected to 16S rRNA analysis and confirmed to be of the *M. caseolyticus* species. Further analysis with PFGE revealed that thirteen distinct strains of *M. caseolyticus* were isolated. Therefore, the developed PCR-based method offers a rapid and efficient tool for targeting *M. caseolyticus* strains within complex microbial communities as well as discriminating this species from species of its sister genus *Staphylococcus.*

Keywords: flavour development ,rapid detection, complex microbial communities, PCR-based method

Exploring biodiversity in microbial ecosystems along the food chain

P1.11

Microbial ecology of the biofi lms presents at a pork meat industry with recurrent contamination of final products by *Listeria monocytogenes*

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Listeria monocytogenes remains one of the most important foodborne pathogens. The European Union (EU) has seen an increase in notifications of listeriosis outbreaks and sporadic cases. The European Food Safety Authority informed that, in 2013, 1763 confirmed human cases of listeriosis were reported in 27 member states. Almost all cases (99,1%) were hospitalized, which is the highest proportion of hospitalized cases of all zoonosis under EU surveillance. From December 2017, an enormous outbreak of listeriosis started in South Africa, with nearly 950 laboratory-confirmed cases and 180 deaths reported to the National Institute of Communicable Diseases (NICD). The current data show that *L. monocytogenes* is still a contemporary problem and it is difficult to control. Concretely, its ability to form biofilms makes its elimination a challenge for food companies. In natural environments, such as in food industry, biofilms are formed by multiple bacterial species. Mixed-species biofilms have been found to be more resistant to disinfectants and sanitizers than the rarely occurring, more investigated, mono-species biofilms.
The aim of this study was to analyze the ecology of biofilms from real work surfaces. The ultimate goal is to evaluate the effect of this surrounding microbiota on *Listeria monocytogenes*' growth.
This microbial ecology study was realized from a real pork meat cutting plant established in Spain. The analysis of the microbiota was conducted using sensors of surfaces. The sensors consisted in metal discs, which were included magnetically in 13 different points of the meat cutting plant (cutting board, trolley, soil, etc.). This evaluation of the microbiota present in the different work surfaces was carried out for 6 months. Finally, 595 microorganisms were isolated from the PCA (Plate Count Agar) and MRS (Man, Rogosa and Sharpe agar) culture media. Catalase, oxidase and Gram stain tests were also included. From these isolates, 199 microorganisms with different profiles were identified, including aerobic, lactic and yeast bacteria through API® systems (API 20E, API 20 NE, API 50 CHL, API 20 C AUX) and Crystal GP®. The largely predominant bacteria were *Pseudomonas sp.*, *Bacillus sp.* and *Leuconostoc mesenteroides ssp. cremoris.* The yeasts as *Candida zeylanoides* represented 30 % of the isolated microorganisms. This whole study was also contrasted with a metagenomic study of the 13 sensors to confirm the presence of the identified bacterial groups.

Keywords: Listeria monocytogenes, biofilms, microbial ecology, pork meat industry, contamination

Exploring biodiversity in microbial ecosystems along the food chain

P1.12

Functional properties of yeast strains isolated from raw ewe's soft-cheese

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The term probiotic is currently used to name 'live microorganisms when are administered in adequate amounts, confer a health benefit on the host, mainly in digestive and immune system'. While many bacteria species have been characterized as probiotics, only one yeast strain, *Saccharomyces cerevisiae* var. *boulardii*, has been recognized as part of this group of microorganisms. However, recent researches have shown that other yeast species commonly found in fermented food as *Kluyveromyces*spp. and *Pichia kudriavzeii* can act as potential probiotic. Due to the interest of the food industry in microorganisms with functional properties, the aim of the present study was to evaluate the probiotic potential of yeast isolated from traditional soft cheese. The identification of isolates was performed by ISSR-PCR, targeting $(GTG)_5$ microsatellite and gene sequencing of the 26S rRNA. Yeasts isolates with different ISSR-PCR profile and origin were tested for their ability to survive to low pH of the stomach in the range of pH from 2 to 3 for 3 hours and grow in YPD agar supplemented with bile salt at 1% and 2% (w/v). To evaluate the survival to entire gastrointestinal transit conditions, yeast isolates were exposure to simulated gastric juice at final pH adjusted to 2.5 for 2 hours followed by 6 hours in simulated intestinal juice at pH 8. Furthermore, antioxidant capacity was determined as described Chen *et al.* (2010; IJDT 63(1), 47-54) and finally the potential of adhesion of each strain to intestinal epithelial cells was evaluated by auto-aggregation and hydrophobicity test as described by Binetti *et al.* (2013; JAM 115(2), 434-444). A total of 167 strains were isolated from different soft chesses from Extremadura region. *D. hansenii* and *K. marxianus* were found to be the dominant species, followed in lower proportion by *Candida* spp. *and Pichia* spp. Almost 49% of the examined yeast isolates showed good adaption to the *in-vitro* gastrointestinal conditions studied. *Pichia fermentans* showed the highest capacity (59.65%) to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl). Regarding the auto-aggregation and hydrophobicity ability, three strains of *D. hansenii*, two *K. marxianus* and a *K. lactis* showed values higher than 75% in both assays. In conclusion, although further in vitro and in vivo investigations are required, these six yeast strains are promising as potential candidates to be used as probiotics in traditional soft cheese productions.

Keywords: Yeast, probiotics, gastrointestinal transit, raw ewe´s soft cheese

Exploring biodiversity in microbial ecosystems along the food chain

P1.13

Lactic acid bacteria and yeasts diversity in spontaneously fermented grain amaranth *(Amaranthus hybridus)* products

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Grain amaranth (*Amaranthus hybridus*) is an underutilised pseudo-cereal whose potentials are enormous, yet unexploited, in the Nigerian food industry. Limited research has been carried out on the production of pseudo-cereal-based foods for nutrition and food security. Lactic acid bacteria (LAB) and Yeasts diversity in spontaneously fermented grain amaranth (*Amaranthus hybridus*) products were investigated. Grain amaranth (*Amaranthus hybridus*) was spontaneously-fermented, a weaning porridge (Amaranth-*ogi*) and a non-alcoholic beverage (Amaranth-*kunu*) were developed. Lactic acid bacteria (LAB) and Yeast cultures were isolated, screened and identified based on morphological, biochemical and sugar utilisation patterns. The temperature, pH and Total Titratable Acidity (TTA) of Amaranth-*ogi* and Amaranth-*kunu* were also determined using standard methods. The pH of Amaranth-*ogi* samples ranged from 4.50 - 4.74, while Amaranth-k*unu* samples was 4.49 - 5.79. The pH of the samples decreased with increased fermentation time, TTA and temperature increased with increased fermentation time while the mesophilic bacterial count followed the normal bacterial growth curve. *Lactobacillus plantarum*, *Saccharomyces cerevisae*, *Saccharomyces exiguus*, *Pichia membranefaciens*, and *Schizosaccharomyces pombe* were isolated from the Amaranth-*ogi* and Amaranth-*kunu* samples. A non-alcoholic beverage (Amaranth-*kunu*) and breakfast porridge (Amaranth-*ogi*) were developed from *Amaranthus hybridus*. The Lactic acid bacteria (LAB) and Yeast cultures in the Amaranth-*ogi* and Amaranth-*kunu* could be applied as starter cultures for future large-scale production of Amaranth-*ogi* and Amaranth-*kunu* with improved quality, thereby, contributing to the SDG-2, zero hunger.

Keywords: Lactic acid bacteria, Yeasts, Fermentation, Amaranthus hybridus, Amaranth-ogi and Amaranth-kunu.

Exploring biodiversity in microbial ecosystems along the food chain

P1.14

Traceability of *Listeria monocytogenes* strains along food processing chains

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Listeria monocytogenes (*Lm*) contamination is one of the leading microbiological causes of food recalls. The global improvement of hygienic practices in food industry, including both better cleaning and disinfection methods and a more widespread use of refrigeration, reduced the prevalence of most foodborne diseases. Nevertheless such treatments may be ineffective on *Lm*, in fact due to its physiological characteristics, such as growth at low temperatures, tolerance to low pH, high salt concentration and heat treatment *Lm* may well survive in the industrial food environment in which a wide range of potential microbial competitors were eliminated. There is marked strain variation in how the environment influences *Lm* survival under stress conditions and unequivocal identification of *Lm* isolates is essential in public health management, for surveillance, epidemiological tracking, and outbreak investigation purposes. Four hundred strains were isolated from food matrices and food industries over four years (2013-2016) and identified as *Lm* with microbiological procedures. The survey included 87 plants of food processing (including meat, dairy, vegetable and fish processing plants) all located in the Trentino region (North-East of Italy). From *Lm* isolate collection, 323 were typed using a 2bRAD-based Next-Generation Sequencing (NGS) approach applying a scheme developed and tested previously (Pauletto et al 2016, doi: 10.1111/1755-0998.12495). The complete dataset comprised 355 unique genotypes including the 323 isolates, two reference strains (ATCC19117 and Scott A) and 30 isolates of *Lm* belonging to the previous study. The analysis of the 355 unique genotypes with Phylovitz software identified 38 different Rad-Types (RTs), of which 24 belonged to lineage II, 11 to lineage I and one to lineage III. The RT1 resulted the most represented with 98 isolates. Five RT were isolated in all the four years (RT1, 2, 5, 6, 9). These data were used to define the population structure and to evaluate the distribution of *Lm* strains in the different plants in order to make a hypothesis about the potential contamination sources. The recurrence of RT in plants in different years confirms the ability of *Lm* to persist in food industry environment.

Keywords: Listeria monocytogenes, food processing plants, molecular typing, traceability

Exploring biodiversity in microbial ecosystems along the food chain

P1.15

The effect of growth conditions on the biofilm formation of *Listeria monocytogenes*

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Listeria monocytogenes is ubiquitous in nature and a major concern for the food industry, since it is the causal agent of the serious foodborne illness listeriosis. This organism can be introduced through many routes to food-processing environments and may become established on food-processing equipment. Subsequently, food products may become contaminated during processing. Biofilms are regarded as important with respect to the survival and growth of microorganisms in the food industry. Consequently, microorganisms growing in biofilms are protected against cleaning and disinfection and are difficult to eradicate. *L. monocytogenes* may grow in biofilms that protect them against environmental stress and can be isolated from surfaces after cleaning and disinfection. In this study, a total of nine *L. monocytogenes* strains isolated from the meat industry were studied for their capability to form a biofilm. The biofilm forming behavior of *L. monocytogenes* strains was determined in two different media such as Tryptone soya yeast extract broth (TSYEB) or Brain-heart infusion broth (BHIB) at temperatures 7 ºC, 25 ºC, 37 ºC, 42 ºC after 5 days. The method used to assess biofilm formation was crystal violet staining. All strains were able to form biofilm, but the growth condition affected the levels formed. Biofilm formation of *L. monocytogenes* strains significantly increased with temperature and the highest biofilm formation was observed at 37 ºC. The incubation temperature was the most significant factor influencing the biofilm production levels, and also the nutritive medium used was important factor.

Keywords: biofilm formation, Listeria monocytogenes

Exploring biodiversity in microbial ecosystems along the food chain

P1.16

Adhesion of *Candida* spp. and *Pichia* spp. to stainless steel surfaces under various growth conditions

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Microbial adhesion and biofilm formation to surfaces is of great environmental, medical and industrial importance and consequently draws considerable attention in the last decades. The persistence of microorganisms in biofilms is a serious hygienic problem in the food industry, causing processing and post-processing cross-contamination leading to reduced product shelf life and effectiveness of sanitizing treatments as well as potentially affecting the consumer's health. Despite the research efforts devoted on bacterial adhesion, very little information is available on the adhesion behaviors of *Candida* spp. and *Pichia* spp. onto stainless steel surfaces, although these yeasts are usually contaminants in the food industry. Hence, in this study we investigated the impact of growth medium and temperature on *Candida* and *Pichia* adherence using stainless steel (AISI 304) discs with different degrees of surface roughness (Ra = 25.20 - 961.9 nm). The adhesion of the yeast strains to stainless steel surfaces grown in Malt Extract broth (MEB) or YPD broth at three temperatures (7°C, 37°C, 43°C for *Candida* strains and 7°C, 27°C, 32°C for *Pichia* strains) was assessed by crystal violet staining. The results showed that the nutrient content of medium significantly influenced the quantity of adhered cells by the tested yeasts. Adhesion of *C. albicans* and *C. glabrata* on stainless steel surfaces were significantly higher in MEB, whereas for *C. parapsilosis* and *C. krusei* it was YPD broth. In the case with *P. pijperi* and *P. membranifaciens*, YPD broth was more effective in promoting adhesion than MEB. On the other hand, our data indicated that temperature is a very important factor which considerably affects the adhesion of these yeast. There was also significant difference in cell adhesion on all types of stainless steel surfaces for all tested yeast. An understanding of adhesion behavior of *Candida* spp. and *Pichia* spp. under different environmental conditions is key to the development of effective preventive measures against biofilm-associated infection.

Keywords: adhesion, yeast, stainless steel surfaces, growth medium, temperature

Exploring biodiversity in microbial ecosystems along the food chain

P1.17

Metagenomic analysis of the spoilage microbiota of Asian seafood

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Introduction: Seafood products are generally subject to fast spoilage. The huge variety in packaging and processing methods likely results in a variety in dominant spoilage flora as well. Classical isolation and identification of spoiling microorganisms is a slow and labor intensive process and it is subject to biases. Metagenomics enables analysis of the spoilage flora more cheaply and less labor intensive, inevitably leading to establishment of product segment associated microbiota.

Purpose: The current study is to analyze the dominant spoilage flora in seafood from Asian market stored under "wet market conditions" (2 days at 30℃) and at "refrigerator conditions" (30 days at 10℃).

Methods: Multiple samples (15 in total) were bought in Thailand, Singapore and Indonesia. 85% of the samples were bought in the supermarket (65% from refrigerator section, 20% from the freezer) and 15% from the wet market. Samples were frozen after purchasing at -28°C. Frozen samples were shipped to the laboratory in the Netherlands and stored at -28°C until analyzing. Seven samples were stored for 30 days at 10°C and eight samples were stored for 2 days at 30°C. At the end of incubation time the total plate count, pH and water activity (aw) were measured. The microflora was analyzed by metagenomics.

Results: Leuconostoc mesenteroides was the most dominant species in seafood stored for 30 days at 10°C.
Bacillus paraflexus was the most dominant species in seafood stored for 2 days at 30°C. Overall the main flora of seafood obtained from the Asian market contains lactic acid bacteria, *Bacillus*, *Brochotrix* and *Sp. psychrophila*. There was no obvious difference in type of product or the history of the samples (refrigerated/frozen or wet market).

Significance: A better understanding of the dominant spoilage flora will lead to better and specific interventions to elongate shelf life.

Keywords: Seafood, Metagenomics, Spoilage

Exploring biodiversity in microbial ecosystems along the food chain

P1.18

Molecular characterization of lactic acid bacteria isolated from fresh fruits and vegetables

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Background & Objectives: Lactic acid bacteria (LAB) have been studied for their commercial potential in food preservation and health benefits. Identification of LAB on the basis of carbohydrate fermentation patterns is unreliable and not accurate enough to distinguish closely related strains. This study investigated the diversity of LAB isolated from fresh fruits and vegetables using Molecular techniques with the view of identifying LAB with anti-microbial potentials.

Method(s) & Results: LAB associated with fresh fruits and vegetables were investigated using a polyphasic approach that encompassed both classical microbiological and DNA-based methods in order to get an overview of the important bacteria. Isolates were identified based upon the sequences as Weissella cibaria (5 isolates, 27.78%), Weissella kimchi (5, 27.78%), Weissella paramensenteroides (3, 16.67%), Lactobacillus plantarum (2, 11.11%), Pediococcus pentosaceus (2, 11.11%) and Lactobacillus pentosus (1, 5.56%) from fresh vegetable; while Weissella cibaria (4, 18.18%), Weissella confusa (3, 13.64%), Leuconostoc paramensenteroides (1, 4.55%), Lactobacillus plantarum (8, 36.36%), Lactobacillus paraplantarum (1, 4.55%) and Lactobacillus pentosus (1, 4.55%) were identified from fresh fruits.

Conclusions: This study shows that LAB can be quickly and holistically characterized by molecular methods to specie level by nested PCR analysis of isolate genomic DNA using universal 16S rRNA primers and LAB specific primer.

Keywords: Nested PCR; Molecular characterization; 16S rRNA gene; Lactic acid bacteria.

Exploring biodiversity in microbial ecosystems along the food chain

P1.19

Specification of lactic acid bacteria and yeasts in traditional Austrian sourdoughs

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Traditional sourdough is characterized by the absence of starter cultures and its regular propagation and back-slopping. Due to the fermentation by Lactic Acid Bacteria (LAB) and yeasts, sourdough breads exhibits some major advantages, like improved baking properties, nutritional benefits, superior shelf life, and sensory quality.
In Austria, data on the diversity of LAB and yeasts in traditional sourdoughs is still lacking. Therefore, the aim of this study was to characterize the microbiota of traditional rye and wheat sourdoughs regarding the previously mentioned microorganisms. Samples were obtained from artisan bakeries as well as households.
To cultivate a broad spectrum of LAB, six culture media, differing in their composition, were used. Enumeration revealed various counts ranging from less than 10^6 to more than 10^9 cfu g^{-1} sample. Compared to yeasts, LAB were present in doughs at a ratio of up to 1:1000. Due to different morphologies, LAB isolates were selected and further analyzed by 16S rDNA-sequencing and MALDI-TOF MS (mass spectrometry). This led to the detection of five different genera: *Lactobacillus*, *Weissella*, *Leuconostoc*, *Pediococcus*, and *Streptococcus*. Within these genera, *Lactobacillus* showed the highest diversity. Furthermore, the application of rep-PCR revealed differences on subspecies/strain level.
For the enumeration and isolation of yeasts, four media were used. Further, 26S rDNA-sequencing as well as MALDI-TOF MS were applied for identification. This led to the detection of the genera *Saccharomyces*, *Candida*, *Pichia*, and *Kazachstania*, whereat *Saccharomyces cerevisiae* was the most abundant species.
This work forms the basis for the preparation of bakery products by sourdough fermentation, whereat certain isolates should be used to gain superior baking properties.

Keywords: traditional sourdough, lactic acid bacteria, yeasts

Exploring biodiversity in microbial ecosystems along the food chain

P1.20

Dynamics and biodiversity of bacterial and yeast communities during fermentation of cocoa beans

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Forastero hybrid cocoa bean fermentations were carried out in Box (B) and in Heap (H) with or without the inoculation of *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* as starter cultures. Bacteria, yeasts and microbial metabolites (volatile and non-volatile organic compounds) were monitored during fermentation in order to assess the link between microbiota and the release of metabolites during this process. The presence of starter cultures was detected during the first two days of fermentations by means of culture-dependent analysis. However, it did not show statistical difference in any physico-chemical or microbiological analysis. Plate counts revealed the dominance of yeasts at the beginning of the fermentation followed by acetic acid bacteria (AAB) and lactic acid bacteria (LAB). *Hanseniaspora opuntiae, S. cerevisiae, Pichia, Acetobacter pasteurianus* and *Lactobacillus fermentum* were the most abundant OTUs during both fermentation processes (B and H), reporting different relative abundances. Only the diversity of fungal species indicated a higher level of complexity in B compared to H fermentations ($P < 0.05$) and also revealed a statistically significant difference between starter cultures initially inoculated ($P < 0.01$). However, the analysis of microbial metabolites indicated different distribution of volatile and non-volatile compounds between the two procedures B and H ($P < 0.05$), rather than between the inoculated and non-inoculated fermentations. Box fermentations showed a faster carbohydrate metabolism and higher production of organic acid compounds than in heap fermentations, which boosted the formation of alcohols and esters. Overall, the microbial dynamics and associations between bacteria, yeast and metabolites were found to depend on the type of fermentation. In spite of the limited effectiveness of the starter strains inoculated, this study provides new information on the microbial development of Box and Heap cocoa fermentations, by coupling for the first time yeast/bacteria amplicon-based sequencing data with microbial metabolites detection. The information so far available suggests that microbial communities have been an important factor in the evolution of aroma compounds. Understanding the pathways taken place during the formation of aroma by micro-organisms could be used to improve fermentation processes and to enhance chocolate quality.

Keywords: Cocoa beans; fermentation; yeast; bacteria; volatile and non-volatile organic compounds

Exploring biodiversity in microbial ecosystems along the food chain

P1.21

Myopathies of broiler chickens breasts affect microbial targets and shelf life

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White Striping (WS) and Wooden Breast (WB) are emerging issues of the broiler production affecting the quality of *Pectoralis major* muscle. Breasts present white stripes in the case of WS and are macroscopically pale, hard, and with bulging areas in the case of WB. Both myopathies decrease product commercial value depending on their degree. Histopathological examination reveal lipidosis, fibrosis, necrosis and regeneration of muscle fibres. Myopathies also lead to changes on qualitative attributes as final pH, color, chemical composition, in particular a decrease in protein content and an increase in lipid content. Few information are available on the microbial ecology of WS and WB stored under refrigeration. Thus, this work aimed to explore changes of several microbial targets as total viable count (TVC), *Pseudomonas*, LAB (lactic acid bacteria), *Enterobacteriaceae* and hydrogen sulphide-producing bacteria. The observations were carried out on 144 *Pectoralis major*: 48 without myopathy (N), 48 of WB and 48 with WS. All breasts were stored at 4°C for 10 days in plastic film, without compositional modifications of the air, and exposed to artificial light. Samples were analysed at six different times, every 2 days, during the refrigeration period. A multivariate statistical approach allowed the definition of the microbial and physicochemical changes of breasts during the shelf life. Permutational multivariate analysis of variance and nonparametric-combination test display changes on microbial profiles and targets with the exception of H_2S-producing bacteria. Non-metric Multi-Dimensional Scaling plot and Hierarchical Cluster analysis clearly show that normal samples had different behaviours than WB and WS. Estimated growth parameters obtained using Baranyi and Roberts models described a shorter Lag phase (TVC and Pseudomonas) on breasts without myopathies. Considering the acceptable threshold of 7.3 $\log_{10}$ colony forming unit g^{-1} for *Pseudomonas*, WB samples showed 32 h increased shelf life compared to breasts without abnormalities. Distance-based multivariate analysis DISTLM using forward selection procedure extrapolated the physicochemical features significant in explaining the variation of microbial data. Shear force, water and crude protein contents were the most relevant selected features. These results suggested that, due to their different muscle structure and composition, breasts with myopathies can harbour different microbial populations.

Keywords: Broiler; White Striping; Wooden Breast; multivariate analysis

Exploring biodiversity in microbial ecosystems along the food chain

P1.22

Microbiological quality of sea urchin *Paracentrotus lividus* – Influence of harvesting waters contamination and potential as antimicrobial resistance vehicles

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Sea urchin *Paracentrotus lividus* is a grazer-feeding echinoderm. Gonads are the only edible part of sea urchins and they are usually consumed raw. Although sea urchins are included in legislation regulating bivalve safety, few studies have been carried out to evaluate sea urchin microbial contamination. The aim of this work was to study the levels of bacterial contamination on sea urchin gonads, coelomic fluid, superficial biofilm and water column associated during all seasons, and evaluate the potential of sea urchin as vehicle for antibiotic resistance.

Sea urchin and water column were collect between September 2016 and May 2017 from two natural nurseries located in the north of Portugal (n=8). Total amount of aerobic bacteria, Enterobacteriaceae, *Escherichia coli*, *Enterococcus* spp. coagulase-positive staphylococci, *Clostridium perfringens*, *Salmonella* spp. and *Listeria monocytogenes* were determined following international standard methods. Aerobic bacterial counts and faecal indicators were evaluated on superficial biofilm and coelomic fluid samples. Study of antibiotic resistance was performed in *E. coli* and *Enterococcus* spp. isolated from all samples following CLSI guidelines. An isolate was considered multidrug resistant (MDR) when was not susceptible to 3 or more antibiotics from different families.

All sea urchin seasonal samples presented results for *E. coli*, *Salmonella* spp. and *L. monocytogenes* within the sanitary limits imposed by legislation. *Salmonella* spp. was isolated from harvesting water. Levels of aerobic bacteria on superficial biofilm were 2 log higher than in the internal content samples of sea urchins. Water contamination was not reflected on gonad or coelomic fluid bacteria levels. From all *E. coli* isolated 40% were MDR. 88.5% of them were isolated from water samples including 3 strains producers of beta-lactamases of broad spectrum (ESBL). *Enterococcus* spp. isolated presented a lower level of MDR (26.8%) with the majority of *Enterococcus* MDR being isolated from water samples (81.8%) and none isolated from gonads. Resistance to carbapenems and vancomycin were not detected, respectively, in *E. coli* and *Enterococcus* spp. studied. Although harvesting waters represents a major threat to sea urchin safety, levels of bacteria on sea urchin gonads were satisfactory and a low prevalence of MDR bacteria were detected on this food matrix, characterizing them with low potential to be carriers of antibiotic resistance transmission.

Keywords: Sea urchin, MDR, antimicrobial resistance, faecal contamination, food safety

Exploring biodiversity in microbial ecosystems along the food chain

P1.23

Effect of temperature treatment on postharvest quality of the cherry tomato (*Lycopersicon esculentum* var. *cerasiforme*)

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Heat accumulation in the field accelerates softening and shortens the shelf life of cherry tomatoes. The purpose of this study was to investigate the effect of temperature treatment after harvest on the postharvest quality and microbial communities of cherry tomatoes. Regarding fruit quality properties, a 10° C treatment after harvest caused a delay in increasing weight loss, firmness, electrolyte leakage and decay rate. After the temperature treatment, an approximately 1-2 log reduction on total viable cells, fungi, Enterobacteriaceae and coliforms was observed in the 10 °C treatment group compared with those at 20 and 30 °C. However, total viable cells and decay rate remarkably increased at 10 °C treatment after 26 days. The microbial communities in cherry tomatoes after temperature treatment were composed mainly of *Staphylococcus xylosus* and *Bacillus pumilus.* During storage, the relative abundance of *Staphylococcus xylosus* and *Bacillus pumilus* decreased while *Pseudomonas fluorescens, Rahnella aquatilis* and *Leuconostoc mesenteroid* increased during late storage. The rapid increase of total aerobic cells and decay rate in the 10 °C treatment group may be associated with a marked increase in *Pseudomonas fluorescens. The results* from this study demonstrate the effectiveness of pre-cooling to suppress the softening and indicate an impact on microbial community composition and dynamics during storage.

Keywords: Cherry tomato; Pre-cooling; Postharvest quality; Microbial community composition

Exploring biodiversity in microbial ecosystems along the food chain

P1.24

Cabbage to sauerkraut: Characterization of bacterial ecosystem dynamics throughout the natural fermentation

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To produce sauerkraut, cabbage leaves, previously cut into strips, are salted and placed under anaerobic conditions in containers, so that the natural lactic fermentation can start. Described in literature, the spontaneous fermentation occurs in three different phases of pH lowering and a succession of species of *Leuconostoc*, *Lactobacillus* and *Pediococcus* manages these phases. The quality of sauerkraut obtained in this way depends on the substrate, the production environment, but above all on the natural bacterial population. Currently, there is little information on the bacterial ecosystems of cabbage and sauerkraut. To achieve a better control of product quality, it is necessary to determine the diversity of the bacterial population in cabbage and its dynamics during the fermentation in relation to physico-chemical changes.

Throughout the fermentation, 54 samples were taken (6 fermentation stages*9 replicates). The physico-chemical monitoring of fermentation was carried out by measuring pH, titratable acidity, lactic acid and acetic acid levels, glucose and fructose concentrations. To characterize the bacterial diversity, the 16S rDNA of 29 samples (4 fermentation stages) was amplified for Illumina Hiseq sequencing. Reads were then clustered into Operational Taxonomic Units (OTU). Abundance of bacterial species was quantified by qPCR.

The 29 samples provided 1,221,090 bacterial 16S rRNA sequences, which were analyzed and clustered into 111 OTUs. The bacterial diversity of salted cabbage was mainly composed of *Proteobacteria* species such as *Pectobacterium carotovorum, Pantoea agglomerans* or even *Acinetobacter rhizosphaerae*. At the beginning of fermentation, *Leuconostoc mesenteroides* was dominant and remain during the two first stages of fermentation. Combined to this *Leuconostoc* species, other bacteria, such as *Lactobacillus curvatus* or *Leuconostoc fallax*, were predominant and certainly contributed to the fermentation process. At the end of the fermentation, changes in bacterial diversity were observed. *Lactobacillus silagei*, *Pediococcus parvulus* and *Lactobacillus plantarum* were predominant whereas they were subdominant at the beginning of fermentation.

The development of lactic acid bacteria, leading to the acidification of products, makes sauerkraut unfavourable to the development of *Proteobacteria* and more specifically *Enterobacteria*.

Keywords: Sauerkraut, Spontaneous fermentation, natural bacterial ecosystem, lactic acid

Exploring biodiversity in microbial ecosystems along the food chain

P1.25

Enterobacteriaceae in the production chain of organic lettuce (*Lactuca sativa* L.) grown in Sao Paulo, Brazil

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The consumption of organic produce has increased worldwide over the past decades, due to its association with a healthy diet without chemical hazards. On the other hand, several foodborne illnesses have been associated to the consumption of these products, which can become contaminated with pathogenic microorganisms along the production chain. The aim of this study was to enumerate and identify *Enterobacteriaceae* in different points of the production of organic lettuce grown in eight certified farms, located in Sao Paulo, Brazil. The presence of *Salmonella* spp. using conventional and molecular (real-time PCR) methods was also investigated. Samples of fertilizer (n=24), irrigation water (n=24), washing water (n=24) and lettuces harvested directly from the field (n=40) and after a washing step at the farms (n=40) were collected in these farms. Total counts of *Enterobacteriaceae* were obtained by plating on violet red bile glucose agar. The identification (genus and species) of these bacteria, conducted for samples from three farms, was performed by using a MALDI-TOF MS Biotyper™. The average counts of *Enterobacteriaceae* were: fertilizer (4.9±2.1 log CFU/g), irrigation water (2.2±0.7 log CFU/mL), lettuces from the field (4.6±0.8 log CFU/g) and washed lettuces (4.1±1.1 log CFU/g). Only one farm presented counts in the wash water (2.03±0.1 log CFU/mL). No correlation was observed between *Enterobacteriaceae* counts in samples of fertilizer, irrigation and wash water with those obtained in lettuce samples. Regarding the identification of these bacteria, it was possible to identify 24 species. *Hafnia alvei* was the most prevalent bacteria identified in lettuce samples collected from the field (30.8%) and after washing step (36.7%). In fertilizers, irrigation and washing water, the most prevalent bacteria were *Citrobacter sedlakii* (33.3%), *Morganella morganii* (42.9%) and *Enterobacter asburiae* (60.0%), respectively. *Salmonella* spp. was not isolated from any sample but was detected by real-time PCR in one sample of washed lettuce. Despite the low occurrence of *Salmonella* spp., other *Enterobacteriaceae* species were detected in the production chain, indicating conditions for the presence of enteropathogens, which may pose health risks to consumers.

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Keywords: Enterobacteriaceae, lettuce, organic agriculture, Salmonella spp.

Exploring biodiversity in microbial ecosystems along the food chain

P1.26

Biodiversity of cheese-damaging clostridia in milk and cheese

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Clostridia cause severe spoilage by producing excessive amounts of gas and butyric acid during ripening of hard and semi-hard cheese. In previous studies, more than 500 isolates from milk and cheese had been assigned to the genus *Clostridium*. Of these isolates, 140 strains from different origin were selected for further analyses. Differentiation on the species level was performed using 16S rDNA sequencing and revealed *C. tyrobutyricum* as the predominant species (66,4 %) followed by *C. sporogenes* (11,4 %) and *C. bifermentans* (7,9 %). *C. tyrobutyricum* is considered the main cause of clostridial cheese spoilage. However, differences have been observed among clostridia on a lower taxonomic level suggesting characteristic properties on the subspecies or strain level. The aim of this study was to differentiate dairy-relevant clostridia on the subspecies level. For this purpose, MALDI TOF MS profiling was compared with nucleic acid-based approaches and phenotypic properties of pure clostridial spore suspensions. Interestingly, similar clustering of isolates of *C. tyrobutyricum* was observed according to patterns obtained from ribosomal protein and DNA analyses. Taking into account also the phenotypic variations, differentiation of clostridia on the subspecies level may be useful to identify potential contamination routes or assess the possible impact of clostridia on cheese quality.

Keywords: Clostridium, late-blowing, milk, cheese, typing

Exploring biodiversity in microbial ecosystems along the food chain

P1.27

Microbiological ecology of millets sourdough

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Background & Objectives: Millet is an underutilized cereal grown in Nigeria and sourdough fermentation was identified as one of the processing method that may transform it to acceptable and convenient foods. Insight into the microbial diversity and population dynamics during millet fermentation is an initial but imperative step in the development of starter cultures and standardization of millet for large scale production of millet sourdough baked products.

Method(s) and Results: In this study, two varieties of millet flour were spontaneously fermented for 72 h to obtain sourdough and samples taken at 12 h intervals for analysis. Microorganisms associated with spontaneous fermentation of the millets were investigated using a polyphasic approach that encompassed both classical microbiological and DNA-based methods in order to get an overview of the important bacteria and yeast. Sequencing of the 16S rRNA encoding genes clearly revealed that a cocktail of microorganisms was associated with the millets sourdough, they are LAB *(Leuconostoc lactis, Lactobacillus curvatus, Pediococcus pentosaceus, Lactobacillus plantarum, Leuconostoc citruem, Lactococcus lactis, Enterococcus sp. and Weisella confusa),* yeast *(Pichia kudriavzeii, Candida inconspicua, and Candida glabrata)* and other organisms including *Klebsiella sp.* and *Cronobacter helveticus*.

Conclusions: The study revealed the microbial ecology, succession pattern and frequency of occurrence of the isolated organisms during fermentation of the millet sourdough.

Significance and Impact of the Study: This study forms an essential first step towards the development of starter cultures to produce millet sourdough of consistent quality, an intermediate product for the development of sourdough bread.

Keywords: Millet, Spontaneous fermentation, Sourdough, Microbial profile

Exploring biodiversity in microbial ecosystems along the food chain

P1.28

Quantifying LAB populations in raw milk and dairy products through a Real Time PCR assay

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Raw milk, and its derived products, are a complex environment with microbial populations belonging to several different taxonomic groups. Until now, knowledge on the identification of microorganisms in these matrices has been gained mainly through culturing methods and, possibly, with phenotypic typing analysis. However, often these techniques lack a sufficient discrimination power and do not allow to consider those bacteria adverse to cultural growth, leading to problems of underestimation of the microbial populations. In the last years, NGS allowed a more accurate estimation of the microbial diversity. However, this technology requires costs and times of analysis that not all the laboratories are able to bear. For these reasons we developed a qReal Time PCR assay to allow the rapid quantitative estimation of the main LAB populations that are naturally present in raw milk and have beneficial effects on the cheesemaking process. In particular, we focused on *Streptococcus thermophilus*, *Leuconostoc mesenteroides*, *Lactococcus lactis*, *Lactococcus garviae*, and *Lactobacillus* spp., and consequently five pairs of specific primers were identified after bibliographic research and *in silico* testing. The reactions were initially tested on certified strains grown at their optimum conditions to ensure an acceptable CFU/mL quantitation. The reactions were carried out using the SsoAdvanced™ Universal SYBR® Green Supermix (Biorad). Evaluation of positive reactions was pointed out by observation of amplification plot and melting curve data. Standard curves were generated by plotting the cycle threshold values (Ct) of the qPCR perfomed on diluition series of purified DNA from the different strains against their correspondent log input cells mL^{-1}. Cells concentrations were calculated by the viable cell plate count method, plating tenfold diluition of the cultures on BHI, incubating for 2 days and determining CFU in duplicate. Subsequently the assay was tested on field samples of raw milk, curd, and cheese in different moments of the aging. The developed method will have the double function of i) assessment of the suitability of raw milk for cheesemaking due to the presence of non-starter LAB populations beneficial for the process, ii) monitoring of the dynamics of the considered populations throughout the aging, to help understanding how they may influence the final quality of the product.

Keywords: dairy; enumeration; PCR; quantification; Lactic Acid Bacteria; Real-time

Exploring biodiversity in microbial ecosystems along the food chain

P1.29

The effect of different dry ageing processes on spoilage microbiota of beef

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Ageing is a process that increase the tenderness of the meat due to the post-mortem action of endogenous muscular enzymes. This process can be performed by three different methods: wet aging, dry ageing and special bag. Dry ageing is one of the main types of ageing used to produce unique flavoured, value-added beef. Recently, a new dry ageing process using a highly moisture-permeable bag was introduced to the market to improve the traditional unpackaged dry aging. However, there are few reports about the impact of these processes on the natural microbiota of beef. Therefore, the aim of this study was to investigate the combined effects of two ageing methods (dry and special bag), two relative humidities (65 and 85%) and two ageing periods (21 and 42 days) on the microbiological characteristics of the surface and inner part of the meat. Analysis of the aerobic mesophilic microorganisms, psychrotrophics, enterobacteriaceae, lactic acid bacteria and yeast and mould were performed at the beginning and at the end of each ageing time. At 85% of relative humidity the highest counts for all microbial groups were observed. After 21 days aerobic mesophilic and psychrotrophic counts reached values > 7 log cfu/g in both ageing processes. At 65% of relative humidity, lactic acid bacteria and enterobacteriaceae counts remained under the limit of detection (1 log cfu/g) throughout the storage for both ageing methods. Higher counts of psychrotrophics and yeasts and moulds, were obtained on the surface of the samples aged in special bag (4.7 and 3.2 log cfu/g), when compared to dry aged (2.8 and 2.1 log cfu/g). On the other hand, aerobic mesophilic counts were higher in the dry ageing (4.12 log cfu/g) than in the special bag process (1.39 log cfu/g) at 21 days. When the inner part of the meat at 65% of relative humidity was evaluated, dry ageing resulted in higher counts of psychrotrophic than special bag at both ageing times. In conclusion, in the experimental conditions carried out in this study, the ageing processes at 85% of relative humidity are unworkable. However, at 65% of relative humidity, both the dry ageing and special bag processes resulted in counts < 5.7 log cfu/g for up to 42 days.

Keywords: dry ageing, special bag, beef, psychrotrophic, latic acid bacteria

Exploring biodiversity in microbial ecosystems along the food chain

P1.30

A survey of the microbiota associated with ready-to-eat mixed salads from field to retail

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Food of non-animal origin is a major component of the human diet and has been considered to pose a low risk regarding bacteriological safety. However, the numbers of outbreaks caused by food borne pathogens linked to the consumption of fresh vegetables have increased around the world. Among these, *Salmonella* spp., STEC and *Listeria monocytogenes* are the most frequently identified causative pathogens. Food processors, especially those who produce ready-to-eat (RTE) products like mixed salads, need to be vigilant of these pathogenic bacteria. This work aimed to determine the occurrence of human pathogens and the diversity of microbiota in a traditional mixed salad processing facility over twelve months. A total of 861 samples, including 734 environmental samples (swabs, contact plates, water- and air samples) and 127 product samples along the chain from field to retail were collected and investigated. Only 4 of 734 environmental samples were positive for *L. monocytogenes* (serovar IVb/IIb) and 2 were positive for enteropathogenic *E. coli* (O63:H6 and O96:H7). *Salmonella* serovars and shiga toxin-producing *E. coli* were not detected. However, three product samples were positive for shiga toxin-producing *E. coli* (O146:[H28]) or *L. monocytogenes* (serovar IVb/IIb). The second aim of this study was to determine possible influences of the processing environment on the initial microbiota of fresh cut salad. For this, the microbiota of three different salads (*Lactuca sativa* var. *longifolia*, *Cichorium endivia* and *Lactuca sativa* var. *crispa*) was analyzed by next generation, 16S Amplicon sequencing of samples from the production process on the field, or taken during storage, processing and after packaging. The 16S rRNA gene sequencing analysis of the microbiota showed that core microbiota was shared by most of the samples and included *Proteobacteria* (47-91 %), *Firmicutes* (1-20 %), *Bacteroidetes* (2-19 %) and *Actinobacteria* (5-22 %). Moreover, the microbiota was unique for the different salad plants and stable after storage and processing (cutting away of the outer leaves). Nevertheless, the washing process significantly changed the composition of microbiota with a shift to pseudomonads. Our findings indicate that only low levels of pathogens were detected in the food chain of RTE mixed salads during harvesting, processing and distribution. Moreover, our results of microbiota analyses highlighted the impact of washing water for the composition of microbiota at retail level.

Keywords: Microbiota, human pathogens, ready to eat mixed salads

Exploring biodiversity in microbial ecosystems along the food chain

P1.31

Biodiversity and microbial interactions of predominant bacterial taxa from high bacterial count raw cow´s milk from the bulk tank

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Cold storage of raw milk in the bulk tank after milking leads to conditions that select for psychrotrophic bacteria, which are well adapted to cold temperatures and are able to outgrow the accompanying microbiota. They pose a serious problem for quality and shelf life of processed milk and dairy products because they are able to produce extracellular lipases and thermostable proteases, which may withstand even ultra-high temperature processes.

Data from earlier studies suggest that single fast growing, psychrotrophic bacterial populations dominate raw milk from the bulk tank with high microbial load. In contrast to this, we could demonstrate that the majority of raw milk samples with high microbial load are colonised with a bacterial community of high diversity.

For this, we characterised the contaminants from 48 raw milk samples with high microbial load (>100.000 cfu/mL) from different dairy farms. Total bacterial counts and bacterial diversity were determined by a cultivation approach at 30°C and 10°C and by culture-independent analyses of 16S rRNA sequences from DNA extracts as well. Diversity of bacterial communities was calculated with Shannon diversity index and Equitability.

The dominating bacteria were identified as species of the phyla *Gammaproteobacteria* and *Firmicutes.* Most of the *Gammaproteobacteria* were psychrotrophic and possibly related to poor hygiene of the milking machines, while the majority of the *Firmicutes* were mesophilic and potential mastitis pathogens, which showed high persistence in the cooled raw milk storage tanks. For samples with high diversity, species of the phyla *Bacteroidetes* and *Actinobacteria* had a higher abundance as well.

We could detect microbial interactions between several isolates. Interactions were detected using a drop assay and co-culture systems, which showed growth supporting as well as inhibiting interactions between bacterial populations. This demonstrates that the raw milk microbiota represents complex and divers communities with inhibiting or growth-supporting interactions between bacterial populations, which may promote microbial diversification of raw milk microbiota.

Keywords: raw milk, microbial load, diversity, psychrotrophic bacteria, co-culture

Exploring biodiversity in microbial ecosystems along the food chain

P1.32

Effects of bacteriocin AS-48 and high hydrostatic pressure on microbial load and bacterial diversity of refrigerated vegetable cream

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A commercial refrigerated vegetable cream containing pumpkin and carrots as main ingredients was stored under refrigeration for 30 days without treatment (controls), supplemented with bacteriocin AS-48, treated by high hydrostatic pressure (HP, 600 MPa, 8 min, 55ºC) or a combination of bacteriocin and HP. At day 2, half of the samples were incubated for 24 h at room temperature (simulating a temperature abuse event) and then refrigerated. Total viable counts and bacterial diversity were determined. Bacteriocin did reduce viable counts, but HP treatment (singly r in combination) was the most effective. Viable counts increased in controls during temperature abuse, but not in samples treated with bacteriocin, HP or both. The initial microbiota of control samples was composed mainly by *Proteobacteria* (75.54%), followed by *Firmicutes* (20.47%) and *Actinobacteria* (3.50%). *Proteobacteria* became the predominant group during refrigerated storage (86.21 to 99.34%). After simulation of a 24-h temperature abuse event, control samples had lower relative abundances of *Firmicutes* during storage and higher relative abundances of *Proteobacteria*, *Bacteroidetes* and *Actinobacteria*. Samples treated by high hydrostatic pressure had lower relative abundances of *Firmicutes* during storage compared to untreated controls. Samples treated with bacteriocin singly or in combination with high pressure had similar relative abundances for the main phyla during storage. Analysis at genus level revealed *Bacillus* as the predominant member among *Firmicutes* in control samples during storage and also in many of the samples treated by high pressure, bacteriocin or their combination. *Pseudomonas* had higher relative abundances during late storage. The relative abundances of *Acinetobacter* and *Methylobacterium* typically increased during the temperature abuse event.

Keywords: vegetable cream; bacteriocin; high hydrostatic pressure; biodiversity

Exploring biodiversity in microbial ecosystems along the food chain

P1.33

Diversity and biotechnological potential of yeast isolates from kefirs

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Kefir grains consist with characteristic microflora including lactic acid bacteria, acetic acid bacteria, and yeasts. According to FAO/WHO Food Standards 1 g of kefir should contain a minimum of 10^4 cfu of yeasts. Despite numerous reports concerning the nature of symbiotic systems in kefir grains, little is known about kefir yeasts and their biotechnological potential.
The yeast cultures were isolated from natural kefir products within their shelf life period. The isolates of pure cultures were identified by the ITS region sequence analysis. All sequences of the identified strains were deponated to GenBank data. They were identified as *Candida inconspicua, Debaryomyces hansenii*, *Kazachstania unispora, Kluyveromyces marxianus, Zygotorulaspora florentina* and *Trichosporon domesticum.* The studies confirmed the occurrence of *Z. florentina* and *T. domesticum* yeast in kefir samples for the first time. The strains were characterized considering their biotechnological potential. Studied kefir yeast strains were distinguished by the ability to exopolysaccharides (EPS) biosynthesis. The highest total production of EPS (2,98 g L^{-1}) was observed during incubation of *Debaryomyces hansenii* strain in mineral medium containing maltose. Other carbon sources (glucose, saccharose, lactose, glicerol and sobitol) significantly reduced the efficiency of EPS biosynthesis. There was also the great ability to lipid biosynthesis observed, therefore some of the yeast can be a source of single cell oils (SCO). The strains were also able to utilize potato wastewater. Our earlier study confirmed that wastewater can be inexpensive and valuable source of nitrogen, potassium, phosphorus and other elements in yeast cultures. The biomass of *T. domesticum* yeast was characterized by the highest (33% CDW) content of lipids and the lipid yield exceeded 4.8 g L^{-1}. Lipids of all examined yeast isolates demonstrated a large share of C18 fatty acids, among which oleic acid (C18:1, *cis-9*) was predominant. The lipid fraction of *Trichosporon domesticum* biomass was exceptionally characterized by the highest contribution of linoleic acid (C18:2 *, cis-9,12*) and *Kazachstania unispora* by palmitoleic acid (C16:1, *cis-7*). The fatty acid composition of the kefir yeast lipids confirms its nutrition value or application in biodiesel production depending on the strain and culture conditions.

Keywords: kefir, yeast, single cell oils, exopolysaccharides

Exploring biodiversity in microbial ecosystems along the food chain

P1.34

Brazilian artisanal cheeses: A source of lactic acid bacteria with biotechnological and biopreservative properties

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As the majority of traditional foods, Brazilian artisanal cheeses (BAC) present a huge microbial diversity, mainly represented by lactic acid bacteria (LAB). These LAB may present interesting technological properties due to their ability to predominate and resist to the fermentation process of milk and dairy products. The aim of this study was to evaluate biotechnological and biopreservative properties of industrial relevance from LABs isolated from BAC. A total of 220 presumptive LAB strains previously analyzed and showing high enzymatic (proteolytic and lipolytic) and inhibitory activities against *Listeria monocytogenes* and *Staphylococcus aureus* was assessed with respect to their ability to produce diacetyl (precursor of aromatic compounds), exopolyssacarides (from sucrose, fructose, lactose, and glucose), which is relevant for sensorial and textural features of fermented food, and bacteriocins, an important biopreservative. These strains were isolated from samples of BAC produced in different regions: Northeast ("Coalho" cheese; n=53) and ("Manteiga"; n=8); Midweast ("Caipira"; n=39); Southeast ("Araxá"; n=16), ("Campo das Vertentes"; n= 36), ("Cerrado"; n= 2) and ("Serro"; n= 23) and South cheeses ("Colonial"; n= 12) and ("Serrano"; n= 9). The production of diacetyl was indicated by a pinkish ring formation in tubes, being classified according to their intensity. The evaluation of exopolysaccharide production (EXP) was assessed by the ability of the strains to produce slimy colonies (EXP-positive). The bacteriocin production was assessed by the spot-on-the-lawn method against two enterotoxigenic *S. aureus* and two *L. monocytogenes* (isolated from raw milk) strains. Then, the treatment of cell-free supernatants was done with four enzymes: α-chymotrypsin from bovine pancreas type II, *Streptomyces griseus* protease type XIV, trypsin and proteinase K, in order to indicate the proteinaceous nature of the antimicrobial compound. The absence of halos after enzymatic treatment indicated the presence of bacteriocins. In summary, 59.54%, 28.18%, 25.91%, 29.10% and 33.64% of the strains showed high dyacetil and exopolysaccharide production from sucrose, fructose, lactose and glucose, respectively. A total of 12.27% were bacteriocin positive, being isolated from "Manteiga", "Colonial", "Serro" and "Caipira" cheeses. This study shows that BAC are a good source of wild LAB strains with interesting biotechnological and biopreservative properties.

Keywords: Artisanal cheeses, Endogenous microbiota, Raw Milk, Technological properties

Exploring biodiversity in microbial ecosystems along the food chain

P1.35

Assessment of probiotic potential of wild lactic acid bacteria isolated from Brazilian artisanal cheeses

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The increasing interest in products with functional properties has encouraged the search for new strains in natural sources. Traditional products, especially artisanal cheeses, have been the target of such studies because they present a wide microbial diversity, composed mainly of wild acid lactic bacteria (LAB) which stand out due to their singular metabolic characteristics resulting from resistance to food fermentation stresseses. The aim of this study was to evaluate the probiotic potential properties of 220 presumptive LAB strains that presented high enzymatic (proteolytic and lipolytic) and inhibitory activities against *Listeria monocytogenes* and *Staphylococcus aureus*. These strains were isolated from samples of Brazilian artisanal cheeses produced in different regions: Northeast ("Coalho" cheese; n=53) and ("Manteiga"; n=8); Midweast ("Caipira"; n=39); Southeast ("Araxá"; n=16), ("Campo das Vertentes"; n= 36), ("Cerrado"; n= 2) and ("Serro"; n= 23) and South cheeses ("Colonial"; n= 12) and ("Serrano"; n= 9). Resistance to low pH values and bile salts, hydrophobicity and autoaggregation assays, antibiogram, hemolytic activity and resistance to the simulated conditions of gastrointestinal tract (GIT) were assessed. In summary, 71 (32.27%) strains were resistant to at least one acid condition and submitted to bile salt tests and 22 strains showing high survival to 0.4% bile salt were submitted to adhesion assays. The values of autoaggregation (68.46 - 99.02%) were considered moderate/high and interesting for technological and therapeutic purposes due to their ability to prevent pathogenic adhesion to the intestinal mucosa. Hydrophobicity values varied greatly between strains (4.97 to 64.25%) and 7 strains presented values higher than 40%, indicating good potential for application as probiotics because hydrophobic cells exert an important role in the activation of the immune system of the intestinal mucosa. Most of the strains (88.81%) showed a good survival after passage through the simulated GIT. None of the strains showed hemolytic activity, but all strains were resistant to vancomycin and streptomycin, 91.30% to ciprofloxacin and 82.61% to gentamicin. Eve though the strains presented potential probiotic properties, before their commercial application, further studies are needed to assess their virulence factors and in vivo capability.

Keywords: Artisanal cheeses, Endogenous microbiota, Raw Milk, Probiotic properties

Exploring biodiversity in microbial ecosystems along the food chain

P1.36

Persistent spore formers constitute a widespread phenomenon in dairy food production lines

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Spore forming bacteria are of particular relevance in food as they may constitute a health risk or serve as hygiene indicators. However, many dairy products such as extended shelf life (ESL) milk or milk powder are manufactured without UHT treatment and spores are not fully eliminated. In case of ESL milk psychrotolerant spore formers such as *Paenibacillus* and *Bacillus cereus* are of particular interest, as they can grow during refrigeration and lead to premature spoilage. For milk powder thermophilic spore formers have gained increasing attention as they are able to proliferate at 50-70°C, the common temperatures of e.g. evaporation processes. As spores are introduced into the process via the raw material, but may also be a result of recontamination events, detailed knowledge on contamination routes and influence of process parameters on the development of spore counts is needed to set up effective prevention measures.

We comprehensively analysed spore counts and microbiota of bulk tank milk as well as ESL milk of six and milk powders of nine manufacturers to investigate the correlation between raw material and end products. In addition, we performed process controls of four production lines for ESL milk that used pasteurization in combination with either microfiltration or bactofugation for spore reduction. The growth dynamics of thermophilic spore formers during the production of milk powder were investigated in detail for two production lines.

In all cases, the raw material turned out to have almost no impact. The spore counts were exceptionally low and those species responsible for spoiled milk or present in high counts in milk powder were isolated from the raw materials very rarely. Instead, it was observed for milk as well as milk powder lines that identical strains of *B. cereus* or *Anoxybacillus flavithermus* occurred in end products of multiple production batches. They survived cleaning procedures, persisted in the production lines over several months and recontaminated product after a new production had started.

This is evidence that persistent spore formers are a widespread phenomenon in the dairy sector while the impact of production lines (besides filling machines) on microbial recontamination has been largely neglected in the past. Consequently, prevention strategies to limit spore counts and to reduce health risks posed by potential toxin producers such as *B. cereus* need to focus on plant hygiene and optimized sanitation procedures.

Keywords: Spore forming bacteria, persistence, ESL-milk, milk powder

Exploring biodiversity in microbial ecosystems along the food chain

P1.37

Utilisation of DNA sequencing to investigate the dynamic dairy microbiota from farm bulk tank milk to milk powder

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Microorganisms from the environment can enter the dairy food chain at various stages during milk collection and processing, with potential associated quality and safety-related implications. The tracking of these microorganisms has been greatly aided by high throughput DNA sequencing (HTS). While HTS has been used to assess the impact of seasonality, bulk tank storage, and transport on the bacterial populations in raw milk, the technology has not been used to examine the multiple stages across the process from raw milk on farms to final powdered product at the processing facility. This study addressed this topic and in addition investigated the impact of temporal storage, transport, and processing on bacterial populations in dairy.

The study utilized 16S rRNA amplicon sequencing of metagenomic DNA extracted from fresh, early and late lactation (i.e. seasonal) milk samples collected from farm bulk tanks, collection tankers, whole milk silos, skimmed milk silos, and powder samples. Shotgun metagenomic sequencing was also performed on a subset of representative samples.

Analysis of HTS data revealed that the microbiota of the powder generated during the early lactation period was very different from the starting raw milk. Two thermophilic genera, predicted to originate from within the processing plant, were dominant. In contrast, the late lactation powder microbiota resembled its raw milk inputs, and was dominated by spoilage associated psychrophilic bacteria. While 16S rRNA analysis provided a useful overview of the bacteria present in samples, it did not facilitate differentiation of taxa to the species level. Shotgun metagenomic analysis provided results that were consistent 16S rRNA analysis but with the added value that species level classification was provided.

This study demonstrates that HTS can be used to trace microorganisms through a processing run, classify them to species level and, in the process, highlights that across different processing runs, different sources of contaminating microbes may be relevant. HTS allows detailed investigation of the potential impact of the species present.

Keywords: DNA sequencing, raw milk, processing, milk powder, microbiota, food chain, farm, processing facility

Exploring biodiversity in microbial ecosystems along the food chain

P1.38

Characterisation of microbial isolates of soak-water of "ofada" rice

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"Ofada" (*Oriza glaberrima*) is a popular local rice cultivar in Nigeria that is relished for its flavour and aroma which develops during the traditional processing method. The microbial community of the soak-water was investigated at 24h intervals for 120h by the traditional cultural and molecular methods. Three major clusters of bacteria namely; lactic acid bactreia , *Bacillus* spp and the *enterobacteriacaea* while clusters of fungi (*yeast*, *Aspergillus* and *mucor species*) were isolated from the soak-water by the traditional cultural method. The Sequencing of their 16S rRNA genes revealed a cocktail of
bacteria with *Enterococcus feacium* being the predominant bacteria while sequencing of the yeast's ITS 5 genes revealed *Pichia kudriavezii* as the predominant yeast. The neighbour joining Phylogenetic analysis of the sequenced bacteriashowed that *Enterococcus feacium, Enterococcus lactis and Enterocccus duran* were closely related.

Keywords: Molecular, Characterisation, "Ofada" Rice, Isolates, Phylogenetic tree

Exploring biodiversity in microbial ecosystems along the food chain

P1.39

Microbiological characterization of Salame Piemonte IGP

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Salame Piemonte is an typical fermented sausages of Nord-West of Italy that is preserved by protected geographical indication (PGI). These kind of products constitute a significant part of the Mediterranean diet and have a long tradition originating from Europe. Their microbiota is specific of the region or area where they are produced and it is essential in the better management of microbial resources to protect the sensory characteristics of the product.
With the aim of the selection of autochthonous starter cultures for this local fermented sausage, the ecology and microbial dynamics during the fermentation process of three different productions from the same factory were evaluated. The study of fermented sausages has been carried out by culture-dependent and independent methods in addition to volatilome profile, chemicals and sensory analysis.
Preliminary data suggests that coagulase-negative cocci (CNC), lactic acid bacteria (LAB) and yeast are the principal groups that conduct the fermentation process. LAB showed a fast growth increase in the first 4 days of maturation, while in the successive period they slowed down their propagation showing a costant microbial count. CNC and yeast showed a slower growth rate and in particular CNC showed, after 8 days, a soft decrease of the microbial count.
Molecular techniques carried out for the characterization of the isolates and the application of metagenomics approaches helped in the understanding of the correlation between strains and their respective metabolic activity impacting the specific sensory taste of the studied product.

Keywords: Salame Piemonte PGI, Ecology, LAB, Molecular techniques

Exploring biodiversity in microbial ecosystems along the food chain

P1.40

Identification and probiotic characterization of *Lactobacillus* Isolated from water buffalo's dairy products

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Products containing probiotic micro-organisms are now an important product group for consumers who want to be healthy. Buffalo milk and its products are thought to be probiotic sources since they contain lactic acid bacteria.
In this study, a total of 502 isolates obtained from 23 samples of buffalo milk products collected from different cities of Turkey. Gram staining, catalase test and oxidation-fermentation test were applied to these isolates. Species-based identification of 145 *Lactobacillus* isolates were made by phenotypically.
Of the probable probiotic isolates of the *Lactobacillus* species that have been identified, 53 selected isolates have examined for viability in different conditions, primarily at low pH, in the presence of bile and phenol which are found in the gastrointestinal environment. According to results 26 isolates were further analysed for antibiotic susceptibility, bile salt hydrolase activity and cholesterol assimilation.
Of the all 26 isolates were proven that having probiotic properties. Two strains (isolate 33 and isolate 43) were not resistant to any antibiotics used in the study, it was decided that they could be used as probiotic strains in further studies.
As a result, buffalo dairy products have been found to be a good source of probiotic microorganisms. This work has opened the way for the use of buffalo milk products as an alternative source for probiotic strains.

Keywords: Water Buffalo´s Dairy Products, Probiotic, Lactobacillus

Exploring biodiversity in microbial ecosystems along the food chain

P1.42

The role of lactic acid bacteria in Indonesian indigenous fermented foods: Current status

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The Indonesian indigenous fermented foods were typically characterized by spontaneous fermentation. Salt was mostly used as selecting agent which enabled to inhibit the growth of both spoilage and pathogenic microorganisms during the process. There were plenty sorts of fermented foods from Indonesia as products of lactic acid bacteria (LAB) activities. They were categorized into the following groups: fermented fruits, vegetables, fishes, cassava tubers, rice, milk and soybeans.The role of LAB in various Indonesian indigenous fermented foods will be discussed in this paper, from tempoyak in South Sumatera to tempe in Yogyakarta and from wadi in Banjarmasin to peda in Lombok. In conclusion, the genus Lactobacillus played the most important role in Indonesian fermented foods and followed by Pediococcus, Streptococcus, Leuconostoc, Enterococcus and Weissella.

Keywords: fermented foods, lactic acid bacteria, spontaneous fermentation

Exploring biodiversity in microbial ecosystems along the food chain

P1.43

A pilot study of the role of corn dextrin and milk peptides supplementation on faecal microbiota in healthy adults

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The gastrointestinal microbiota has an important role in human health. Dietary interventions are of great interest to modulate the composition and metabolic functions of the gut microbial communities and to improve health, and prevent or treat diseases. Consumption of prebiotics is one dietary strategy for beneficial manipulation of the gut microbiota, because it allows increasing the fibre intake, especially in people with western dietary habits, who do not take the recommended daily amount of fiber. Interestingly, milk peptides can also positively affect the beneficial gut microorganisms. The present work is a pilot study aimed to investigate the effect of a prebiotic supplementation on composition and metabolic activity of microorganisms living in the human gut. In this trial, 12 healthy subjects received 10g/die of supplement Biotransit®, composed by corn derived dextrin and milk peptides, produced and marketed in Italy by Depofarma (Italy), for 4 weeks with a 2 weeks washout. Outcome measures were assessed at four time points (before the supplementation T0-1, T0-2, at the end of intervention, T30 and after washout, T45), including gut microbiota profiling by 16S rRNA gene sequencing and intestinal functional metabolism measuring faecal Short Chain Fatty Acid concentrations (SCFAs). The effects of the Biotransit supplementation on bifidobacteria were also assessed with culture dependent techniques. Gut microbiota analysis revealed that Biotransit® supplementation after 30 days did not exert effects on the overall gut microbiota structure. Although no significant differences on alpha diversity were obtained, we observed an increase of diversity after 30 days of treatment. Beta diversity analysis, calculated on Bray-Curtis distances revealed significant differences comparing T0 vs T45 and T30 vs T45. Interestingly, at T45, we found an enrichment of *Porphyromonadaceae*. Biotransit® induced quantitative changes in cultivable bifidobacteria with increased amount at T45, even if the total number of species has not been influenced. Biotransit® supplementation is also associated to an increase total SCFAs concentration in T30 and T45, in particular related to acetate, propionate and butyrate ($p < 0.05$). Future study will be aimed to follow the time course of the persistence of this effect after the end of treatment.

Keywords: Microbiota profile, peptides, fibers, Bifidobacterium, SCFAs

Exploring biodiversity in microbial ecosystems along the food chain

P1.44

Understanding the phylogenetic structure of silage microbial communities and its impact on raw cow milk microbiota in dairy farms

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Milk composition and microbiological quality highly impact its suitability for processing. In dairy farms, although grass and corn silages harbour complex microbial communities, their specific contributions in shaping the raw milk microbial structure is unknown. This study aimed to investigate the impact of silage feeding practices on milk microbial composition. Dairy farms (24) implementing different silage feeding typologies, namely either hay, or silages of inoculated grass and corn (GICI), or non-inoculated grass and corn (GCNI), or non-inoculated grass and inoculated corn (GNICI), or non-inoculated grass (GNI) were selected. Accordingly, hay, silages (GI: inoculated grass, GNI, CI: inoculated corn, CNI: non-inoculated corn) and associated raw milk samples were collected. Their bacterial and fungal communities were separately analysed by high-throughput sequencing of the 16S and ITS2 rRNA gene respectively. Lower richness and diversity levels were observed in GI compared to GNI, while CI displayed higher richness and lower diversity compared to CNI according to Chao1, Shannon and Simpson indices. Principal coordinate analysis based on weighted-UniFrac metric (WU-PCoA) revealed significant differences ($p < 0.001$) between hay and silages (GI, GNI, CI, CNI), and between CI and CNI, whereas GI and GNI had similar phylogenetic structures. However, significantly lower abundances ($p < 0.01$) of *Pediococcus* and some *Lactobacilliaceae*, or higher abundances of *Candida* and *Debaryomyces* were observed in GI compared to GNI. In raw milk, *Enterobacteriaceae* (>52%) and *Pseudomonadaceae* (>15%) for bacteria, or *Engyodontium* (>70%), *Candida* and *Geotrichum* (>5%) for fungi were the most prevalent. Milk from hay farms showed different phylogenetic structures from those of silage farms as revealed by WU-PCoA. Although GICI, GCNI, GNICI and GNI milk showed similar community structures, linear discriminant analysis effect size identified bacterial and fungal biomarkers among them. Overall, shared taxa proportions between milk and associated silages were generally higher in silage farms (52-81% for bacteria and 59-70% for fungi) compared to hay (~58%). More importantly, highest and smallest proportions of shared taxa within silage typologies were found in GICI and GCNI for bacteria, or GNI and GCNI for fungi, respectively. Our results demonstrate the role of inoculated and non-inoculated silages as major microbial contaminant sources of the raw milk in dairy farm environment.

Keywords: milk microbiota, silage microbiota, inoculant, propidium monoazide, metataxonomics

Exploring biodiversity in microbial ecosystems along the food chain

P1.45

Microbiological characterization of "innovative" alheiras produced in Portugal

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Nutrition and health concerns are having an increasingly significant influence on the consumers' food choices. In response, the food industry has begun to offer a wider variety of products that reflect changing in consumer tastes and preferences. In addition to traditional *alheiras* made with pork and/or poultry meats, other varieties of *alheiras* ("innovative") made from codfish, mushrooms, tofu, soy and vegetables were lunched in the Portuguese market in an attempt to reach those consumers with "healthier and cleaner" lifestyles without losing tradition.

Even though there is already extensive scientific knowledge on fermentations and behaviour of pathogens in traditional *alheiras* little is known about these new types of *alheiras*. The objective of this study was the characterization of these new products, with respect to their microbiological safety. Therefore, twelve different products, available on the Portuguese market, were submitted to microbiological characterization, in order to increase knowledge on possible risks these new products may represent. Detection of sulphite reducing *Clostridium* spores, *Listeria monocytogenes* and *Salmonella* spp. as well as enumeration of *Enterobacteriaceae*, *Enterococci*, *Escherichia coli*, lactic acid bacteria, *L. monocytogenes*, total counts at 30ºC, *Staphylococcus aureus*, yeast and moulds were performed according to ISO standards.

The results showed that *Enterobacteriaceae*, lactic acid bacteria and yeasts and moulds, were the prevalent microbiota of "innovative" *alheiras.* Sulphite reducing *Clostridium* spores, *E. coli*, *L. monocytogenes*, *Salmonella* spp. or *S. aureus* were not detected in any sample.

In conclusion, unlike traditional *alheiras* which often contain pathogenic agents, no harmful organisms were found in these new products, even being produced by the same companies.

Keywords: Fermented foods; Innovative alheiras; Microbiological safety

Exploring biodiversity in microbial ecosystems along the food chain

P1.46

Induced fermentation of natural black Kalamata olives by selected strains of yeasts with technological and probiotic traits

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Use of starter cultures originating from the olive's autochthonous microbiota in table olive processing may contribute to the development of a novel final product with functional characteristics. Yeasts have been studied by several authors for their technological properties and their probiotic potential. Specific strains are able to produce enzymes such as β-glucosidase which cause the degradation of oleuropein from olive drupes, while others have shown high survival potential during GI tract simulation conditions. The aim of this work was to study the microbiological and physicochemical changes during Kalamata natural black olive fermentation inoculated with 5 different yeast strains previously isolated from the microbiota of fermented olives and selected for their technological and probiotic properties. Olives were fermented in 7% w/v NaCl brine solution (control) or brine inoculated individually with 5 yeast strains belonging to the species *Pichia kluyveri* Y5, *Metschnikowia pulcherrima* Y14, *Pichia guilliermondii* Y22, *Saccharomyces cerevisiae* Y34 and *Candida molendinolei* Y45 to a final concentration of 10^6 CFU/mL in each case. Changes in microbiological counts, pH, and acidity were analyzed for a period of 150 days. The survival of the inoculated strains was estimated by DGGE fingerprinting in the end of the process. Results showed that yeast populations ranged from 3.5 to 4.7 $\log_{10}$ CFU/g and 3.7 to 5.3 $\log_{10}$ CFU/mL on olive drupes and in the brines, respectively. Moreover, pH values ranged between 4.1-4.7 and 3.8-4.7 for the olives and the brines, respectively, while acidity in the brines ranged between 0.25 and 0.59% (w/v, lactic acid). The results from the DGGE fingerprinting at the end of fermentation (150 days) showed the survival of the starters into the brines, with the exception of Y5 that could not be recovered, whereas none of the strains survived on the olive surface.

Keywords: black olives, yeasts, fermentation, DGGE fingerprinting

Exploring biodiversity in microbial ecosystems along the food chain

P1.47

Development of a predictive growth model for seasonal and geographical comparison of microbial ecology in two Irish bakeries

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Low-moisture foods frequently contain spore-forming bacteria displaying physiological diversity, longevity and tolerance to adverse ecological conditions. This is an important quality and safety challenge in the food industry. The present study was conducted to determine the base-line microbiome of two artisanal Irish bakeries in two different geographical locations, denoted as *A* and *B*, using classical culture-based techniques mainly focusing the spore formers. Surface swab samples, dried food ingredients and baked goods from these manufacturing environments were collected on-site. Microbiological analysis targeted total viable counts (TVC), and species from the genera *Bacillus*, *Clostridium*, *Listeria*, *Staphylococcus*, *Salmonella*, Lactic Acid Bacteria, Coliforms along with Yeast and Moulds. Preliminary findings recorded Yeast and Moulds to be dominant (70%) whereas the microbial consortia varied through spring, summer and winter. Dominant levels of yeast and moulds in Bakery *A* reflected their role in shaping site-influencing product-specific characteristics. Conversely, levels of spore-forming *Bacillus* and *Clostridium* in all seasons were higher in Bakery *B* (3.80 x 10^6 CFU/ml) *versus* Bakery *A* (3.41 x 10^6 CFU/ml). The numbers of spore-forming *Bacillus* and *Clostridium* were highest in summer (3.03 x 10^6 CFU/ml) followed by spring (2.69 x 10^6 CFU/ml) and winter (1.50 x 10^6 CFU/ml). The diverse and variable microflora detected in both sites highlighted differences between the suppliers and products as well as time-temperature of sampling w.r.t the production times in the facilities. The results validate the microbial diversity noted as a function of location and season. A mathematical model was derived from quantitative assessments of the intrinsic microbiome recorded from both sites. The quantitative microbial ecology model will be applied to support safety and quality improvements in low-moisture food production environments in addition to contributing to the evaluation of the process controls and identification of the hot-spots in the production facilities.

Keywords: Microbiome,Spore-formers,Food Processing environment,Dry Foods,Low-moisture foods,Predictive model

Exploring biodiversity in microbial ecosystems along the food chain

P1.48

Pasture feeding of dairy cows improve the milk and cheese microbiota

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IIn this work, we studied the effects of pasture feeding on dairy cows' milk and cheese microbiota. The study involved 12 Brown Swiss cows farmed indoor and clustered in two groups. Six cows remained in the farm, the others were moved from July to September, to a temporary summer farm located at 1860 m.a.s.l., where cows where free to graze on the alpine pasture. Milk was collected every month from June to October, during cow milking for a total of 60 samples. 2 Liters of milk from each cow, were used for mini-cheese processing. Milk samples and cheeses were analysed for microbial counts and 200 milk isolated strains have been identified at species level, in order to compare the milk microbial population, and its evolution in the cheese, under different conditions of farming management and diet. Metagenomics analysis of the 60 milk samples by MySeq Illumina are in progress. Most of the microbial groups analyzed by plate counting (lactic acid bacteria, propionibacteria and bifidobacteria) were significantly higher in milk and cheese samples from cows moved to the alpine pasture. When the cows moved back to the indoor farm, the milk and cheese microbiota showed no more differences compared to the cows that were indoor housed during the whole summer, suggesting that the effects of summer alpine pasture disappeared when the cows moved back to an indoor farming.
The 16S rDNA partial sequencing showed a picture of the milk microbial ecology that was strongly and positively affected by the pasture feeding. During the summer alpine pasture, milk was characterized by 25% of *Lactococcus lactis* and 19% of *Lactobacillus paracasei*, which are desired species for their dairy technological attributes. *Bifidobacterium crudilactis* and *Acidipropionibacterium jensenii*, which are known for their healthy properties, represented 25% of the total population. Conversely, when cows were housed indoor during summer, the milk microbiota contained 36% of *Enterococcus faecalis*, which is not always desired owing to its ability to carry antibiotic resistances. In addition, *Lactococcus lactis* and *Lactobacillus paracasei*, which were dominant in milk during the summer pasture, decreased to 12% and 7%, respectively. *Acidipropionibacterium jensenii* was not found anymore, and *Bifidobacterium crudilactis* was only present in traces (2%). Finally, *Staphylococcus aureus,* which is responsible of mastitis, was detected only in milk samples from indoor farmed cows (10% of the total population).

Keywords: milk, cheese, microbiota, pasture feeding, dairy cow, bifidobacteria

Exploring biodiversity in microbial ecosystems along the food chain

P1.49

Application of *Lactobacillus rhamnosus* GG in functional fermented maize products

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Due to consumer´s demands for healthier foods, the industry is directing development of new products towards the area of functional foods. Research in the area of functional foods has moved progressively towards development of dietary supplementation, introducing the concept of probiotics and prebiotics that may affect gut microbial composition. Worldwide, probiotic bacteria are most frequently included in the composition of dairy sector that represents the largest food market. This may be a great opportunity for non-dairy food and beverage probiotics sector with a diversity of new products expected in the future.
Thus, the aim of our work was to evaluate growth and metabolic characteristics of selected lactic acid bacteria (LAB) in maize substrates as a basis for the design of functional foods. Although milk is the most typical growth medium for the growth of LAB, mixed Fresco culture showed good growth in all prepared mashes (specific growth rates ranged from 1.02 to 1.30 h^{-1}). Addition of flavouring compounds increased the growth rates about 10 - 18 % in comparison to those added with sucrose. The highest specific growth rate was calculated in milk chocolate mash (μ = 1.30 $^{-1}$). During the fermentation process the application of 5% (v/v) starter culture of Fresco resulted in reaching the pH levels of 4.16 - 4.85 representing a decrease of 1.8 - 2.5 and 1.5 - 1.8 unit in water and milk products, respectively. Added concentration of probiotic strain *Lactobacillus rhamnosus* GG decreased in average about 1 log order during period of 14 days (6 ±0.5 °C) but not under the legislation limit (6 log CFU ml^{-1}). Based on the overall acceptance of the designed products, mashes right after the fermentation process and those after storage period (14 d) exhibited an attractive sensory acceptability (2.8 - 3.6).
This work was supported by the VEGA project No. 1/0050/17.

Keywords: Lactobacillus rhamnosus GG, functional products, maize, overall acceptability

Exploring biodiversity in microbial ecosystems along the food chain

P1.50

Antimicrobial, antioxydant, and dental anticaries characteristics of marshmallow with betel of chew extract addition

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The research objective was to determine functional characteristics of *marshmallow* with betel of chew extract addition which consisted of 10 treatment levels: E1 (10 %), E2 (20 %), E3 (30 %), E4 (40 %), E5 (50 %), E6 (60 %), E7 (70 %), E8 (80 %), E9 (90 %) and E10 (100 %) dissolved in 100 mL water used for gelatin soaking and 3 best treatments will be taken later. Determination of the best treatment is decided from organoleptical analysis based on the panelists preference. The three best treatments based on panelists preference were E4 (*marshmallow* + 40% betel of chew extract), E6 (*marshmallow* + 60% betel of chew extract) and E8 (*marshmallow* + 80% betel of chew extract). The testing conducted in this reserach were antibacterial analysis, antioxidant analysis and anti dental caries analysis, respectively. The best treatment was E8 (*marshmallow* + 80% betel of chew extract) having antibacterial activity to *Streptococus mutans* of 2.15 mm, antioxidant activity (IC_{50} value of DPPH test) of 2.78 mg/ml, and dental caries value of 4.0 and caries inhibition percentage of 83%, respectively.

Keywords: betel of chew extract, marshmallow, functional, antibacteria, antioxidant

Exploring biodiversity in microbial ecosystems along the food chain

P1.51

Effect of a traditional jaggery based distilled liquor (Chulai) on the gut bacterial profile and fecal metabolites of the tea-tribal population of Assam (India)

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Alcoholic beverages are consumed worldwide and categorized as either beer, wine or distilled spirit. It has been established that the gut microbial changes is associated with the pathogenesis of alcohol-related liver diseases. Assam, a major north-eastern state of India, is home to a number of tribal populations of which the tea-tribes form a major community. These tea-tribal communities consume a form of distilled alcoholic liquor from fermented jaggery (*Chulai*) whose chemical composition and health effects are unknown. In this study, we illustrate the effect of daily intake of *Chulai* on the gut bacterial profile (GBP) of the tea-tribal communities and correlate it with the changes in their faecal metabolite profile and biochemical biomarkers of the population. The GBP of *Chulai* drinkers (n=25) and non-drinking healthy individuals (n=25) were analysed by Next Generation Sequencing (NGS) using Illumina Miseq platform. NGS data revealed that the bacterial phyla *Verrucomicrobia* and *Fusobacteria* were significantly altered in the *Chulai* drinkers. GC-MS based faecal metabolite profiling identified 23 metabolites to be significantly altered in the *Chulai* drinkers. Additionally, metabolites such as Cholan-24-oic acid, 2-Pyrrolidone-5-carboxylic acid and Hexanedioic acid could be correlated with the significantly altered bacterial genera (*Sutterella* and *Streptococcus*). Significant difference was also observed in the serum biochemical parameters such as triglyceride, total cholesterol and liver marker enzymes. It is suggested that consumption of *Chulai* alters the GBP leading to gut dysbiosis. Additionally, alterations in faecal metabolites observed in *Chulai* drinkers may possibly correlate with the changes in their GBP and biochemical markers which suggest progression of alcoholic liver injury.

Keywords: alcoholic beverages; GC-MS analysis; metabolites; Next Generation Sequencing; liver disease

Exploring biodiversity in microbial ecosystems along the food chain

P1.52

Examination of microbiological activities in an Austrian beet sugar factory

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Microorganisms enter and circulate in the beet sugar production process through different ways and may cause a variety of processing problems as well as economically significant sucrose losses due to their metabolism. In this context, the extraction and juice purification processing steps are of crucial relevance. Acidic metabolites produced usually reduce the pH-value, thereby resulting in hydrolytic cleavage of sucrose and indirect sucrose losses. In the light of ongoing changes in the agricultural regulatory framework (i.e. expiration of the European Sugar Market Regime in October 2017) and concomitantly resulting competition on the market, European sugar producers seek to maximize the sugar yield while simultaneously minimizing the input of energy and other resources. This study was carried out to assess and to monitor the presence as well as the activity of microorganisms in the sugar production process in relation to seasonal factors. For this purpose, the microbial load in the extraction area and the juice purification section of an Austrian sugar factory was investigated during the so-called sugar campaign 2017-2018. Samples were collected at different sampling points along the extraction as well as the juice purification process throughout originating from two consecutive sampling days within three months. These samples were analysed with a collection of different culture media in order to determine presumptive microorganisms known as predominant for this type of production. Subsequently, at least optically different and microscopically pre-selected colonies were isolated from each culture medium per trial period and further subjected to MALDI-TOF mass spectrometry to identify the microorganisms to the species level. In parallel, the collected product samples were analysed by means of HPLC in order to evaluate microbiological growth performance based on the metabolites (butyric acid, lactic acid, acetic acid, ethanol as well as mono- and disaccharides) formed.
Preliminary results demonstrate that the most common bacteria in the analysed samples belonged to the genus Bacillus and Lactobacillus. The analyses are still ongoing and further results, especially about the seasonal influence will be presented on the conference.

Keywords: sugar beet, extraction, juice purification, Bacillus, Lactobacillus, microbiological metabolites

Exploring biodiversity in microbial ecosystems along the food chain

P1.53

Microbial quality and mineral proximate composition of Wara, African soft cheese, produced from goat milk using different unconventional coagulants

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Wara, African Soft Cheese, is a nutritious unripened cheese consumed in several parts of West Africa. It is produced by the addition of extract from *Calotropis procera* to non-pasteurized whole milk from cattle and heating until the milk curdles. In the present study, *wara* was produced from non-conventional milk, goat milk and coagulated with extracts from *C. procera*, *Carica papaya*, lemon juice and steep water from fermented maize, sorghum and millet. Microbial analysis revealed the presence of the following organisms in the *wara* samples: *Lactobacillus casei*, *Lactococcus lactics*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus* and *Pseudomonas aeruginosa*. However, only *Lactobacillus casei* and *Lactococcus lactics* were isolated in *wara* sample produced with lemon juice as the coagulant. Also, the following fungi; *Aspergillus niger*, *A. fumigatus*, *Rhizopus stolonifer*, *Penicillium chrysogenum* and *Saccharomyces* sp were found to be distributed in the wara samples obtained from goat milk. Mineral and proximate analyses also showed a significant differences in the parameters analysed. Calcium (668mg/100g) was highest occurring mineral in the entire cheese sample while Zinc (0.90mg/100g) was the least. *Wara* produced from goat milk coagulated with *Carica papaya* extract had the highest protein content (24.90%) while the least protein (16.22) was recorded in sample produced with steep water from maize as the coagulating agent. The result from this study revealed that different coagulating agent used in the production of *wara* significantly affects the microbial quality and mineral proximate composition of the *wara*. Goat milk can also be used to produce acceptable *wara*.

Keywords: Wara, coagulating agent, microbial, mineral, proximate

Exploring biodiversity in microbial ecosystems along the food chain

P1.54

Foodresistome: Meeting the need to evaluate a bacterial DNA extraction protocol from a rainbow trout filet

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Bacterial acquisition of antibiotic resistance is a growing concern. The role of food in the transfer of resistant bacteria or antimicrobial resistance genes (ARG) in the human gut microbiota is yet to be explored as it is insufficiently characterized. Considering this antimicrobial resistance genes transfer, the environment may impact the exposition level of food. In this context, the aquaculture sector seems to be particularly interesting. The ponds in which the fishes are bred are exposed to the contaminants in the upstream river water that flow through them. The aquaculture sector also has a good societal acceptation and the world production has been evaluated by the FAO to represent 77 million tonnes in 2015.

This project is focused on rainbow trout filet in order to provide a more precise picture of the links between the nature and concentration of the antimicrobial residues, the diversity of the bacterial community and the antimicrobial resistance genes on the rainbow trout filet. Fishes and filets will be obtained at the end of the breeding process and in processing plants respectively. The ARG will be detected by qPCR in the bacterial community characterized by 16S metabarcoding and the concentration of antimicrobial residues will be quantified using chromatography and mass spectrometry.

Considering that the extraction of bacterial DNA from this food matrix is a key point, a calibrating protocol has been developed in order to assess the qualitative and quantitative significance of the DNA extraction method. This protocol included contaminations of rainbow trout filets with various calibrated concentrations of Brochotrix thermosphacta. This species which is rarely found on the fish skin microbiota was used as a marker independent from the commensal communities. The B. thermosphacta and total viable counts were followed from the samples to the DNA extraction. The DNA was used for B. thermosphacta and total bacterial number quantification through two specific qPCR. The aims were first to evaluate the loss in total viable count induced by the protocol and second, to determine the detection threshold.

The precise knowledge of the biases induced by this protocol will allow better analyses of the results obtained with the metabarcoding and qPCR approach.

Keywords: aquaculture, antimicrobial resistance gene, DNA extraction

Exploring biodiversity in microbial ecosystems along the food chain

P1.55

Suitability of commercial dairy starter cultures for fermentation of non-dairy milks

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The demand for vegetarian and vegan alternatives to dairy products is constantly and significantly growing all over the world. Fermented plant based foods (yogurt and cheese analogues) offer attractive prospects within the market of non-dairy products, since they can be valuable sources of good quality fats, proteins, dietary fiber, and other phytochemicals important for human health. The choice of lactic acid bacteria for fermentation of milk analogues is limited to strains that can ferment the sugars typically found in the raw materials (stachylose in soy for example).

The objective of the present study was to evaluate the technological potential of traditional yogurt bacteria in view of their application as starter cultures for the production of non-dairy fermented foods. Commercial starter cultures were grown in pasteurized retail non-dairy milk (soy, rice, oat, coconut and almond) and bacterial growth was studied using isothermal batch microcalorimeter TAMIII. Maximal specific growth rates, heat produced during different growth stages and lag-phases duration were determined by processing calorimetric curves. In parallel to calorimetric measurements the changes of concentrations of carbohydrates and lactic acid were determined and pH measurements were carried out in order to obtain additional information for the interpretation of calorimetric power-time curves. The sensory characteristics of fermented plant-milk based yogurts were evaluated by a trained panel of assessors using descriptive analysis for their sensory qualities including aroma, colour/appearance, flavor, texture, and overall acceptability. Gas chromatography coupled with mass spectrometry and olfactometry (GC-MS and GC-O) were applied to describe the production of aroma compounds.

As a result of this study, suitable starter cultures were selected for the fermentation of each non-dairy milk. It was found, that starter cultures can produce several undesired off-flavors and -aromas, which indicates that dairy starters cannot be directly used for the fermentation of non-dairy analogues. As a casein matrix is not formed during the fermentation of plant milks, the formation of desired texture relies on the starters' ability to produce polysaccharides or on the addition of suitable thickening agents. Further studies are required to select suitable stabilizers for each non-dairy yogurt.

Keywords: plant milk, microcalorimetry, starter cultures

Exploring biodiversity in microbial ecosystems along the food chain

P1.56

Characterization of El-Guedid, a traditional salted/dried meat product from Algeria

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El-Guedid is an Algerian traditional meat-based product that is prepared from red meats. It is an old practice of preservation of meat which was wide-spread before the use of refrigeration. It consists in cutting the meat in pieces before salting. The meat is then exposed to the sun for several days until complete drying, and is preserved in hermetically closed jars, away from air and humidity for several months.

Three batches of fresh sheep meat were homemade in the area of Constantine. The batches were sampled at different times of the process up to three months. Microbial analyses were performed on different media. Physicochemical analysis included the measure of pH, moisture and fat content.

Results indicated a decrease in moisture to 13-15% at three months and the pH value remained about 6.1. Microbial analyses revealed the absence of pathogenic bacteria such as *Salmonella* and *Listeria*. But *Staphylococcus aureus* was detected in the fresh meat of two batches and could persist up to one month of storage. The numbers of *Enterobacteriaceae* were variable from one batch to another from 1.8 to 4.6 log CFU/g in fresh meat to 2.7 to under the detection threshold in the products after 3 months of storage. The counts of total aerobic mesophilic bacteria and lactic acid bacteria (LAB) varied from 4.8 to 7.1 log CFU/g and from 2.0 to 5.6 CFU/g, respectively in initial and final samples. The counts of coagulase negative staphylococci (CNS), which could have a potential technological interest, varied from 5.4 to 6.6 log CFU/g and from 5.3 CFU/g to under the detection threshold, respectively in initial and final samples. The identification of LAB and CNS at the species level was achieved by molecular methods. The *El-Guedid* products studied did not present hygienic problem and among the potential pathogens researched, except for *S. aureus* which was found but its level was under the detection threshold after three months of storage. The LAB and CNS constitute an important microbial population in *El-Guedid*. Their characterization could lead to the development of competitive indigenous starters.

Keywords: meat, salting, drying, safety

Exploring biodiversity in microbial ecosystems along the food chain

P1.57

Isolation and characterization of *Lactococcus lactis* bacteriophages from raw milk samples

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Bacteriophages (phages) infecting lactic acid bacteria are widespread in dairy environments. Therefore these phages can cause severe fermentation delays or even total failures of fermentation batches in dairies. The main starter bacteria affected by phage attacks are mesophilic *Lactococcus lactis* cultures. Lactococcal phages of the common 936 phage group can reveal extraordinary high thermal resistance phenotypes. For these thermo-resistant phages, the "mild" thermal treatment of raw milk during standard pasteurization is not sufficient for phage inactivation. Therefore - when present in raw milk - these phages can easily be transferred into the dairy environment. Hence raw milk might be a critical source for phages, but the dissemination of dairy phages in raw milk has only been studied occasionally. In these studies, lactococcal phages were detected in 10% of raw milk samples with maximal titers of up to 10^4 plaque-forming units (pfu) mL^{-1}.

Due to the lack of appropriate lactococcal strains isolated from environments outside of the dairies, we screened 52 raw milk samples with a representative set of *L. lactis* strains from starter cultures. Lactococcal phages were detected in 35% of the raw milk samples with titers ranging from 10 to up to 10^6 pfu mL^{-1}. When analysed by transmission electron microscopy, all isolated raw milk phages revealed morphologies differing from the typical 936 phage morphotype. By DNA sequence analysis it was shown that the raw milk phages represent a remarkably diverse group of lactococcal phages. The majority of these phages showed limited similarity to members of the P335 group of phages. It was remarkable that some of these lactococcal raw milk phages did also show similarity to the recently described new group of *Streptococcus thermophilus* "hybrid" phages.

In contrast to the 936 phages, the raw milk phages did not reveal high thermal stabilities. We also studied the biological characteristics of representatives of the raw milk phages. The phages were not well adapted to the lactococcal starter strains used in our study, as they revealed atypical propagation kinetics regarding adsorption efficiencies, latent phases and burst sizes resulting in delayed lysis of host cultures. According to these data, it can be concluded that i) raw milk phages may not be regarded as a major source of dairy phages and that ii) they are not well adapted to the characteristics of industrial milk fermentation.

Keywords: Lactococcus lactis, bacteriophage, raw milk

Exploring biodiversity in microbial ecosystems along the food chain

P1.58

Selection of fast acidifying starters for rye and wheat sourdoughs

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Sourdough fermentation parameters, rheological properties of the dough and sensory characteristics of bread are dependent on the starter cultures used for fermentation. A good starter should assure fast acidification, allow easy handling of the dough and provide desired aroma and flavour to the final product. In order to select new fast acidifying starters, spontaneous wheat and rye sourdoughs were renewed for two weeks with regular refrigeration between fermentation cycles. Three rye flours and three wheat flours from different mills were used as raw materials.

During the two weeks of daily propagation pH, total titratable acidity and the content of sugars and organic acids were measured after each cycle to evaluate the maturity of the sourdoughs. As for microbiological analysis, the numbers of lactic acid bacteria and yeasts were determined by plating and the composition of microbial communities was described by metagenomic sequencing of 16S ja ITS gene amplicons. The carbohydrate fermentation profiles of the isolated potential starter strains were determined by API CH 50 tests. Isothermal microcalorimetry (IMC) experiments were performed to describe the length of lag phase, maximal specific growth rates and heat produced during different growth stages.

It was found that the composition of the bacterial communities in mature sourdoughs greatly depended on the origin of flour. As refrigeration of sourdoughs is a common procedure in many bakeries, it was determined that selected lactic acid bacteria remained viable at colder temperature and were able to begin acidifying the sourdough shortly after inoculation with fresh flour and water, which describes a short lag phase. The analysis of IMC power-time curves illustrated high adaptation to the sourdough environment, as the starters were able to utilize the carbohydrates in flour during the first few hours of incubation and produce high concentrations of organic acids. The API CH 50 tests confirmed, that isolated lactic acid bacteria were able to ferment a majority of substrates available in flour. As a result of this study new fast acidifying lactic acid bacteria strains were isolated for further evaluation in baking experiments.

Keywords: sourdough, lactic acid bacteria, 16S, isothermal microcalorimetry

Exploring biodiversity in microbial ecosystems along the food chain

P1.59

How the different characteristics of an industrial production chain can influence the microbial fingerprint of cooked ham?

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Sliced cooked ham is characterized by a variability of the initial raw foodstuff (meat origin, seasonal variations) together with variable process parameters. Despite a cooking phase, a remaining microbiota can develop during the process and thus during storage under refrigerated conditions. Some of these bacterial species can induce spoilage such as pH decrease and gas production (sometimes off-odors).
This study aims to map the bacterial fingerprint of an industrial production chain of cooked ham, according to process parameters. The bacterial communities of ham packaged under modified atmosphere were characterized using a global metagenetic approach based on *gyrB* gene analyses combined with organoleptic characterization (pH, volatile compounds analysis).
Following a complete factorial design, sampling was organized in four campaigns, spread over a year to take into account seasonal variability. Around 400 packed hams were produced in one single industrial unit, taking into account different process characteristics: raw meat origin, time and type of transportation of raw meat, churning time, storage time after cooking, slicing line, and O2 permeability of packaging. For each condition of production, samples were collected and analyzed at three different stages of aging: just after packaging (T0), at the use-by-date (UBD) and 15 days after the UBD (UBD+15d).
The metagenetic analysis revealed that the main process parameters influencing the microbiota (diversity, richness and evenness) at UBD and UBD+15d were the slicing line, the O2 permeability of packaging and the churning time. These parameters also influenced the quantities and the nature of volatile spoilage molecules, the bacterial load and the final pH.
Cooked ham microbiota is composed of firmicutes and proteobacteria, with a dominance of firmicutes. However, the presence of proteobacteria is mainly correlated to the use of a film with higher O2 permeability for packaging. An analysis at the species' level revealed that the slicing line strongly influences the diversity of both dominant and sub-dominant species for both phylum. The other parameters mainly influence the abundance of the microbiota. The different microbiotas were also correlated with some discriminant volatile compounds.
Our large scale analysis allowed us to conclude that the process steps have a strong influence on the diversity of ham microbiota. This global approach can thus be helpful for further process piloting.

Keywords: metagenetic - microbial fingerprint - production chain

Exploring biodiversity in microbial ecosystems along the food chain

P1.60

Dairy farm of origin is a dominant source of background microflora in artisan cheeses from Canada

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Cheese is a unique food in that its characteristics are partially determined by its unique microbiome, giving cheeses an individual "terroir". It is therefore of interest to determine how much of this microflora is independent from added starter-cultures, and where these native microorganisms come from. The objective of this project was therefore to investigate the relative abundance of non-starter bacteria in artisan cheeses, and to determine how much of this microflora may be related to the dairy farm environment. Environmental samples were collected from a dairy farm in British Columbia, Canada, and included clover pasture (n=20), hay pasture (n=20), hay (n=20), straw bedding (n=19), cow feces (n=20), and udder swabs (n=19). Artisan cheeses produced solely from milk from the same farm were also collected and included a pasteurized milk brie (n=15), and non-pasteurized casacadia (n=8), gruyere (n=9), and cheddar (n=12) styles. Microbial community analysis was conducted by sequencing 16S rRNA extracted from the samples. Operational taxonomic units (OTUs) were determined based on 97% similarity between the sequences. Background microflora of cheeses was considered all observed OTUs not identified as the common starters *Lactobacillus*, *Lactococcus*, *Streptococcus*, or *Leuconostoc*. The cheeses showed a significantly lower number of OTUs (49 ± 17) compared to the dairy farm environment (199 ± 53; Kruskal-Wallis test; $p < 0.001$), but most (> 99.5%) of the background microflora in the cheeses were also observed in the dairy farm environment. These common OTUs were most often observed in the pasture, or in the straw bedding, and suggest that the dairy farm environment may be a dominant source of background microflora in artisan cheeses. Counter-intuitively, the relative proportion of background microflora was significantly higher in the pasteurized brie (31.2% ± 24.8%) compared to the non-pasteurized cheeses: 8.2% ± 23.0%, 8.8% ± 7.5%, and 1.5% ± 0.7% in cheddar, gruyere, and cascadia, respectively (Kruskal-Wallis test; $p < 0.05$); though large variation was observed between samples. This suggests that pasteurization does not necessarily remove all the native microflora, and that cheese style may play a role in relative abundance of the background microflora. This work suggests that the dairy farm environment may be a dominant source for background microflora in artisan cheeses, and supports the idea that cheeses possess a terroir related to their place of origin.

Keywords: cheese microbiome, background microflora, pasteurization

Exploring biodiversity in microbial ecosystems along the food chain

P1.61

Genetic engineering strategies for the bioproduction of conjugated linoleic acid from *Lactobacillus delbrueckii* subsp. *bulgaricus*

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Conjugated linoleic acid (CLA) has attracted attention in recent decades because of its health benefits. CLA is an intermediate product of the biohydrogenation pathway of linoleic acid in bacteria. The biotransformation of linoleic acid into CLA by microorganisms is a potentilly useful industrial process. Several bacterial species capable of efficiently converting linoleic acid to CLA have been widely reported in the literature, among them *Lactobacillus delbrueckii* subsp. *bulgaricus*. Recent researches has proposed a hypothesis of a multi-component enzymatic system consisting at least of three enzymes involving the biohydrogenation process of linoleic acid. The genome sequencing of *L. delbrueckii* subsp. *bulgaricus* 2230 revealed only the gene capable of encoding an oleate hydratase (*oleA*). Associated to previous studies with the same strain, these findings suggest a potential catalytic promiscuity of the oleate hydratase present in the the *L. delbrueckii* subsp. *bulgaricus* 2230 genome. The *oleA* gene of *L. delbrueckii* subsp. *bulgaricus* 2230 was identified, cloned into expression vectors and introduced into the parental strain *L. delbrueckii* subsp. *bulgaricus* 2230 as an extra copy under the control of constitutive promoter and in the receptor strain *Escherichia coli* BL21 (DE3) under the control of an inducible promoter. The *E. coli* insert will be used for *in vivo* heterologous expression assays as well as for subsequente purification of the recombinant protein to be used in *in vitro* assays for the characterization of the enzymatic activity. The recombinant strain of *L. delbrueckii* subsp. *bulgaricus* expressing constitutively *oleA* will be used for phenotypic analyzes, among which an evaluation of the CLA potential production in standard culture medium for cultivation of *Lactobacillus* sp. compared to the production obtained in fermented whey-based.

Keywords: Oleate hydratase; biohydrogenation; lactic acid bacteria

Exploring biodiversity in microbial ecosystems along the food chain

P1.62

Development of novel biofunctional foods and total quality enhancement of traditional dairy products by suitable management of their microbial ecology - BIO TRUST

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The objective of the current project is to develop new bioactive dairy products and enhance the quality and safety of traditional Greek dairy products by suitable management of their microbial ecology. The main aims of the project are to a) improve the traditional dairy products' overall quality by extension of shelf life, enhance organoleptic characteristics, improve hygiene/ safety level, standardization/ stabilization of the production process and minimize product returns/recalls, b) develop novel fermented dairy products with traditional character and 'distinctive' organoleptic properties, supporting the innovative production along with the 'nationality' and authenticity of the product, company and/ or area of production, and c) develop novel bio-functional dairy products with desirable organoleptic characteristics and high added value due to the dominance of 'active' lactic acid bacteria (LAB) with known bio-protective properties *in situ* and probiotic properties *in vitro*.

A variety of indigenous LAB is being isolated from different sources (high quality milk, whey, fresh curd of cheese and/ or final Greek cheeses) and explored at phenotypic, biochemical, molecular and proteomic level for the development of novel specific bacterial cultures. LAB with explicit technological, bioprotective and/or probiotic properties are consequently exploited for the formulation of a highly competitive and desirable predominant microbiota culture of 'specific composition'. These novel LAB cultures are then being applied in fermented dairy products on industrial scale. Target products of this project are Feta and White Brine cheeses produced by Hellenic Dairies-TYRAS and acid coagulated fresh cheeses (i.e. Galotyri, Tsalafouti) and Anthotyros (a fresh whey cheese) produced by SKARFI, exploring in parallel the microbial spoilage and potential microbial hazards associated with each product. The new cultures are expected to be able to postpone spoilage, eliminate or inactivate the growth of *L. monocytogenes* and other pathogens that might be present due to cross contamination and confer increased biofunctional/ probiotic properties at the novel product.

The main innovation of this project lies in the fact that the novel lactic bio-functional and/ or probiotic cultures include competitive LAB strains with excellent adaptation, resistance and long-term survival in the food environment, preserving in parallel the traditional organoleptic character of each product.

Keywords: Microbial ecology,Dairy products,New cultures,Safety,Biofunctional foods,Gastronomic identity

Exploring biodiversity in microbial ecosystems along the food chain

P1.63

Staphylococcus aureus and background microbiota of dairy samples determined by culture method and by metagenomic based on 16S rRNA

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Dairy products are highly susceptible to contamination by pathogenic and spoilage microorganisms. To avoid significant losses for the dairy industry, it is very important to evaluate the background microbiota of samples harboring pathogenic microorganisms, to better understand the factors that favor their survival and persistence. To aid in the elucidation of these microbial communities, metagenomic analysis is a powerful tool that targets the genetic information from a community of microorganisms present in a sample. For this study, two groups of samples positive for *Staphylococcus aureus* by classic technique (surface plating on Baird Parker agar and phenotypic confirmation) were also evaluated for background microbiota by both, culture and 16S rRNA metagenomic.analysis. For metagenomics, total DNA was extracted with the PowerLyzer® PowerSoil® DNA isolation kit (MoBio®) and DNA libraries were prepared according to the protocol of Illumina®. DNA sequencing was done with the Illumina® MiSeq platform and bioinformatic analysis were performed with the dedicated software from Illumina®. The presence of *S. aureus* was confirmed by metagenomics in one group of samples, which included: whey curd, fresh cheese and mozzarella packed cheese. The background microbiota of these samples determined by culture approach was composed of *Leuconostoc mesenteroides* and *Streptococcus salivarius.* The results of 16S rRNA metagenomic analyses were superior to show the microbial diversity of samples and detected mainly *Lactococcus* sp. (55.3%), *Bacillus* sp. (16.1%), Vagococcus sp. (2.5%), *Escherichia* sp. (2.1%), *Enterococcus* sp. (1.5%) and Enterobacter sp. (1.2%). FAPESP: 2012/50507-1

Keywords: Dairy, Staphylococcus aureus, background microbiota, metagenomics

Exploring biodiversity in microbial ecosystems along the food chain

P1.64

Enumeration, isolation and identifi cation of lactic acid bacteria from the Italian traditional fermented milk "Gioddu"

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In many areas of the world traditional dairy products rely on spontaneous milk fermentations which result in a large variety of products with different flavours and containing important beneficial microorganisms. Gioddu, a traditional fermented milk, is prepared in the very restricted areas of north Sardinia (Italy) from sheep or goat milk and it is a typical meal of shepherds. Gioddu is prepared as follows: the row milk is added with calf rennet and maintained firstly for 30 min at 30-40°C. then for one-two days at room temperature. This preparation is used as natural starter culture ("*sa madrighe*") and added at a rate of 1-2% to fresh milk previously boiled for 5 min and cooled to about 30-40 °C. After 8 hours the Gioddu is ready. Gioddu can be obtained also after adding boiled milk with the previous day's Gioddu production. The objective of this study was to investigate the diversity of lactic acid bacteria (LAB) communities in 5 different samples of Gioddu and *madrighe* collected from 3 different areas in Sardinia, with culture dependent and independent techniques.
The LAB counts in these samples varied from 10^5 and 10^9 ufc/ml. Fermented milks had almost identical mean numbers of LAB. In total, 100 isolates were obtained from these samples using MRS agar and M17 agar incubated at 25, 37 and 42°C in order to widen the possibility to isolate different strains. Each isolate was considered to be presumptive LAB based on gram-positive and catalase-negative properties and on RAPD-PCR. Basing on RAPD-PCR database the following species have been presumptively identified *Lactococcus lactis, Streptococcus thermophilus, Leuconostoc mesenteroides, Enterococcus* spp. and *Lactobacillus paracasei.* LH-PCR fingerprinting, associated to the sequencing of 16S rRNA genes, was used for the characterization of the bacterial community and to confirm identification of species in the 5 Gioddu and Gioddu starters tested. The resulting LAB confirmed the results of RAPD-PCR with two main LAB associations: in 2 samples the prevalent species were *Lactococcus/Streptococcus, Leuconostoc, Enterococcus* while in the other 3 samples *Lactococcus, Streptococcus, Leuconostoc.* These results can help to understand more about Gioddu which is a traditional fermented product until now poorly investigated. Due to the importance of finding new probiotics, further studies will include challenges from a phylogenetic point of view and for screening the functional properties of the strains isolated.

Keywords: Gioddu, lactic acid bacteria, fermented milk, Sardinia

Exploring biodiversity in microbial ecosystems along the food chain

P1.66

Microbial diversity of Brazilian artisanal cheeses from different regions

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Brazilian artisanal cheeses are mostly produced from raw milk, with regional differences in the technology of production. In this study, the microbial ecology of different types of artisanal cheeses produced in Brazil (n=578) was investigated by 16S rRNA gene targeted high-throughput sequencing. Different types of cheeses (Marajó, Butter, Curd, Caipira, Araxá, Campo das Vertentes, Canastra, Cerrado, Serro, Colonial and Serrano), coming from North, Northeast, Central, Southeast and South regions were analyzed. Differences in the cheese microbiota were observed as affected by the producing region and production technology. Lactic acid bacteria (LAB) dominated in all cheeses although the presence of contaminants such as *Enterobacteriaceae* and *Staphylococcus* also occurred in North, Northeast and Central cheeses. The most abundant genera of LAB were *Streptococcus*, *Leuconostoc*, *Lactococcus* and *Lactobacillus*, with variable abundance according to the cheese type and the region of production. The present study provides a microbiological mapping of Brazilian artisanal cheeses and shows the impact of geographic origin and production technology on the microbiota.

Keywords: Artisanal cheese, cheese microbiota, 16S rRNA, metagenomics.

Exploring biodiversity in microbial ecosystems along the food chain

P1.67

Microbiological profile of fermented cabbage juice and its change during the storage

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Last years of the twentieth century were marked with increased worldwide interest in functional products containing probiotic bacteria. Published research confirms the potential of agro-food by-products to be used as a good healthy constituent of functional foods containing probiotics. Fermented cabbage juice is still insufficiently exploited vegetable resource and agricultural by-product, recently recognized as a great medium for lactic acid production. Therefore, microbiological profile of fermented cabbage juice, cultivar Futoški, was investigated. The fermentation process was carried out under industrial conditions using different combinations of NaCl (7%, 8%), starter culture (1 dl) and temperature (18°C, 22°C). Furthermore, the influence of fermentation process conditions on the following parameters was analyzed: the number of lactic acid bacteria, aerobic mesophilic bacteria, aerobic spore-forming bacteria, yeasts and molds. In addition, the change in the content of lactic acid bacteria in fermented cabbage juice was monitored during the storage of 35 days at 4°C and -20°C. Higher number of lactic acid bacteria and aerobic mesophilic bacteria was measured in the fermented cabbage juice sample obtained using 8% of NaCl at 22°C (2.55×10^6 and 2.45×10^6 CFU/g, respectively) in comparison to that obtained using 7% of NaCl at 18°C. On the other hand, cabbage juice sample obtained using 8% of NaCl at 22°C had lower number of yeasts (4.8×10^5 CFU/g) and molds (270 CFU/g). The number of aerobic spore-forming bacteria was < 10 CFU/g in both samples. Results also showed the change during the storage in terms of a drop in the number of lactic acid bacteria in fermented cabbage juice. The number of lactic acid bacteria was higher in the samples kept at temperature of 4°C. In conclusion, this research represents a pioneering work to further investigations directed towards the bioactive properties of fermented cabbage juice for the purpose of new functional product design and development.

Keywords: Cabbage juice; Fermentation; Lactic acid bacteria

Exploring biodiversity in microbial ecosystems along the food chain

P1.69

Salmonella prevalence and concentration in poultry litter from the Southern United States

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Poultry houses in the United States produce 10.2 million tons of litter each year. This high nitrogen containing by-product is frequently applied to agricultural lands as an alternative to synthetic fertilizers but little is known about the prevalence, concentration, or survival of *Salmonella* in poultry manure used as a soil amendment.

This study determined *Salmonella* concentration and prevalence in raw (untreated) poultry litter from farms in the southern United States in order to inform future regulatory efforts on establishing application-to-harvest interval(s) when amending soils with raw poultry manure to grow produce covered under the FDA's Produce Safety Rule.

Poultry litter was collected from 12 broiler and breeder farms in the US states of Florida (1 farm), Georgia (2), Alabama (8), and Texas (1). Seven samples (30 g each) from three to four litter piles at every farm were collected from poultry houses or dry stack sheds. Samples (30 g) were selectively enriched (Rappaport-Vassiliadis [RV] and Tetrathionate [TT] broths) and screened for *Salmonella* presence by streaking onto Xylose-Lysine-Tergitol 4 agar (XLT4). All presumptive colonies were PCR confirmed as *Salmonella*. The concentration of *Salmonella* was determined through concurrent enrichment and serial dilution in MPN reservoirs at the time of screening, which was paused using refrigeration, until *Salmonella* screening was complete. When a sample was confirmed positive for *Salmonella* processing of the RV and TT broth MPN reservoirs resumed. Following enrichment, 10 µL from each MPN reservoir was streaked onto XLT4; all presumptive positive *Salmonella* colonies are PCR confirmed and MPNs calculated.

Of the 37 piles sampled from 12 farms, 13 piles (35%) from 6 farms (50%) were positive for *Salmonella*. Within positive piles, the number of positive samples ranged from 1 to 7 out of 7 collected. The minimum and maximum MPN/g were 1.6 and >280,000, respectively; mean and median MPN/g *Salmonella* for positive samples were 101,331.6 and 2,850.0, respectively.

Salmonella was present on half the farms sampled, in less than half of poultry litter samples collected. Concentrations were highly variable. Contaminated, untreated, poultry litter may pose a health risk if untreated poultry manure contacts produce and the application-to-harvest interval is insufficient to allow for adequate pathogen die-off.

Keywords: Salmonella, poultry litter, produce

Exploring biodiversity in microbial ecosystems along the food chain

P1.70

Isolation and evaluation of probiotic properties of lactic acid bacteria on poultry

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Performance and productivity in the poultry industry lately is predicated on the use of antimicrobials, which has led to various negative impacts; including the emergence of a variety of pathogens and bacterial resistance. Probiotics are now accepted as safe and effective alternatives which can sustain or promote growth performance and prevent disease. This study was designed to isolate and evaluate the probiotic properties of LAB on poultry. Strains of *Enterococcus faecium* and *Pediococcus acidilactici* were isolated from yoghurt and goat milk samples by colonial, morphological, and biochemical methods, and further characterized for probiotic properties. *In vivo* evaluation of the isolates was conducted by their effect on the body weight gain and immune response of the birds. A total of 20 day-old Cobb broiler chicks were purchased from a local hatchery and randomly divided in 3 groups. The study group were administered the LAB strains isolated, the commercial group with commercial probiotic (Protexin®) while the control group with antibiotic (Renaflox) alongside commercial feeds for 35 days. Different productive, economic and hematological measures were determined and postmortem examination conducted for side effects. Results obtained reveal that the livability of study and commercial groups was 100.0% while 83.0% was recorded for the control group. The mean body weight in the study, commercial and control groups were 2503.00±36.95gm, 2531.25±34.98gm and 2293.75±42.97gm. There was a statistically significant ($P< 0.05$) increase in weight gain observed in probiotic (commercial and study) supplemented birds than birds not supplemented with probiotic (control). Dressing percentage measured was74.53±1.84%, 75.21±0.89% and 72.69±1.27% for the study, commercial and control groups respectively. Nevertheless, the measured giblets percentage was highest for the study group (4.88±0.01%), as the average weight relative to total live weight of gizzard and spleen were also highest in study group (1.27±0.13% and 0.08±0.01% respectively). The hematological parameters (RBC, WBC, Hb, PCV and ESR) evaluated were high in the study and commercial groups than the control group. The postmortem analysis of organs; hearts, spleens, gizzards and meats showed no significant differences ($P> 0.05$) among all the groups. The effects of probiotic supplementation have significant effect on growth performance, internal organs weight, meat yield and hemato-biochemical parameters in poultry.

Keywords: Lactic acid bacteria, probiotics, poultry, antibiotics

Exploring biodiversity in microbial ecosystems along the food chain

P1.71

Evaluation of scaling effects on microbiological and physico-chemical characteristics of raw fermented sausages

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In literature, very few studies of fermented sausages, also known as salami, focus on an industrial scale volume i.e. upwards of 400 kg per production day. Research projects rather focus on smaller productions comparable to artisanal practice. Agroscope, Switzerland are heading the construction of a pilot plant biosafety level 3 lab in Posieux, Switzerland which will produce cheese and meat fermented goods with microbial safety being of up most concern. Therefore, it is vital to investigate the microbial ecology in fermented meat production across ascending (industry) or descending (pilot and lab) scales of production. Use of different quantities, technologies and process steps may have an influence on the final product for its physico-chemical and microbial safety attributes. In this study a number of comparative studies were carried out on the scaling effects of meat fermentation and drying. Three major influences were investigated (ripening chamber, production scale, salami diameter). Comparative analysis of physico-chemical, culture dependent and MALDI-TOF methods were applied to investigate the microbial ecology during ripening across all three scales.
A difference in production scale technologies did have an effect on the texture and salt content of the salami on the other side the result of MALDI-TOF showed the microbial ecology in the raw material, as well as how the addition of a starter culture influences and change it during salami fermentation and ripening.

Keywords: raw sausage, fermentation, microbial ecology

Exploring biodiversity in microbial ecosystems along the food chain

P1.72

Bacterial community composition and prevalence of antibiotic resistance in milking machine biofilms

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Biofilms on milking machines are a source of contamination of raw milk and its products. They can also facilitate the transmission of mastitis pathogens within herds. Due to the close contact between bacterial cells within a biofilm, we analyzed the relation between bacterial population density and the abundance of antibiotic resistance genes. Our aim was to reveal the impact of biofilms on horizontal gene transfer.

Swab samples of different parts of the milking machine of a German dairy farm were investigated by culture-dependent and independent methods. Spots in the milking system with enhanced microbial colonization were identified by colony counting on selective and non-selective media. The fraction of antibiotic resistant cells was quantified on media containing different β-lactams and tetracycline. Isolates were identified by 16S rRNA gene sequencing to assess the overall bacterial diversity and to identify dominating bacterial groups and antibiotic resistant isolates. DNA was extracted directly from each swab sample, and different groups of antibiotic resistance genes were quantified in these extracts by RT-qPCR.

Different parts of the milking machine displayed high biofilm cell density. A high bacterial diversity, also of antibiotic resistant strains, was detected for the phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. Different antibiotic resistance genes were detected and quantified by RT-qPCR. They were correlated with bacterial densities at the sampling points.

Differences between cultural and molecular microbial counts will be elucidated. The impact of bacterial cell density on the abundance of resistant cells and antibiotic resistance genes will be discussed.

Keywords: Dairy biofilm, milking machine, community composition, antibiotic resistance

Exploring biodiversity in microbial ecosystems along the food chain

P1.73

Biodiversity of food borne microbes in palm wine: An African naturally fermented alcoholic beverage

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Palm wine is an alcoholic beverage obtained from the sap of various types of palm tree like palmyra, date palms and coconut palms. It is common in many parts of Africa, Asia and south America where it has been given different names in various locations/ regions. Several species of bacteria and yeasts have been reported to originate from this rich alcoholic beverage like *Saccharomyces cerevisiae* species, *Candida sp*. and *Endomycopsis* sp. (Amoa-Awua *et*. *al*. 2006; Ayogu, 1999).
Several researchers have reported its microbial biodiversity *Saccharomyces cerevisiae* was reported to dominate the yeast biota, *Lactobacillus plantarum* and *Leuconostoc mesenteroides* were the dominated lactic acid bacteria while acetic acid bacteria were isolated only after the third day when levels of alcohol had become substantial and all these are industrially ustilized for biotechnological purposes (Nwachkuw *et*. *al*., 2006).
Palm wine has served has the origin of so many microbes of biotechnological usefulness and more focus on this will really be of high economic advantage.

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Keywords: palm wine, biodiversity

Exploring biodiversity in microbial ecosystems along the food chain

P1.74

Microbiological quality of frozen organic chicken meats

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Organic food market have grown dramatically over the last decade, particularly in developing and developed countries. The aim of this study was to determine the microbiological quality of frozen chicken meats produced organically at retail level.
A total of 240 frozen chicken meat samples (produced organically) including chicken drumstick, breast and leg quarter were collected from supermarkets in the province of Diyarbakir, and some online shopping markets in Turkey. The numbers of total mesophilic aerobic bacteria, psychrophilic aerobic bacteria, coliform bacteria, *E. coli* biotype 1, mold and yeast in the samples were determined by pour plating, whereas the Pseudomonas spp. count determined by spread plating.
Of the 240 organic chicken meat part samples analyzed, 100 %, 100 %, 100 %, 81,66 %, 54,16 %, 34,16 % and 83,33 % were detected to be contaminated with mesophilic aerobic bacteria, psychrophilic aerobic bacteria, *Pseudomonas* spp., coliform bacteria, *E. coli* biotype 1, mold and yeast, respectively. The mean count (log10 cfu/g) of mesophilic aerobic bacteria, psychrophilic aerobic bacteria, coliform bacteria, *E. coli* biotype 1, *Pseudomonas* spp., mold and yeast were determined as 4.99±0.80, 5.29±0.96, 3.53±0.92, 2.45±0.65, 4.63±1.10, 2.03±0.42, 3.68±1.13, respectively.
This study results showed that chicken meat parts produced organically could be contaminated with some important spoilage and indicator microorganisms. Therefore, consumers should not be considered perfectly safe the organic chicken meats at retail level.

Keywords: Organic, Chicken meat, Frozen, Microbiological quality, Food safety

Exploring biodiversity in microbial ecosystems along the food chain

P1.75

Austrian hard cheese production: The impact of autochthonous facilityspecific microbiota

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Cheese ripening involves the development of a complex and dynamic microbiome that establishes throughout the process and strongly influences the characteristics of the final products. This microbiome also influences the safety of cheeses, as microorganisms can act as a natural barrier for pathogens and spoilage microorganisms. The food processing environment can act as a source of natural microbial inoculation, especially in traditionally manufactured products.
Austrian Vorarlberger Bergkäse (VB) is an artisanal hard cheese produced in the western part of Austria (Vorarlberg) and has a protected designation of origin (PDO). VB is brined either in a brine bath or by dry salting surface treatment and no other treatment, such as the addition of external ripening cultures, is applied during ripening, which last from three to up to 18 months.
Composition of bacterial communities present on VB rinds and on several environmental surfaces (air filter, floor, racks, shelves and wall) from two different ripening cellars (that contain short- or long-ripened VB) was investigated by 16S rRNA gene cloning and Sanger sequencing, yielding near full-length high-quality 16S rRNA sequences per clone.
VB production conditions (long ripening time, high salt concentration and low temperatures) favored the growth of psychro- and halotolerant bacteria, such as *Halomonas, Oceanisphaera, Pseudoalteromonas* and *Psychrobacter*. *Halomonas* was the most abundant genera in the study and was present on VB rinds and environmental surfaces, although 99% similarity-OTU level analysis revealed that not all *Halomonas* OTUs seem to colonize both environments.
Analysis of OTUs shared between different surfaces suggests that VB rind bacteria are inoculated naturally during the ripening from the processing environment and that cheese surfaces exert selective pressure on these communities, as only certain bacterial groups such as *Brevibacterium*, *Staphylococcus equorum*, *Halomonas boliviensis*, *Corynebacterium*, *Leucobacter* and *Sphingobacterium* seem to be able to flourish.
The processing environment and cheese rind microbiota may be considered as a whole with the goal of better defining the events that take place during cheese production. This study shows for the first time the relationship between cheese rinds and facility-specific microbiota from Austrian Vorarlberger Bergkäse and the importance of non-inoculated autochthonous microbiota dominating VB rinds.

Keywords: cheese microbiota, food processing environment, microbial ecology, DNA sequencing

Exploring biodiversity in microbial ecosystems along the food chain

P1.76

Microbial diversity in breadsticks production as revealed by culturedependent and culture-independent approaches

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Breadsticks are bakery products of Italian origin (first reports of their production dates back to 17th century), which market is increasing in Europe and worldwide as appetizers or as alternative to bread. The different existing technology processes impact on their characteristics, e.g. texture. However, little is known about the microbiological scenario which develops during production, which could take several hours from the preparation of the dough to baking and could impact on other characteristics, such as aroma or digestibility.

The aim of the present study was the investigation of the microbial dynamics during the industrial production processes of breadsticks from wheat (*Triticum aestivum*) and organically produced spelt (*Triticum spelta*) flours.

Three different productions for each type of product were sampled at the company at different time points and culture-dependent and culture-independent analyses were performed to unveil the microbial diversity, characterise the cultivable strains, and determine which was the impact of commercial sourdough and yeasts, raw material and environment.

At the last sampling point, just before baking, yeast counts usually reached 8 log, while lactic acid bacteria (LAB) counts varied depending on the medium used for the determination (4 modified SDB and 3 modified MRS-based media), but were generally as high as 5-6 log.

The culture-independent technique PCR-DGGE was applied to either DNA isolated directly from the unbaked doughs and to DNA isolated from all colonies grown on the diverse culture media. As for yeasts, *Saccharomyces cerevisiae* was the dominant species, while, for LAB, a complementary view was obtained with the two approaches, suggesting that culturable LAB were not representative of the whole population.

A total of 206 LAB colonies were isolated and, after de-replication with REP-PCR (GTG5), identified by 16S rRNA gene sequencing. A relatively high biodiversity among strains belonging to different heterofermentative LAB species was revealed, suggesting that strains derived from commercial natural yeast preparations contributed, but were not dominant, in the different fermentations. Further work will be focused on the characterization of selected LAB strains, to evaluate their technological impact and role in sensorial properties of breadsticks.

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Keywords: breadsticks, dough microbiota, culture-dependent analysis, molecular identification

Exploring biodiversity in microbial ecosystems along the food chain

P1.77

Microbial composition of kombucha determined using amplicon and shotgun metagenomics

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Kombucha, a fermented tea generated from the symbiotic culture of yeasts and bacteria, has gained worldwide popularity in recent years due to its potential benefits to human health. As a result, many studies have attempted to characterise both its biochemical properties and microbial composition. To date, culture-dependent methods and amplicon-based sequencing have been used to study the Kombucha microbiome. However, these methods have limited taxonomic resolution and do not allow functional profiling of microbial communities. Here, we have applied a combination of whole metagenome sequencing (WMS) and amplicon (16S rRNA and Internal Transcribed Spacers (ITS)) sequencing to investigate both the bacterial and fungal communities of Kombucha. We have determined taxonomic and functional profiles at three different time points (days 3, 10 and 15) of fermentation. The 16S analysis indicated that *Komagataeibacter* was the dominant bacterial genus (>85%) in all samples at all time points, while the ITS analysis indicated that the yeast community was dominated by the genus *Zygosaccharomyces* (>95%). The WMS results showed strong agreement with these amplicon analysis results. Furthermore, we recovered three nearly complete bacterial genomes from the Komagataeibacter genus and one fungal genome of Zygosaccharomyces bailii and determined their genomic properties. Our results suggest that interactions between these dominant species may establish and sustain a functionally stable and low diversity ecosystem in Kombucha throughout the fermentation process.
Overall, this study provides a detailed description of the functional and taxonomic characteristics of the Kombucha microbial community.

Keywords: Kombucha, shotgun metagenomics, next generation sequencing, fermented tea, bacteria, yeast

Exploring biodiversity in microbial ecosystems along the food chain

P1.78

Prevalence, contamination and molecular detection of virulence genes of *E. coli* O157:H7 from vended cattle milk in Kwara State, Nigeria

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Contamination of raw milk by zoonotic pathogens it is a major public health concern presenting food safety threats to consumers. Reports of *E. coli* O157:H7 contamination of vended milk and milk products in Nigeria are scare creating a knowledge gap.
A cross-sectional study was carried out to determine the quality, prevalence and virulence characteristics of *E. coli* O157 in raw cattle milk processed and vended in Kwara state, Nigeria.

Methods: Total aerobic plate count (TAPC), Total coliform count (TCC), Methylene blue reductase test (MBRT), and California mastitis test (CMT) were carried out to determine the quality and wholesomeness of randomly collected raw cattle milk (n = 1255), across various markets (n = 10) in the major zones (Central, North & South) of the State. Isolation of *E. coli* O157 was done using standard microbiological procedures while $eaeA_{O157}$, Stx_1 and Stx_2 virulence genes *E. coli* O157: H7 were detected by PCR.
Mean TAPC ranged from 8.5 ± 1.4 logcfu/ml to 11.5 ± 0.6 logcfu/ml while all TCC were higher than logcfu 5. CMT revealed that 58.5% and 41.5% of milk samples were positive and negative respectively for mastitis causing organisms. The overall prevalence of *E. coli* spp was 844 (68.9%) and 35 (2.9%) for *E. coli* O157:H7. The virulence genes $eaeA_{O157}$ and Stx_1 were detected in 28 E. coli O157 isolates. Detection of Stx_2 is still ongoing
Our study determined the presence of pathogenic *E. coli* and largely poor quality of vended raw milk in the markets. In view of public safety, the need to demonstrate a high level of hygienic practice during milk processing - while enlightenment of processors on the need for standard hygiene - is required.

Keywords: Milk contamination, Prevalence, E. coli O157, virulence, Kwara State, Nigeria

Exploring biodiversity in microbial ecosystems along the food chain

P1.79

Measuring the response of Listeria monocytogenes single-strain biofilms to sanitisers used in ready-to-eat food processing environments using the CO_2 evolution measurement system (CEMS)

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Biofilms are omnipresent in the food processing environment and grow and proliferate under various conditions. Dissimilar growth conditions result in diverse biofilm structures and communities that respond differently to sanitation efforts. In this study, the response of *L. monocytogenes* monospecie biofilms, to sanitation chemicals was evaluated using a CO_2 evolution measurement system (CEMS). The CEMS is an effective method with which to study biofilms under flow conditions, since it accurately simulates conditions in water drain environment. Protocol development was conducted since *L. monocytogenes* biofilms have not been studied using this system. Four sanitisers representing quaternary ammonium compounds, peracetic acid and alternative chemicals were evaluated using their manufacturer prescribed minimum concentration and contact time. Responses were classified as the biofilm displaying development of resistance over time or being eradicated. Peracetic acid sanitiser and the proprietary QAC chemical showed no bactericidal effect. A general use QAC and proprietary QAC-free chemical yielded satisfactory results. This has laid the foundation in which to study the effect that a common industry practice, namely flushing sanitisers down drains without detergent washing, has on the persistence of *L. monocytogenes* biofilms.

Keywords: Listeria monocytogenes biofilms; sanitisers; resistance; drain biofilms, CEMS

Exploring biodiversity in microbial ecosystems along the food chain

P1.80

Occurrence of *Salmonella* spp. in backyard eggs available in Portugal and Romania

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Salmonella is one of the most frequently isolated foodborne pathogens. There were more than 94,500 human cases of salmonellosis reported in the EU in 2016 and foodborne infections caused by *Salmonella* Enteritidis have increased by 3% since 2014. Although a low prevalence of individual egg contamination was observed (0.29%), *Salmonella* in eggs still represents the highest risk agent/food combination (67 outbreaks). It is important to point out that this prevalence was calculated based on a low number of samples tested in each country and a great variability between countries was reported. Most of the data on the prevalence of *Salmonella* in eggs relates to eggs from henhouses kept in industrial settings and sold at commercial establishments. As consumers seek products promoted as “natural,” “organic” and “humanely-raised,” there is an increased trend for the consumption of eggs from backyard chickens. In the scope of SafeConsumE project (http://www.safeconsume.eu/), presence of *Salmonella* spp. was investigated in eggs collected from 50 domestic henhouses (two eggs/henhouse) located in Portugal and 54 located in Romania. *Salmonella* was detected in 10% of Portuguese and 42.6% of Romanian henhouses. In agreement with previous studies, more eggshells were positive for *Salmonella* spp. than the yolks. No correlation was found between visible faecal contamination and presence of *Salmonella* spp.. Although further investigation is still needed, these preliminary results highlight the need to inform consumers about the risks these eggs may represent.

Keywords: backyard eggs; Salmonella

Exploring biodiversity in microbial ecosystems along the food chain

P1.81

Occurrence and concentrations of mesophilic and thermophilic, aerobic and anaerobic sporeforming bacteria throughout the gelatin processing chain in different seasons

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Gelatine is a protein of animal origin extracted by the partial hydrolysis of collagen through thermal and chemical processes. After that, the extracted product is purified and subsequently dried. Despite the drastic conditions applied during processing, gelatin may present high bacterial loads depending on the processing conditions. The objective of this work was to determine the concentrations and incidence of sporeforming bacteria throughout the production process of gelatin. The samples were collected during different seasons of the year. The samples collected were analyzed for the presence/counts of aerobic (mesophilic and thermophilic), anaerobic (mesophilic) and *Bacillus cereus*. Sampling was conducted during summer (January and February), autumn (March and May) and spring (September and November). The analyses were performed according to the methods described in the Compendium of Methods for the Microbiological Examination of Foods. A high count of sporeforming bacteria was found in samples collected at the centrifugation step, except for *B. cereus* which presented high count in the ultrafiltration step. The mesophilic aerobic sporeforming bacteria were the most predominant group in six from fourteen stages together with the mesophilic anaerobic, which also predominated in other six stages of gelatin productive chain. In summer (February), there was a high incidence of *Bacillus cereus* group (37%) and thermophilic aerobic sporeforming bacteria (33%). A high incidence of aerobic mesophilic (51%) and anaerobic (56%) sporeforming bacteria was found in autumn (March) and spring (September). Moreover, the mesophilic aerobic sporeforming bacteria were the most frequent bacterial group in four months (January, March, May and November). *B. cereus* was the only bacterial group that was not predominant in any of gelatin processing stages. In the dried gelatin the highest loads of thermophilic aerobic (1.61 log UFC/g) and by mesophilic aerobic (1.22 log UFC/g) sporeforming bacteria were found in samples collected in March and November, respectively. The results indicate that, despite drastic conditions such as low pH, high temperatures and drying, sporeforming microorganisms are able to survive in all seasons of the year, but with predominance in the summer months.

Keywords: gelatin, sporeforming bacteria

Exploring biodiversity in microbial ecosystems along the food chain

P1.82

Fermentable dietary fibers differentially alter the rat gut microbiome

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Dietary fibers are associated with numerous health benefits, including reduced systemic inflammation and improved glucose homeostasis. However, the mechanisms by which these fibers influence health and the differences in physiological outcomes between distinct fiber sources are still poorly understood. To address this, we used a multifactorial study design to investigate the effects of low-amylose whole grain corn (WG), high-amylose resistant corn starch (RS), or their combination (WG+RS) on the physiology and gut microbiota of Sprague-Dawley rats fed either moderate- or high-fat diets. Rats fed WG+RS had the highest cecal butyrate concentrations, while RS-fed animals had the highest acetate and propionate levels. To identify bacteria that were responsive to the different diets, we examined the cecal microbial communities by 16S rRNA marker gene and metatranscriptome sequencing. Both the RS and WG+RS diets led to a dramatic shift in bacterial populations, characterized by a decreased phylogenetic richness and enrichment of the uncultured S24-7 family in the *Bacteroidetes* phylum. The WG diet lead to an enrichment of gut *Lactobacillus,* primarily *Lactobacillus johnsonii* and *Lactobacillus intestinalis*, but otherwise did not greatly affect cecal community structure. Interestingly, *Lactobacillus murinus,* another predominant member of the lactobacilli in the rat intestine, was unresponsive to WG consumption. The identities of these bacteria were confirmed upon culture-based enrichments from rat cecal contents. According to the cecal metatranscriptomes, *Lactobacillus*-associated alpha-galactosidases were expressed in rats fed the WG diets. Transcripts for amylopullulanases were upregulated and might serve an essential function in the breakdown of the plant-associated polysaccharides. Overall, these results show that complex dietary fibers maintain bacterial diversity in the digestive tract, while also enriching for specific glycan-degrading bacteria associated with health benefits.

Keywords: Lactobacillus, starch, fiber, microbiome, diet, gut, nutrition, fermentation

Exploring biodiversity in microbial ecosystems along the food chain

P1.83

Microbial diversity of Brazilian native fish during chilled and frozen storage

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Pacu (*Piaractus mesopotamics*), patinga (female *Piaractus mesopotamics x* male *Piaractus brachypomus*) and tambacu (*female Colossoma macropomum x male Piaractus mesopotamics*) highlight as native species of significant economic importance for Brazilian fish farming, being well accepted by the consumers. Spoilage Specific Organisms are directly influenced by storage conditions of the product and the development of undesiderate microbes, being responsible for the production of undesirable off-flavors, associated with spoilage. Thus, this study aimed to assess the microbiota involved in the spoilage of pacu, patinga and tambacuand in order to understand how they can affect the quality of these fishes. Changes in bacterial diversity of these three fish species, during ice or frozen based storage for one year, was studied through 16S rRNA amplicon based sequencing. The development of volatile organic compounds (VOCs) as well as the production of microbial metabolites assessed by nuclear magnetic resonance (NMR) were also investigated. The microbiota of the Brazilian fish was composed mainly by *Pseudomonas fragi, Brochothrix thermosphacta, Acinetobacter, Acinetobacter johnsonii, Bacillus, Lactobacillus plantarum, Kocuria* and *Enterococcus.* No significant differences were observed between fish species ($P< 0.05$). The results showed the presence of methane, propanoate, butanoate, sulfur, arginine and proline in higher amounts in frozen stored samples compared to ice stored samples. *Kocuria*, *P. fragi*, *L. plantarum, Enterococcus* and *Acinetobacter* were positively correlated with the metabolic pathways with the ether lipid metabolism. *B. thermosphacta* and *P. fragi* were related with metabolic pathways involved in aminoacid metabolism (arginine, alanine and proline metabolism). *P. fragi* was the most prevalent spoilage bacteria at in both conditions followed by *B. thermosphacta*, which can contribute to determine the final characteristics of the products. Moreover, the mains OTUs identified in fish samples were positively correlated with the production of off-flavour. In particular *P. fragi* was linked with the presence of hexanol, nonanal, octenol and ethyl-hexanol. Improved storage conditions is needed, possibly coupled with antimicrobial packaging in order to achieve a simultaneous inhibition of more spoilage microbial groups and to preserve the microbiological quality of fish during storage.

Keywords: Microbial ecology, Fish spoilage, 16S rRNA sequencing, volatile organic compounds (VOCs).

Exploring biodiversity in microbial ecosystems along the food chain

P1.84

Populations of mesophilic and thermophilic sporeforming bacteria and *B. cereus* during fermentation and drying of cocoa

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Cocoa pre-processing comprises fermentation and drying process of the seeds on farms. Fermentation and drying are performed without aseptic conditions, providing a high microbial contamination. Among these microorganisms, sporeforming bacteria (SFB) can survive the processing and be found in the final products.
The aim of this study was to investigate the incidence of SFB, including *B. cereus* (BC) throughout the pre-processing steps of cocoa.
A total of 180 samples were collected in different steps of cocoa pre-processing chain in two Brazilian farms (01 and 02). Samples were collected after the opening of the fruit (n=20), once a day during fermentation (n=100) and once a day during drying (n=60). Counts of mesophilic aerobic sporeforming bacteria (MAS), thermophilic aerobic sporeforming bacteria (TAS) and *Bacillus cereus* (BC) were performed after heat shock (80ºC/30min, 100ºC/20min, 110ºC/10min e 70ºC/15min), on Tryptone Glucose Extract agar (TGE), Dextrose Tryptone agar (DTA) and Mannitol Egg Yolk Polymyxin agar (MYP), respectively. The enumeration of mesophilic anaerobic sporeformers (MAnS) was performed using the most probable number (MPN) technique and PE-2 Medium (PE-2).
The highest counts (6 log spores/g) of SFB were observed in the samples collected during drying process. Populations of BC increased from 0.3 log spores/g to 0.8 spores/g log during drying process in farm 01 and remained constant (average value of 2.0 log spores/g) in farm 02. The counts of TAS was low in farm 01 (< 1.0 log spores/g) and reached 2.5 log spores/g in farm 02 (end of fermentation process). High counts were observed in MAS population of farm 02 (6.4 log spores/g in the end of fermentation). In the end of pre-processing, SFB were found in high concentration (5.8 log spores/g) and MAS was the dominant microbial group (2.0 and 5.8 log spores/g, for farms 01 and 02, respectively).
High population of SFB after fermentation and drying is a concern for industry, because the use of contaminated raw materials may impact on the microbiological quality and safety of thermally processed cocoa-based foods.

Keywords: cocoa, sporeforming bacteria, Bacillus cereus, prevalence, fermentation, drying

Exploring biodiversity in microbial ecosystems along the food chain

P1.85

Microbial diversity of the kayseri soudjouk, a fermented meat product with protected geographical indication

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Analysis of the fermented food products with the newly developed techniques in recent years enables recognizing the complex microbial relationships and revealing the effects of these on the product. In this study, it is aimed to determine the microbial diversity of Kayseri soudjouk by both culture-dependent and culture-independent methods. In the production of the traditional Kayseri soudjouk, spontaneous fermentation takes place, which allows the development of a complex microflora and rich flavor components preferable by the customers. In this work, instead of only the final product, the entire fermentation process is aimed to be examined to investigate the microbial profile in detail during fermentation. For this purpose, soudjouk samples will be obtained from local companies that produce traditional Kayseri soudjouk during the fermentation period (0, 1, 3, 5, 7, 11, 16 and 21 days). Basic chemical analysis (moisture, salt, pH and fat) and basic microbiological analyses (total mesophilic aerobic bacteria, coliforms, lactic acid bacteria (LAB), mold-yeast, pathogenic microorganism (*E. coli*, *Salmonella*, *Listeria*)) will be performed on the obtained soudjouk samples. Lactic acid bacteria and yeasts will be isolated by culture-based methods and purified. Then the purified microorganisms will be grouped genotypically using Rep-PCR. The representative microorganisms selected according to their profile in Rep-PCR will be identified by sequencing. For identification, the 16S rDNA region in bacteria and 26S rRNA for yeasts will be amplified in PCR. The amplified DNA fragments will be purified and sequenced. In order to determine the microbial diversity by culture-independent methods, DNA samples will be isolated directly from the soudjouk samples. The DNA samples will be subjected to high-throughput sequencing (HTS) analysis using the Illumina MySeq system. Carrying out both the culture-dependent and independent analyses will allow the determination of the microbial profile in detail and isolation of the effective microorganisms in the fermentation of the traditional Kayseri soudjouk.

Keywords: Traditional Kayseri fermented soudjouk, microbial diversity, HTS (high-throughput sequencing), lacti

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P1.86

Utilization of Streptococcus thermophilus 84C and *Lactobacillus brevis* DSM 32386 as promising strains for production of GABAenriched cheese

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Gamma-aminobutyric acid (GABA) plays a fundamental role in brain function through the gut:brain axis system, regulates the immune system and inflammatory processes and influences host energy metabolism. GABA is present at low concentration in foods, especially foods fermented with certain lactic acid bacteria. In this study we used the GABA-producing *Streptococcus thermophilus* 84C and *Lactobacillus brevis* DSM 32386 as starter and adjunct cultures respectively during experimental cheese production and investigated their ability to produce GABA *in situ* during cheese ripening.

Four experimental semi-hard cheeses were manufactured in triplicate during three consecutive weeks for a total of 36 samples: control (CTRL) cheese contained a commercial starter strain; 84C cheese contained *S. thermophilus* 84C; CTRL-DSM cheese contained the commercial starter strain and *L. brevis* DSM 32386; 84C-DSM cheese contained *S. thermophilus* 84C and *L. brevis* DSM 32386. Samples of bulk milk, curd and cheese after 2, 9 and 20 days ripening were processed for microbiological counts. Cheese samples were further analyzed in order to quantify the concentration of GABA and microbial populations. The amino acid composition of all cheese samples was quantified by Ultra High Performance Liquid Cromatography- Orbitrap Q-Exactive Mass Spectrometry (UHPLC-HQOMS). Total genomic DNA was extracted and a fragment of the hypervariable V1-V3 region of the 16S rRNA gene amplified and sequenced. Data did not show any significant difference amongst the four groups in terms of microbiological evolution and composition. We detected very interesting variations of GABA concentrations between the different groups during cheese ripening: after 2 days ripening GABA was detected at very low concentration in all cheese groups, with mean values ranging between 5.4 ± 4.3 and 16 ± 8.2 mg/Kg. After 9 days ripening the concentration of GABA was significantly higher in 84C (84 ± 37 mg/Kg), 84C-DSM (47 ± 22 mg/Kg) and CTRL-DSM (63 ± 19 mg/Kg) cheeses, compared to the CTRL cheese (11 ± 8.1 mg/Kg). At the end of ripening (20 days), 84C-DSM cheese had the highest level of GABA (91 ± 28 mg/Kg), which was not significantly different from 84C (73 ± 24 mg/Kg) and CTRL-DSM (88 ± 24 mg/Kg), but significantly higher than CTRL cheese (11 ± 10 mg/Kg). In conclusion, this study demonstrated that the tested strains *S. thermophilus* 84C and *L. brevis* DSM 32386 are promising candidates for the production of GABA-enriched cheese.

Keywords: GABA producing lactic acid bacteria, GABA-enriched cheese, experimental cheese-making, sequencing,

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P1.87

Effect of olive oil polyphenol dietary supplementation on gut microbiota and on shelf life of broiler chicken breast fillets

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Polyphenols are natural antioxidants and antimicrobials derived from various plant materials, whose beneficial effects on human health have been demonstrated by several epidemiological studies. Olive polyphenols are byproducts of olive oil production that cannot be discarded in the environment due to their polluting effect, but they may be reused as high value food additives. In this study, we investigate the effect of olive oil polyphenol dietary supplementation in broiler chickens in order to evaluate changes in their gut microbiota and breast microbial shelf life. A total of 144 chicks Ross 308 were fed with three dietary treatments from 24 days of age until commercial slaughter (48 d): control diet (L0); L1 diet supplemented with 220 mg/kg of Crude Phenolic Extract (CPE) obtained from olive vegetation water; and L2 diets supplemented with 440 mg CPE/kg. Individual live weight and feed intake were monitored throughout the trial. During rearing, cloacal swabs were collected at 23 d, 34 d and 44 d of age. DNA was extracted from these samples to determine the gut microbiota composition. After slaughtering, the breasts were subjected to microbiological analyses after 0, 2, 4, 6, 8 and 10 d to establish the microbial community and the shelf life following the different dietary treatments.

Growth performance and gut microbiota composition were not significantly affected by the dietary treatments. Nevertheless, the alpha- and the beta-diversity significantly decreased with age. A slight modification of gut microbiota was evident between L0 and L2 animals. Of note, chickens fed with the same diet showed high microbial variability, which might affect the statistical significance of our results. In the breast, few microbial targets (H_2S producing bacteria, *Pseudomonas* and total mesophilic count) increased both in L1 and L2 groups near the end of shelf life (8 d). Taken together, these results show that CPE dietary supplementation does not significantly affect chicken growth performance or their gut microbiota composition. CPE addition seems to reduce the breast shelf life due to the growth of *Pseudomonas* as spoilage organism. Flesh microbial community showed similar behaviours. Further research is needed to investigate the relation between polyphenol dosage and meat shelf life. Remarkably, polyphenols were found in the breasts of chickens belonging both to L1 and L2 groups, which may represent an improvement of the meat nutritional value.

Keywords: gut microbiota, polyphenols, shelf life, broiler

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P1.88

Evaluation of *Bacillus thuringiensis* strains ABTS-351 and ABTS-1857 biopesticides within a simulated gut environment

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Trends in agricultural and environmental regulation favour the adoption of biopesticides, including those based on *Bacillus thuringiensis* (Bt); however phenotypic similarities between Bt-based biopesticides and *B. cereus* species have raised concerns about the safety of commercial Bt on food. As sequencing commercial strains Bt subsp. *kurstaki* ABTS-351 and Bt subsp. *aizawai* ABTS-1857 confirmed an inability to produce the emetic toxin, cereulide, *in vitro* studies were conducted with these strains to assess their potential in causing diarrhoea under the current theoretical model: spore survival through GIT, adhesion, and outgrowth in the intestine. The Simulator of the Human Intestinal Microbial Ecosystem (SHIME®) system was employed to analyse the behaviour of these specific commercial Bt strains in the GIT and their relative impact on colonic microbiota and simulated host gut wall. To ensure worst-case conditions, a TripleSHIME® consisting of stomach/small intestine, proximal colon and distal colon reactors was treated with clindamycin to induce dysbiosis, mimicking an immunocompromised individual. Commercial Bt strains were fed into the SHIME at $4.9X10^8$ CFU/day for two weeks and evaluated for possible germination. An *in vitro* human cell culture model, consisting of a co-culture of Caco-2 cells and THP-1 macrophages, was exposed to spore-free extracts of the model to measure the impact of secondary metabolites on the inflammatory response to LPS. The potential for adhesion and germination of Bt spores was assessed using a Caco-2 cell assay. Neither Bt strain germinated in the upper and lower intestine; rather gastric passage undermined thermotolerance of Bt spores resulting in a 1 log reduction following the standard pasteurization procedure (20' at 80°C) for quantification. Unexpectedly, Bt treatments had minor beneficial effects on recovery of the gut microbiota upon antibiotic-induced dysbiosis. Finally, spore-free extracts of the SHIME® media improved cell integrity (TEER) of a simulated gut wall. Spore adhesion of Bt strain ABTS-351 was extremely low compared to Bt strain ABTS-1857 and *B. cereus*. The pathogenic *B. cereus* strain germinated in contact with Caco-2 cells while neither commercial Bt strain germinated after 4 h. The activity of the commercial strains studied did not conform to the proposed model for a diarrheal event supporting their designation as low risk plant protection products and highlights the need for discerning detection methods.

Keywords: Bacillus thuringiensis, Bacillus cereus, pesticide, SHIME, diarrheal food poisoning, in vitro model

Exploring biodiversity in microbial ecosystems along the food chain

P1.89

Lactobacillus crispatus to produce a functional cheese as dietary strategy to improve woman wellbeing

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Within this research, the suitability of Squacquerone cheese to support the viability of *Lactobacillus crispatus* BC4, a vaginal strain endowed with a strong antimicrobial activity against urogenital pathogens and foodborne microorganisms, in order to recommend a gender food for woman wellbeing, was investigated. The viability of *L. crispatus* BC4, used as adjunct culture in the production of a functional cheese, was evaluated during the product refrigerated storage, also when Squacquerone cheese was subjected to simulated stomach-duodenum passage. Moreover, the effects of the probiotic strain addition were evaluated on product pattern profiles, in terms of proteolysis, lipolysis and volatile molecule profiles. The data obtained underlined that *L. crispatus* BC4 maintained high viability, also in presence of physiological stress conditions, until the end of the refrigerated storage. Moreover, the inclusion of *L. crispatus* BC4 gave raise to cheese products with higher score of overall acceptability when compared to control cheese. Moreover, the survival of *L. crispatus* BC4, carried in Squacquerone cheese, was verified by the patented Simulator of the Human Intestinal Microbial Ecosystem (SHIME). The results highlighted that the vaginal *Lactobacillus* was initially affected by the low pH in the stomach, simulated by the reactor of the SHIME in fed state, while it was resistant to bile salts and pancreatic juices. Although only in vivo trials can confirm the functionality of the produced cheese in vagina environment, these data represent a further tassel to use the Squacquerone cheese produced as gender dietary strategy for woman well-being.
These results suggest that the obtained cheese could represent not only a gender strategy to prevent vaginal dysbiosis, but also a challenge to improve the dairy sector.
The research was supported by a grant obtained at the University of Bologna (Italy), within the Alma Idea Project “Approcci biotecnologici per la produzione di un alimento di genere - Biotechnological approaches to produce a gender food”.

Keywords: Functional Soft cheese, Lactobacillus crispatus, gender food, voletile molecule profiles

Exploring biodiversity in microbial ecosystems along the food chain

P1.90

Isolation and pathogenicity of some rot-causing mycoflora of irish potato (*Solanum tuberosum* L.)

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Irish- potato(*Solanum tuberosum L.*) is a very important tuber crop that is consumed as food globally. It can be eaten boiled, fried, roasted and in stews. It is also cultivated for its use as livestock feed and for industrial purposes. Storage rot constitutes a major constraint to Irish- potato production globally. Therefore, this study was carried out to: isolate and identify some rot-causing mycoflora of Irish potato; determine the moisture content of the potato as well as detect mycotoxins produced by some of the isolated mycoflora.The fungi associated with storage rots of Irish -potato tubers sold in Ibadan,Nigeria were investigated. Irish- potato tubers showing symptom of rots were randomly obtained from five different markets located within the Ibadan metropolis.The study areas were visited three times in three months for collection of rotted potato samples which were selected randomly at intervals. The fungi isolated and their percentage frequencies of occurrence were : *Aspergillus niger*(20.8%), *Aspergillus flavus* (12.5%), *Penicillium* sp.(29.2%), *Allomyces arbuscular*(8.3%), *Rodotorulla* sp.(4.2%), *Tricothecium roseum* (8.3%), *Mucor* sp.(4.2%), *Rhizopus stolonifer* (8.3%) *and Lasiodiplodia theobromae* (4.2%). Pathogenicity test confirmed all the isolated mycoflora as the pathological agents of Irish potato rot. Mycotoxin production levels of *Aspergillus niger*, *A. flavus* and *Penicillium* sp. were determined using thin layer chromatography (TLC) plug agar method. The potential hazards posed by these mycotoxins are discussed. Conclusively, this study highlight some recommendations to reduce storage rot of Irish-potato tubers.

Keywords: Solanum tuberosum L.; fungi; mycotoxin; pathogenicity; rot; thin-layer chromatography.

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P1.91

The effect of the addition of fresh and dried starter cultures on microbiological and chemical parameters of "Chourico", a traditional Portuguese smoked sausage

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The production of bacteriocins by Lactic Acid Bacteria (LAB) is a technology that has attracted the attention of the food industry interested in decreasing chemical additives in their products meeting consumer's expectations for healthier foods. This work aimed to investigate the effect of using a fresh and a dried starter culture of an autochthonous lactic acid bacteria strain, (*Lactobacillus plantarum* ST153Ch), on the physicochemical and microbiological characteristics of "Chouriço Vinha d'Alhos". Simultaneously the antilisterial activity was also investigated, with some samples being inoculated with *Listeria monocytogenes* (ca. 10^7 cfu/g) in a lab. ''Chouriço" produced with the addition of fresh and dried cultures (ca. 10^6 cfu/g) and "Chouriço" control (no starter culture added) were produced by an industrial meat company. All samples were stored at 4 ºC for 105 days and every 15 days the pH, water activity (a_w), humidity, acidity and peroxide index parameters were analysed in triplicate. The detection of L. monocytogenes, Salmonella spp., sulphite reducing clostridia, *Yersinia enterocolitica* and enumeration of *L. monocytogenes*, LAB, moulds, yeasts, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and Enterobacteriaceae was also performed according to ISO methodologies. Results showed that pH values did not change significantly during storage but, as expected, were significantly lower in inoculated samples, and the acidity index was higher in the last sampling points. Peroxide index values were higher in days 30, 45 and 60, in all samples, but decreasing until the end of storage. The lowest values were observed in samples inoculated with the dried culture. Overall, water activity and moisture content did not significantly change. Pathogenic and indicator organisms were not detected or were below acceptable levels for all the samples. In all samples, counts of LAB increased during storage and reached similar values after 90 days (ca. 10^{11} cfu/g). *L. monocytogenes* was reduced by ca. 2.5 log cycles in the inoculated sausages, after 90 days of storage. However, a similar reduction was observed in uninoculated control samples. Further studies are still needed to improve the performance of this antilisterial culture.

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Keywords: Lactic acid bacteria; starter cultures; pathogenic bacteria; smoked meat products,

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P1.92

Exploring the microbiota from cheese brines at Danish dairies by culture-dependent and -independent techniques

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The Danish Danbo cheese is a surface ripened semi-hard cheese, which is submerged in brine for up to 24 hours and subsequently left for ripening. The brining has an important effect on the structural and organoleptic properties of the cheeses and is significant in the regulation of the microbiota on the cheese surface. Even though the microbiota on cheese surfaces has been studied extensively limited knowledge is available on the occurrence of microorganisms in cheese brine. The aim of the present study was to investigate the brine microbiota in four Danish dairies producing Danbo cheese by both culture-dependent and -independent techniques.

The pH of the brines varied from 5.1 to 5.6, lactate from 4.1 to 10.8 g/L and free amino acids from 65 to 224 mg/L. Prokaryotes were isolated on five different media with 0.85-23.0% (w/v) NaCl contents, and eukaryotes on medium with 8% (w/v) NaCl. A total of 31 prokaryotic and eight eukaryotic species were isolated, which included several halotolerant and/or halophilic species. The prokaryotes were dominated by *Tetragenococcus* spp. and *Psychrobacter* spp. (≥6.0 log CFU/mL) followed by *Lactococcus* spp., *Staphylococcus* spp. and *Chromohalobacter* spp. (≥4.5 and < 6.0 log CFU/mL). Among eukaryotes *Debaryomyces hansenii* was dominating (≥3.5 log CFU/mL), followed by *Yamadazyma triangularis* (≥2.5 log CFU/mL) in one of the four dairies. By high-throughput amplicon sequencing of 16S rRNA gene and ITS2 regions for pro- and eukaryotes, respectively, brines from the same dairy clustered together indicating the uniqueness of the dairy brine microbiota. The results obtained by amplicon sequencing fitted, to a great extent, with the culture-dependent technique although unique genera/species were identified with each of the two techniques. Dairy brine handling procedures as e.g. microfiltration were found to influence the brine microbiota. It seems that besides contributing to the structural and organoleptic properties of the cheeses, the brine also serves as an important source for surface inoculation of the un-ripened cheeses with a range of halotolerant and/or halophilic microorganisms, quite likely originating from the sea salt used in the brine. The importance of these species during especially the initial stages of cheese ripening and their influence on cheese quality and safety need to be investigated. Likewise, optimised brine handling procedures and microbial cultures to ensure an optimal brine microbiota should be developed.

Keywords: cheese brine, microbiota, halophilic and halotolerant microorganisms, media, high-throughput sequenc

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P1.93

Presence of pathogenic vibrios in mediterranean mussels and grooved carpet shells collected in three coastal areas of Sardinia (Italy)

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The aim of the present study was to investigate the prevalence and enumeration of Vibrio spp core in naturally contaminated Mediterranean mussels and Grooved carpet shells from three harvesting areas of Sardinia (Italy). Seventy-one samples were collected before and after depuration process in three purification and dispatch centres associated with the main relevant coastal production areas within the Sardinian shellfish production. The preparation of samples for microbiological analysis was carried out according to the ISO 6887-1: 2004. Detection and enumeration of Vibrio spp in relation with salinity, temperature and pH of seawater in the harvesting areas, were performed according to previously published methods. Presumptive identification of Vibrio spp isolates was performed by means of conventional biochemical tests and Single-PCR. Identification and virulence profile of V. cholerae, V. parahemolyticus and V. vulnificus were performed by Multiplex-PCR. Our results confirmed the presence of seawater autochthonous vibrios in Sardinian aquatic habitats. Therefore, the prevalence of Vibrio spp was found to be 14% and 16% in Mediterranean mussels and Grooved carpet shells, respectively. Depuration was unsatisfactory with respect to Vibrio spp. The average contamination levels (mean±s.d.) for Vibrio spp were 1.51 ± 1.09 and 2.00 ± 1.34 Log10 CFU/g in Mediterranean mussels and Grooved carpet shells, respectively. Prevalence of V. parahaemolyticus and V. vulnificus were found to be 18% and 10% in Mediterranean mussels and 27% and 6% in Grooved carpet shells respectively. The contaminated samples revealed the presence of V. parahaemolyticus (n=114), V. cholerae (n=8) and V. vulnificus (n=2). The V. parahaemolyticus isolates carried the tdh or the trh genes. None of the isolates was tdh+/trh+.

Keywords: Mediterranean mussels; Grooved carpet shells; microbiology; Vibrio; Multiplex PCR

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P1.94

Preliminary results on bacteriological and viral investigation combined with phytoplankton and algal biotoxins from a coastal lagoon in the western Mediterranean (Sardinia, Italy)

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The Calich Lagoon is a Mediterranean lagoon of 97 ha located in an area of strong touristic impact. It is inserted in the Regional Natural Park of Porto Conte and plays a central role in contributing to biodiversity, protecting habitats, and managing tourist activities. The lagoon is connected to the sea through a natural channel and receive an extensive freshwater inflow from three fluvial tributaries. The contribution of marine and fresh waters, rich in nutrients, determines a high productivity. Despite its great potential and presence of natural bivalve molluscs populations, the waters of the lagoon have not been yet classified for shellfish farming. In this study, through a multidisciplinary approach, it was valuated the presence of several microbiologic (*Escherichia coli*, *Salmonella* spp, *Vibrio* spp) and viral (*Norovirus* GI e GII) pathogens, in addition to phytoplankton composition and associated algal biotoxins (Paralytic and Diarrethic Shellfish Poisoning). The aim was to provide useful data to improve the knowledge on their spread and to assess the potential risk for public health as well as to implement rational conservation and management strategies in support of future economic and productive activities. During one year, a monthly water sampling was carried out in four stations, representative of different hydrological features (including depth and salinity), for phytoplankton and virus analysis. *Mytilus galloprovincialis* samples were collected seasonally for microbiological, viral and biotoxin investigation. All parameters were analysed with official reference methods according to European legislation. Monitoring started in March 2017, when the lagoon was characterized by hypoxic conditions associated with a bloom of *Ulva* sp., which determined a fish mortality. *Salmonella* spp, *E. coli*, and *Vibrio parahaemolyticus* were detected simultaneity or not, except in summer. *Norovirus* G1 was found in January - February 2018. Algal biotoxins have never been detected. Among potentially harmful microalgae, only *Pseudo-nitzschia* species were present, reaching high values in August 2017. These results showed that potential human poisoning linked to bivalve consummation is a real and continuing problem. The present study will support both the implementation of a pilot action on testing of shellfish farming and the development of natural resource management strategies, strictly dependent on the water quality.

Keywords: Lagoon; mussels; water; phytoplankton; microbiology; virology; biotoxins

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P1.95

Detection and analysis of a prophage-encoded peptidoglycan hydrolase gene in *Lactobacillus helveticus*

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The rapid release of intracellular enzymes upon the cell lysis of starter lactic acid bacteria is an essential step in dairy fermentation, leading to the degradation of proteins, peptides, and lipids in the dairy matrix. Certain strains of *Lactobacillus helveticus* not only have a potent proteolytic system but are also prone to cell lysis. The aim of the present study was to gain insights into the mechanisms that determine the cell lytic properties of *L. helveticus* strains. We used the phenotypic data from a zymogram assay and a lysis assay in lactate buffer in a genome wide association study approach and identified a prophage-encoded putative endolysin gene that was associated with a highly lytic phenotype. The theoretical size of the putative endolysin gene was in accordance with a strong lytic band around 30 kDa on the zymogram gels. Strains possessing the putative endolysin gene showed an average of 95 (± 4) % of lyszed cells after incubation in lactate buffer for 24 h, whereas only 43 (± 11) % of the cells from strains that did not have the endolysin gene in their genome were lysed after 24 h of incubation. The putative endolysin gene was named *lysLH* and synthesized. After the cloning and gene expression of the synthesized gene, the cell wall-lytic activity was confirmed. Transcription analysis of a strain exhibiting the lytic phenotype revealed that *lysLH* is transcribed during the growth of *L. helveticus* FAM8105.

Keywords: Lactobacillus helveticus, cell lysis, endolysin, prophage

Exploring biodiversity in microbial ecosystems along the food chain

P1.96

Yeast diversity in two raw milk cheese technologies twenty years apart

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Due to their important role in the ripening of cheeses, we were interested unrevealing the diversity of yeast species in milks and cheeses and more especially its evolution in twenty years apart. The study takes in account two types of raw milk cheese technologies St-Nectaire and Laguiole. After a numeration of samples on OGA medium, a total of 339 yeast colonies were collected from the surface and the core of cheeses at both periods 1996 and 2016. First, the diversity of yeasts at species level was evaluated by identification combining biochemical characteristics (ID32C Api system, sugar fermentation) and sequencing of the ITS1-5.8S-ITS2 region. In a second way, the diversity at strain level was evaluated by LTR analysis for all strains of the predominant species *Debaryomyces hansenii*. Twenty six species were identified displaying the large diversity of yeast's communities in St-Nectaire and Laguiole cheeses and there was globally no loss in the diversity 20 years apart. The most frequently occuring yeasts common to the both technologies belonged to *D. hansenii, Candida zeylanoides, Torulaspora. delbrueckii, Yarrowia lipolytica* and *Kluyveromyces lactis and Kl. marxianus*. *Candida inconspicua* and *Candida glaebosa* characterized more Laguiole cheeses. However, these predominant species showed some differences in the gap of twenty years. In particular, some species as *Kl. marxianus*, *T. delbrueckii* were not detected in some cheeses from 2016. Differences between the two technologies and sampling periods were more connected in the subdominant populations which came either from milk or from environment.
The isolates of *D. hansenii* were clustered into 20 distinct genomic biotypes suggesting that various strains of *D. hansenii* are involved in the ripening process. We highlighted specific strains of the curd, either specific to the rind or developing as well in both. We found also strains developing rather at the beginning of the ripening of cheese and strains present throughout all the ripening. Finally, we highlighted for each technology some strains common to both manufacturings or isolated in only one of them.
In conclusion, the community of yeasts in cheese is dependant of practices and environment of dairy plants which highlights the link of PDO cheese with the geographic area. Moreover, this large genetical diversity implies a large functionnal diversity which may be exploited for novel interesting strains in industry and starter production.

Keywords: Yeasts, raw milk cheeses, diversity, evolution

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P1.99

Characterisation of halotolerant and halophilic yeasts from cheese brines from Danish dairies producing semi-hard surface-ripened cheese

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Yeasts play an important role in the surface microbiota of cheese, with the most well recognised yeast species being *Debaryomyces hansenii*. The yeasts serve to de-acidify the cheese surface, thereby enhancing the growth of the smear bacteria. Further, yeasts contribute to the cheese ripening by forming aroma compounds by proteolytic and lipolytic activities. Yeasts can be applied as surface-ripening cultures or by old-young smearing, but also the brine could serve as a key source for surface inoculation of the un-ripened cheeses with a range of halotolerant and/or halophilic yeasts. The aim of this study was therefore to isolate, identify and characterise the technological properties of halotolerant and halophilic yeasts from brines used in Danbo cheese production. The growth of the yeasts was investigated at various NaCl concentrations, pH values and temperatures. Yeasts in brines from two dairies were enumerated on MYGP agar added 0.85%, 4%, 8% and 16% NaCl (w/v). Large variations in the yeast counts were observed from different brine samples, even within the same dairies. The highest total yeast counts were obtained at MYGP added 4% and 8% (w/v) NaCl ($9.60*10^3$ to $8.60*10^4$ CFU/ml brine). So far the species were identified as *D. hansenii*, *Kluyveromyces lactis*, *Yamadazyma triangularis*, *Candida intermedia* and *Sterigmatomyces halophilus* by use of 26S rRNA gene sequencing. NaCl tolerance were screened in a spot test on MYGP agar added 0.85-23% NaCl (w/v). Yeast isolates representing the identified species were selected for further investigations of the growth at different combinations of NaCl concentrations, pH and temperatures. All yeast isolates were able to grow at NaCl concentrations from 0.85-10% (w/v) (pH 5-6, 16-25°C), and the majority at 15% (w/v). Further, isolates of *D. hansenii*, *Y. triangularis*, and *S. halophilus* could grow under conditions similar to those in a cheese brine (20% NaCl (w/v), pH 5-6, 16°C) and showed a favourable growth at 8% (w/v) NaCl, corresponding to the cheese surface NaCl concentration. The results indicate that cheese brines harbour a wide range of halotolerant and halophilic yeasts highly capable of growing under conditions similar to cheese brines and cheese surfaces, and hence could be of interest for the dairy industry. The importance of these yeasts, especially their ability to establish on the cheese surface during initial stages of cheese ripening and their potential to inhibit mould, needs to be studied.

Keywords: cheese brine, halophilic and halotolerant yeasts, media, technological properties

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P1.100

Metataxonomics as a tool for investigating foodborne pathogens dynamics in raw milk cheeses

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Microorganisms colonizing food matrices constitute the so-called microbiota, an ecological community of commensals, symbiotic and pathogens sharing an ecological niche and influencing food characteristics.
The study of the ecological dynamics between microbial members is one of the central challenges in food safety. The aim is to predict their impact on the growth and/or survival of pathogenic bacteria that may contaminate food matrices. This topic is particularly relevant when food is prepared starting from ingredients with a high biological risk, as in the case of raw milk.
Predictive microbiology studies have been historically based on predicting the growth dynamics of pathogenic or commensal microorganisms, when variable chemical-physical parameters affecting microbial growth were applied. The limitation of these *in vitro* studies is that the growth dynamic of the targeted microorganism does not take into account the effect of the remaining naturally residing microbial communities in food matrices.
Great advance has been made in this field by the advent of high-throughput next generation sequencing (NGS). These methods allow a detailed study of microbial communities living in food matrices in terms of composition and ecological dynamics. This approach is promising for the implementation of innovative strategies to control the growth of pathogenic microorganisms in food.
In this study, we examined the evolution of the microbial community of the raw milk based Latteria cheese in four different scenarios: cheese artificially contaminated with enterotoxic *Staphylococcus aureus* and *Listeria innocua,* both separately and jointly, and plain cheese (no contamination), each produced in triple biological replicate for robustness. Cheeses were sampled during production (milk and curd) and along all the ripening period (30 days, 10 time points) and residing microbial community was investigated through the analysis of 16S ribosomal RNA (rDNA 16S). A total of 129 samples were pre-processed using QIIME2 in order to obtain a comprehensive feature table. The resulting dataset was used to investigate alpha and beta diversity, to study internal community compositions and to perform differential abundance testing between control cheeses and the three different contaminations over time. Lastly, the ecological interactions within microbial community over time were reconstructed, inferring underlying microbial interactions from abundance tables.

Keywords: Microbiota, raw milk, 16S rDNA, metataxonomics, diversity, interactions

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P1.101

Influence of geographical area of production on the Peruvian Chicha microbial communities composition

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Chicha de Jora is a traditional fermented beverage produced in the Andean region, widely consumed in Peru. The Peruvian Chicha is produced at artisanal level using mainly germinated maize but it can include also other vegetables. In this study, the bacterial community associated with maize-based Chicha made in different Peru regions (Lima, Barranca, Ancash, Huaura, Huacho, Cajatambo, Churin and Huaral) has been investigated. Moreover, to assess the safety for consumers of Chicha the presence of *Salmonella, Listeria monocytogenes* and *Bacillus cereus* was investigated by PCR. Microbial diversity was studied by sequencing the variable V3 and V4 regions of the 16S rRNA gene using the illumina Miseq. The predominant bacterial genera found in Chicha samples included *Lactobacillus*, *Acetobacter* and *Weissella*. Beta diversity analysis based on Bray Curtis similarity index was performed to investigate the differences in the composition of the microbiota among samples from different geographical areas. PCoA based on Bray Curtis distance indicated the existence of a large dissimilarity in microbial composition among samples. In many cases this dissimilarity was observed also in samples taken in the same city. However, the samples coming from Lima and Barranca tended to cluster according to their geographical origin. The Analysis of Molecular Variances (AMOVA) showed that microbial communities exhibited significant differences in their composition when comparing Lima vs Barranca and Lima vs Huaura. All samples were negative to *Listeria* presence while *Salmonella* and *Bacillus cereus* contamination was detected in samples mainly coming from Lima, Huaura and Huacho. The differences observed among the samples are probably the consequence of both the variety of artisanal procedure utilized for Chicha production and the specific composition of the locally adapted microbiota.

Keywords: Chicha, maize, fermented beverage, sequencing

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P1.102

Stress induction of a putative probiotic strain from Korean fermented kimchi, and its anti-obesity effect on diet induced obesity murine model

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Recently numerous reports showed promising anti-obesity efficacy of probiotics when treated in diet induced obese murine model and clinical trials. However, the impact of probiotics on metabolic diseases is still poorly understood. Gut microbiota modulation could be one of the major functional feature of probiotics regarding anti-obesity effect. There are various gene regulation involved for probiotics to adapt to environmental conditions that affect their survivability and colonization in the gastrointestinal tract. This work is focused on probiotic optimization by exposure to different stress conditions prior to administration, and on the possible mechanism involved.

Lactobacillus sakei was grown under different stress conditions such as low pH and physiological concentrations of bile acid. *Lb. rhamnosus* GG (LGG) was used as reference strain. Normal (viable), stress induced strains and heat-killed strain as well as LGG, were administered daily through oral gavage to C57BL6/J mice on a high fat diet (60% fat) *ad libitum*. Mice were housed at 23°C, 55±10 % humidity, in 12h light/dark cycles. The anti-obesity effect of *L. sakei* was monitored every week by measuring body weight gain.

After the *in vivo* experiment, mice were sacrificed and liver, spleen, intestine, adipose tissue and faecal samples were analysed, and microbiota and physiological differences were compared between the groups. Serum was collected and used to analyse immune factors.

Stress induced *L. sakei* showed a different impact on the microbiota and mouse health compared to the negative control and the LGG group. These results suggest that anti-obesity effects occur in a strain specific manner, while the effect of the strains can be improved by exposing them to specific stress conditions prior to administration. In conclusion, stress may enhance strain survivability and performance within the host gastrointestinal tract. However, improving probiotic functions constitutes a special challenge and would justify further research.

Keywords: Lactobacillus, stress, anti-obesity´s

Exploring biodiversity in microbial ecosystems along the food chain

P1.103

Survival strategies of *Listeria monocytogenes* in food processing environments and antimicrobial resistance

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Listeria monocytogenes is able to survive and growth on uncomfortable conditions such as food processing environments -FPE- and infection occurrences. The pathogen is able to create biofilms that are hard to eradicate, thus increasing the risk of listeriosis transmission by food products. Fortunately, treatments with antibiotics are efficient but *L. monocytogenes* can activate molecular mechanisms against stressful conditions.

The aim of this study was to evaluate the biofilm formation and antimicrobial susceptibility of different *L. monocytogenes* genotypes.

Biofilm formation was studied on PVC surfaces and stainless steel coupons. Inoculum concentration was adjusted to 10^8 cfu/ml and was incubated at 37 °C for 48 h. Cells adhered were stained with crystal violet and after washing and re-suspending with ethanol: acetone solution, the biofilm formation was measured at OD_{580}. Moreover antimicrobial susceptibility was studied by E-Test. A total of 21 antibiotics were tested: β-lactamics, glycopeptides, or aminoglucosides families, and also daptomycin, rifampin, tetracyclin, erythromycin, clindamycin and fosfomycin among others.

L. monocytogenes genotypes isolated from surfaces in contact and non-contact with food products were better biofilm formers on PVC surfaces in comparison to stainless steel coupons. Only ST2 and ST388 were intermediate producers of biofilm on PVC. ST1, ST2, ST8 and ST9 were considered non biofilm producers on coupons whereas ST5 tended to develop biofilms. All the strains of *L. monocytogenes* were susceptible to the majority of antibiotics. However, genotypes ST5, ST388 and one isolate of ST9 were resistant to nitrofurans, ST8, ST121 and ST321 to mupirocin and the other isolate of ST9 and ST199 to tetracycline. ST2 and ST388 were the only genotypes resistant to erythromycin and daptomycin respectively. Fosfomycin was the less efficient antibiotic however, ST2 and one isolate of the ST9 showed sensitivity and ST199 an intermediate resistance. ST2, ST321 and ST388 presented resistance to three antibiotics.

In conclusion, the results highlighted the importance of applying good cleaning and disinfection protocols taking in consideration materials and inaccessible sites in FPE surfaces to avoid biofilm formation and also the possible acquisition of disinfectants and antibiotics resistance due to activation of molecular mechanisms against stressful conditions and gen transmission between microorganisms in the biofilm.

Keywords: Stress response, biofilm, resistance, cleaning and disinfection, antibiotics

Exploring biodiversity in microbial ecosystems along the food chain

P1.104

Identification of the sourdough isolate A2 as *Lactobacillus crustorum* using the molecular markers *dnaK* and *rpoA* in addition to 16S rRNA

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Lactic Acid Bacteria (LAB) are known as a functional microorganism group that contain Gram-positive, catalase-negative bacteria that produce lactic acid as the major metabolic end product in carbohydrate fermentation. Among the LAB, *Lactobacillus* is a genus containing a high number of GRAS (Generally Recognized as Safe) species harboring many strains important in food microbiology and human nutrition because of their contribution to fermented food production or due to their probiotic properties. The genomic diversity among lactobacilli is high and their phylogenetic classification is problematic. Generally, 16S rRNA analysis alone is not sufficient for species resolution since this region is highly similar among lactobacilli. Therefore, other phylogenetic markers are usually used additionally. In our previous studies, the bacterial diversity during the fermentation period of the Thracian tarhana, a traditional Turkish fermented food, was determined by culture-based and culture-independent techniques. A *Lactobacillus* sp. was found to be one of the bacterial species effective during the fermentation period. Culture-based methods allowed the species (the isolate A2) to be isolated and purified. 16S sequencing resulted in the identification of the isolate as *Lactobacillus*, but could not allow characterization at the species level. The most similar *Lactobacillus* species were found to be *L. farciminis, L. mindensis* and *L. crustorum.* In this study, it was aimed to further characterize *Lactobacillus* sp. A2 by molecular methods using other informative loci. To allow further characterization, additional genomic regions, the molecular chaperone *dna*K and RNA polymerase alpha subunit *rpo*A were selected for characterization. PCR was performed to amplify *dna*K and *rpo*A. Sequencing of the resulting PCR fragments allowed the isolate to be identified as *L. crustorum*.

Keywords: Lactobacillus, 16S sequencing, Lactobacillus phylogenetic markers, fermented foods, tarhana, sourdou

Exploring biodiversity in microbial ecosystems along the food chain

P1.105

Determination of the microbial profile of the traditional Turkish fermented beverage shalgam during the fermentation period

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Shalgam is a red, sour and turbid fermented beverage produced using black carrot, bulghur flour, turnip (shalgam), water, baker's yeast or sour dough, salt and water. The origin of the remarkable purple-red color of shalgam is the black carrots, which is a carrot variety, Daucus carota ssp. sativus var. atrorubens, known for its rich anthocyanin content. Although the main raw material is black carrots (10-20%), turnip (Brassica rapa) is also added to shalgam at a concentration of about 2% and it positively affects the sensory quality. In shalgam production, generally, sour dough, is used as the main microorganism source for fermentation. The microbial growth is controlled by salt addition at a concentration of 1-2%. Shalgam production does not follow a standart production technique, but is generally performed by two methods; the traditional method involves two stage fermentation (firstly the sourdough fermentation and secondly the carrot fermentation) while the direct method harbors a single stage fermentation. In this study, we aimed to determine the complex microbial profile of shalgam fermentation by both culture dependent and independent methods. To do this, first we will perform the traditional two-stage production method with sourdough fermentation. We will take samples at five points during the fermentation (time zero, after the first stage dough fermentation, and at three points during the carrot fermentation). Basic microbiological analyses will be performed on the samples, which involve total mesophilic aerobic bacteria, coliforms, lactic acid bacteria (LAB) and mold-yeast counts. Lactic acid bacteria and yeasts obtained will be isolated and purified. After DNA extraction, the isolates will be grouped by Rep-PCR and the representative isolates will be identified by 16S and 26S sequencing for bacteria and yeasts, respectively. Culture-based methods will be combined with culture independent HTS (High-throughput sequencing / amplicon sequencing) technique. For this analysis, DNA extraction will be performed directly on the shalgam samples and 16S and 26S sequences will be amplified and high-throughput sequencing will be conducted. The results will elucidate the change of the microbial profile during the fermentation period and the culture-based methods will allow isolation of the microorganisms effective for shalgam production.

Keywords: Shalgam, black carrots, fermented beverage, microbial profile

Exploring biodiversity in microbial ecosystems along the food chain

P1.106

Determination of the microbial diversity of pastirma, a Turkish traditional meat product

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Pastirma is a popular dry-cured meat product of Turkey, produced from whole beef or muscles of water buffalo. Manufacturing involves coating with cemen, a paste made of ground fenugreek, garlic, red pepper and paprika. Pastirma is mostly produced using traditional methods by artisanal manufacturers especially in the Kayseri region of Turkey. During manufacturing, first, the muscles are cured with curing reagents, such as salts, nitrate and nitrite and are then subjected to three drying periods. After the first drying period, the muscles are pressed, and after the second drying period, they are coated with cemen. Pastirma production lasts about 3-4 weeks depending on the size of the muscles used. In this study, we aimed to examine the microbial diversity of pastirma during the production period. To do this, samples will be taken at five different time points during production (time zero, after curing before the first drying, after the first drying, after the second drying and after the third drying) from different manufacturers. During the manufacturing process, pH and water activity will be followed, and microbiological counts of lactic acid bacteria, *Micrococcus/Staphylococcus*, *Enterobacteriaceae* and yeasts-molds will be conducted using MRS (De Man, Rogosa and Sharpe) agar, MSA (mannitol salt agar), VRBD (violet red bile dextrose) agar, and PDA (potato dextrose agar). For culture-based methods, colonies from each agar plate will be purified and stored at -80°C with glycerol. DNA extraction will be performed and both bacterial and yeast isolates will be grouped by Rep-PCR. Representative isolates will be identified using 16S sequencing for bacteria and 26S sequencing for yeasts. For culture-independent methods, DNA will be directly extracted from the meat samples and high-throughput sequencing of 16S and 26S amplicons, for bacteria and yeasts, respectively, will be performed. Both culture-based and culture-independent methods will allow in-depth examination of the microbial diversity of pastirma.

Keywords: Pastirma, dry-cured meat product, microbial diversity, lactic acid bacteria, Micrococcus/Staphylococ

Exploring biodiversity in microbial ecosystems along the food chain

P1.107

Utilization of yeasts starter cultures improved semi dry coffee fermentation

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Coffee fermentation is necessary to remove the mucilage from seeds and reduce water content. *The objective of this study was to evaluate the behavior of Saccharomyces cerevisiae (CCMA 0543), Candida parapsilosis (CCMA 0544), and Torulaspora delbrueckii (CCMA 0684) as starter cultures for semi-dry processed coffee using two inoculation methods: (1) direct inoculation and (2) bucket inoculation. The microbial population was evaluated by real-time polymerase chain reaction (qPCR) and metabolic changes of both inoculation methods during fermentation were evaluated using gas chromatography-mass spectrometry (GC-MS). Acids, pyrazines and pyridines were the main volatile compounds in both green and roasted coffee beans.* The bucket inoculation method favored permanence of the microorganisms (bacteria and yeast) during the coffee process, especially in the treatment inoculated with S. cerevisiae. The starter yeasts-S. cerevisiae (CCMA 0543), C. parapsilosis (CCMA 0544), and T. delbrueckii (CCMA 0684)-worked well with the Catuaí Amarelo coffee variety because they changed the behavior of the microbiota and the chemical composition during the process.

Keywords: coffee fermentation, yeasts, starter cultures, processing, quality

Exploring biodiversity in microbial ecosystems along the food chain

P1.108

Characterization of *Lactobacillus* strains isolated from Italian artisanal sausages: Safety and techno-functional aspects

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Introduction: New *Lactobacillus* isolates from artisanal sausages, with favorable techno-functional characteristic and with demonstrated safety requirements for human use, represents great candidates for innovative sausage production.

Objectives: Isolation, identification and characterization of functional properties of *Lactobacillus* strains.

Material and methods: 1.Strain isolation. *Lactobacillus* strains were isolated from three different sausages artisanally produced through RAPD-fingerprinting analysis and 16S rDNA sequencing. 2.Antimicrobial resistance and biogenic amines production. The Minimal inhibitory concentration (MIC) of different antibiotics was assayed on the selected strains by microdilution in VetMIC plates for LAB (SVA, Uppsala, Sweden). The evaluation of biogenic amines production was performed using the Bover-Cid plate method and HPLC-UV. 3.Techno-functional properties: aroma profile analysis and antimicrobial activity. Volatile organic compounds produced by strains were detected with SPME/GC-MS. Spot test were performed against a foodborne pathogens: *Clostridium sporogenes, Listeria monocytogenes, Salmonella* spp. and *Escherichia coli.*

Results: 210 *Lactobacillus* strains were isolated and after the clustering analysis, 40 different strains were obtained, belonging to two different species: *L.sakei* and *L.curvatus.* 22 strains showed no antibiotic resistance. Vancomycin resistance is the most distributed resistance among isolates. Only one strain was identified as putrescine producer. A great variability was observed among the volatile compound profile of strains, regarding molecules with a relevant impact on the aroma (i.e. 2,3- butanedione, 3-hydroxy-2-butanone, 2-methyl-butanal, 3- methly-butanal, acetic acid, benzaldehyde, benzenacetaldehyde, etc.). The obtained results from the spot test showed an inhibition against gram positive and negative pathogenic bacteria. This activity is going to be further analysed in a meat model.

Conclusion: In the present study 22 *Lactobacillus* strains showed no antibiotic resistance being a suitable candidates as a starter cultures. The data indicated a large variability within the *L. sakei* species, especially regarding the aminoacid metabolism and aroma production. On the whole, some of the isolates can be considered interesting strains to be used as starter cultures for sausage production at industrial level.

Acknowledgements: LONGLIFE, JPI HDHL

Keywords: starter culture, antibiotic resistance, antimicrobial activity, volatile compounds

Exploring biodiversity in microbial ecosystems along the food chain

P1.109

Investigation of growth of microorganisms and product formation during fermentation of *Theobroma cacao* L. seeds with the aim to develop a starter culture with fl avour profi les on demand

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The production of high quality chocolate is an important economic factor worldwide. In Germany about 1 million tons of chocolate are produced annually, which corresponds to 40% of the total European production. Raw cacao of high quality depends, among other things, on post-harvest treatment: fermentation and drying of cacao seeds. Fermentation in particular is essential for the formation of the cocoa aroma as important aroma precursors are produced. Misfermentation can lead to false flavours. The world-wide standard is still a spontaneous fermentation process. It is difficult for chocolate manufacturers to procure high, consistent quality raw cacao.

By using a microbial starter culture, the fermentation process could be better controlled and the formation of certain aroma profiles could be promoted. The development of the taste profile of cocoa is influenced by various factors, such as metabolites of microorganisms formed during fermentation. These can represent flavour precursors with different characters (e.g. fruity, flowery or nutty nuances), which are significantly involved in the formation of the later chocolate flavour during the further processing of the cacao seeds.

Within the framework of the CORNET project “CocoaChain” samples are taken at different locations of spontaneous cacao fermentations in Peru to determine the biodiversity of microorganisms. The isolation and subsequent characterization of yeasts, lactic acid bacteria and acetic acid bacteria using FT-IR should help to determine the influence of the different isolates on aroma formation during cacao fermentation. For this purpose, the aroma profile of different yeast strains is characterized in submerged fermentation with cacao pulp simulation medium on laboratory scale. The aim is the development of a starter culture to influence the production of aroma precursors in cacao fermentations in Peru.

Acknowledgement: We acknowledge the financial support of AiF/FEI within the project Cornet AiF 169 EN/1: “Quality improved cocoa and cocoa-based products with flavour profiles on demand - ‘From farm to chocolate bar’ (CocoaChain)”.
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Keywords: fermentation

Exploring biodiversity in microbial ecosystems along the food chain

P1.110

Lactic acid bacteria fermented milling by-products as basis for breakfast cereals

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By-products from the milling industries derived from the outer layer of grain are commonly used as animal feed, thus resulting in food loss. In this study, the production of breakfast cereals from barley white bran and low-grade wheat flour through applying lactic acid bacteria (LAB) fermentation followed by down-stream processing by extrusion was evaluated. In a multiphasic screening approach, 314 LAB previously isolated from a variety of food fermentations were screened for amylase and peptidase activity, which is essential for degrading the main compounds of milling by-products, and for phytase activity, which was aimed at degrading the antinutritional phytic acid mainly present in the outer layer of grain. 32 LAB strains demonstrated amylase activity on starch agar, 5 peptidase activity on a skim milk agar, and 14 phytase activity on Chalmers-Agar. A selection of 12 active strains where then applied in fermentations (24 h, 30°C, 2:1) of CO_2 treated by-products. A sensorial analysis identified that fermented CO_2 treated by-products were more pleasant in smell than untreated samples. After 24-h fermentation, LAB and yeasts reached 10^9-10^{10} and 10^2-10^4 CFU/ml, respectively, independent of LAB addition, and pH dropped to 3.5-3.8. Fermented by-products with added LAB were rated as fruity, flowery, acidulous, and with a scent of hay in contrast to fermented by-products without LAB addition that were perceived as musty with an off-flavor. Finally, 70 kg barley white bran (CO_2 treated) was successfully fermented at pilot scale using strain JR156 followed by down-stream processing into breakfast cereals with a pleasant taste, thus confirming a high potential for the reduction of food loss in the milling industry.

Keywords: Lactic acid bacteria

Ecology and interactions in food-associated microbial communities

P2.1

Mechanisms by which *Escherichia coli* persist on meat fabrication equipment

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Some Escherichia coli can persist on meat fabrication equipment, thus becoming a recurring source of contamination for meat. This study investigated the potential mechanism by which E. coli persist, by comparing the susceptibility to disinfectants and biofilm forming ability of two populations, recurring and transient, each consisting of 50 E. coli isolated from meat fabrication equipment. Susceptibility to three commercial disinfecting agents, biocide A (a quaternary ammonium compound based sanitizer), B (a sodium hypochlorite based sanitizer), and C (a chlorinated alkaline cleaner) was assessed, by determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). Biofilm developed on polystyrene surface for up to 6 days at 15°C was quantified by crystal violet staining. We found the recurring population did not have higher ($P > 0.05$) MICs or MBCs of any of the three biocides than the transient population, and the maximum MBCs were well within the respective in-use concentrations of the disinfectants. The development of biofilms was time-dependent for both transient and recurring E. coli populations and was correlated with motility and curli expression. By days 2, 4, and 6, 50, 86, 88% of the recurring E. coli, and 58, 82 and 84% of the transient E. coli developed strong biofilms. The transient group had a higher ($P < 0.05$) fraction of isolates that did not form measureable biofilms at all three sampling points. All E. coli in biofilms were able to survive and recover after the treatment of QAC at 200 ppm, irrespective of their groups. The findings of this study suggest that biofilm forming ability may play a role in the persistence of E. coli on equipment surface and the persistence would be unlikely associated with biocide tolerance.

Keywords: Escherichia coli, persistence, biofilm, disinfectant

Ecology and interactions in food-associated microbial communities

P2.2

Influence of dps and ompR genes on tolerance response of *Salmonella* Enteritidis 86 to heterologous stresses after exposure to oregano essential oil in chicken broth

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The survival of *Salmonella Enteritidis* in the food chain is related to its ability to respond to environmental stress. The major concern about this response is the development of tolerance to homologous or heterologous stressing agents, which may affect the efficacy of multiple-hurdles preservation strategies. This study assessed the influence of *ompR* and *dps* genes on tolerance response of *Salmonella Enteritidis* to heterologous stressing agents after exposure to *Origanum vulgare* L. essential oil (OVEO), an emerging food preservative agent. The *strain S. Enteritidis (SE86), involved* in 95% of Brazilian Southwest food-linked outbreaks notified in last decades, and its *isogenic mutants* Δ*dps* and Δ*ompR* (10^6 log CFU/mL) were exposed (18 h, 37 ºC) to OVEO sub-lethal amounts (0.06 µL/mL; based on minimum inhibitory concentration previously determined) in chicken broth. OVEO-adapted cells were inoculated in fresh chicken broth (10^6 log CFU/mL) with sodium chloride (NaCl 5 g/100 mL) or sodium hypochlorite (NaClO 200 ppm). Viable cells were enumerated (log CFU/mL) in 10 minutes-intervals during 240 minutes at 37 °C. To assess the thermo-tolerance cells OVEO-adapted were enumerated in fresh chicken broth incubated at 52 °C. Cells of SE86, Δ*dps* and Δ*ompR* strains non-adapted to OVEO were assessed similarly as controls. The time needed to achieve a 3-log reduction (*t*-3D) was determined using a biphasic model which described the tolerance of each strain to the tested heterologous stressing agents. SE86 OVEO-adapted cells showed higher NaClO- and thermo-tolerance than Δ*dps* and Δ*ompR* OVEO-adapted cells, but lower thermo-tolerance than SE86 non-adapted cells. No difference was observed between the NaClO-tolerance of Δ*dps* OVEO-adapted and Δ*dps* non-adapted cells, while SE86 OVEO-adapted cells showed higher NaClO-tolerance than SE86 non-adapted cells. Δ*ompR* OVEO-adapted cells showed lower NaClO- and thermo-tolerance than Δ*ompR* non-adapted cells. Similar thermo-tolerance was observed in Δ*dps* OVEO-adapted and Δ*dps* non-adapted cells. OVEO-adapted and non-adapted cells of SE86, Δ*dps* and Δ*ompR* showed similar NaCl-tolerance. These results show the influence of *dps* gene on increase of NaClO-tolerance in *S. Enteritidis* after exposure to OVEO.

Keywords: Salmonella enterica, stress response, bacterial adaptation, oregano essential oil

Ecology and interactions in food-associated microbial communities

P2.3

Role of YafQ-DInJ toxin-antitoxin system and indole synthesis by TnaA in heat tolerance of *Escherichia coli*

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Heat-treatment is the most common and useful process to sterilize foods, but over heat treatment can also deteriorate flavor, texture, and nutrients in foods. Mild-temperature treatments can avoid such deterioration but also cause sublethal effect and give a rise of injured but recoverable bacterial cells. Bacterial heat tolerance, including injured cells, is therefore inevitable issue for food safety. Toxin-antitoxin (TA) system is well known as a key factor of bacterial stress tolerance and persister formation, which consists of toxic peptide toxin and cognate immune peptide or non-coding RNA antitoxin. To date, there has been no detailed study on the relations among persister, TA system and heat tolerance. Therefore, we aimed to investigate the role of persister-related TA systems in bacterial heat tolerance.

Persister-related TA gene knockout mutants from *E. coli* single gene knockout collection (Keio collection) were applied to heat tolerance assay using thermal cycler. Some TA systems were suggested to be involved in heat tolerance. In this study, YafQ-DinJ systems were selected for further investigation.

Both *ΔyafQ* and *ΔdinJ* showed higher heat tolerance than wild type, although heat injuries and recoveries were not significantly different from wild type. YafQ functions as endoribonuclease degrading transcripts of such as elongation factors, outer membrane proteins and tryptophanase (*tnaA*). TnaA synthesizes indole from tryptophan, and indole is reported to inhibit persister formation. Interestingly, *ΔtnaA* showed higher heat tolerance than wild type, which is consistent with increase of persister formation by lack of indole synthesis. Also, addition of extracellular indole strongly reduced heat tolerance of all tested strains including *ΔtnaA*. As we expected, additional tryptophan for preculture before heat treatment, significantly reduced heat tolerance of only indole producers such as wild type and *ΔyafQ*, but not *ΔtnaA*.

These results conclude that indole synthesis controlled by YafQ-DinJ is involved in heat tolerance and indole addition can strongly reduce bacterial heat tolerance. However, indole could not be the only one factor of heat tolerance regulated by YafQ-DinJ system since even *ΔyafQ*, that could produce more indole than wild type, indicated higher heat tolerance. Further investigations of YafQ-DinJ system can provide us important and useful information to understand bacterial heat tolerance and to maintain our food safety.

Keywords: toxin-antitoxn, heat tolerance, persister, indole,

Ecology and interactions in food-associated microbial communities

P2.4

Community-level physiological profi ling in microbial communities of broiler cecae

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Poultry production constitutes one of important agricultural output worldwide. It is known that the gut health of broilers is essential for their growth and for providing wholesome products for human consumption. Previously, the microbial diversity of broiler cecae was studied at the microbial genetic level. However, the functional diversity and metabolic activity of broiler cecal microbial communities are not fully investigated. Recently, the EcoPlates™ from Biolog, Inc. have been used for characterizing microbial communities in various environments. In this communication, we applied these plates to physiologically profile cecal microbial communities in broilers. The cecae were aseptically excised from six-week-old birds, and their contents were resuspended in phosphate buffered saline. The EcoPlates were used according to the manufacturer´s instructions. The cultures were incubated at 42 °C for five days in an OmniLog system from Biolog, Inc. Responses of the microbial communities to uses of the various carbon sources were measured on formazin production. The data of average well color development (AWCD) were calculated. The AWCD data for positive substrates showed the sigmoidal curve with three phases: lag, exponential and stationary. The results showed responses of cecal microbial communities to carbon sources differed among broilers. The positive substrate utilizations included β-methyl-*D*-glucoside, *D*-mannitol, *L*-serine, *L*-threonine, glycogen, *D*-cellobiose, glucose-1-phosphate, and α-*D*-lactose. These results provide insight of the potential heterotrophic microbial community in broiler cecae, and a rationale for further evaluation of this technique to assess microbial community characteristics leading to improving management in poultry production.

Keywords: Community-Level Physiological Profiling; Cecae; Microbial communities; Broiler

Ecology and interactions in food-associated microbial communities

P2.5

Antimicrobial resistance in mixed juice treated or not by high hydrostatic Pressure

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A mixed juice was prepared from carrots, red pepper and apples. Pressure treatments (5 min) were applied at 450 and 600 MPa, both at 22 ºC and 50ºC. Controls and treated samples were refrigerated stored for 10 days. Aliquots were plated on non-selective media supplemented or not with benzalkonium chloride (200 mg/l) or cefotaxime (64 mg/l) and McConkey agar supplemented with cefotaxime (64 mg/l) or McConkey broth containing 16 mg/l imipenem. The selected isolates were identified by 16S rDNA sequencing and inspected for antibiotic resistance genes by PCR. Total viable counts in controls increased from 4.84 to 5.83 log CFU/ml during storage. A residual surviving fraction between 1 and 2 log CFU/ml was detected in treated samples on non-selective media, but growth on media supplemented with antimicrobials was only detected in control samples and only in one treated sample. No growth was obtained on imipenem. Of the 110 isolates obtained, 52% belonged to *Pseudomonas*, 25% to *Ewingella* and the rest to *Stenotrophomonas*, *Enterobacteriaceae*, *Bacillus*, *Paenibacillus*, *Rahnella*, *Proteobacterium*, or *Roseomonas*. The following beta-lactamase genes were detected: $bla_{\text{CTX-M}}$ (20 isolates), $bla_{\text{CTX-M2}}$ (5), bla_{TEM} (12), bla_{VIM} (33), bla_{IMP} (11), bla_{NDM} (21), bla_{OXA} (8), $bla_{\text{OXA-48-like}}$ (3) and bla_{KPC} (3). The tetracycline resistance genes *tet*(A), *tet*(E), and *tet* (G) were detected in 10 isolates, while *tet*(B), *tet*(C) and *tet* (D) were detected in 8 isolates. Eleven isolates (including 6 *Pseudomonas*, 4 *Ewingella* and one *Paenibacillus*) tested positive for 4 antibiotic resistance genes, and three (*Ewingella*) tested positive for 5 genes). Multiply resistant *Ewingella* (*E. americana*) persisted in the untreated juice till the end of storage. The only antibiotic-resistant bacterium detected in the pressurized samples was *Paenibacillus*. Results indicate that mixed juices may carry antibiotic-resistant bacteria and also that high-pressure treatments can reduce the risk of antimicrobial resistance in juices.

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Keywords: antimicrobial resistance; high-pressure processing; vegetables

Ecology and interactions in food-associated microbial communities

P2.6

Toxin-antitoxin systems of *Lactobacillus casei* group: Identification and transcriptional activation in response to food-related stress

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Toxin-antitoxin systems (TAS) are two component systems that are widely distributed among all bacterial classes and are involved in the regulation of bacterial growth, death, and in global stress response. TAS mechanism involves a stable toxin, able to kill the cells or to confer growth stasis, and an unstable antitoxin, that can be a protein or a non-coding RNA which inhibits toxin activity. To date, 5 types of TA have been identified. Few studies have addressed the presence and distribution of TAS in food-associated microorganisms, and for this purpose we have sequenced the genome of two strains of *Lactobacillus rhamnosus*, named respectively 1019 and 1473, isolated from long-ripened cheese. In these isolates we have identified the presence of various genes coding for TAS, and focused on Type II, in which both toxin and antitoxin are small interacting proteins and are involved in the persistence phenomena in the presence of antibiotic or specific stress conditions. To understand if there is a link between the activation of these TAS and the environmental changes occurring during food processing, three strains belonging to *L. rhamnosus* and one strain of the closely related species *L. paracasei* were exposed to food-related stresses. Briefly, bacterial cultures were grown in rich media, and transferred to modified culture media exerting the following stresses: osmotic stress (1.5% w/v NaCl), oxidative stress (1mM H2O2), acid stress (pH 4.0), temperature stress (55°C) and nutrient limitation (cheese-mimicking broth). Bacterial viability and transcriptional activation of TAS were measured, showing that activation of TAS in response to the selected conditions is strain-specific, and that among the conditions tested nutrient limitation leads to an upregulation of TAS. Interestingly, thermal stress at 55 °C leads to a temporary drop off of the growth, followed by a recover in bacterial viability both at the optimal growth temperature (37°C) and at 55°C. The study suggests that the activation of TAS could be tightly regulated in response to food processing stress and might be relevant in studying bacterial adaptation in such conditions. This research work was funded by Ministero degli Affari Esteri e della Cooperazione Internazionale, Direzione Generale per la Promozione del Sistema Paese and by CORBEL project, founded from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 654248.
Keywords: Lactobacillus casei group, toxin-antitoxin systems, stress response, gene expression

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P2.7

Anti-quorum sensing effect of Prunella v*ulgaris, Sambucus nigra* and *calendula officinalis*

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Bacteria have a mechanism called quorum sensing (QS) that allows them to communicate with each other. Anti-QS compounds play a part in disrupting this mechanism between microorganisms and controlling them. It is known that QS inhibitors in medical plants are effective in limiting and controlling microorganisms´ activities such as resistance gain, proliferation, and spore formation. In our study, flowers, branch, pulp of plants (*Prunella vulgaris*, *Sambucus nigra*, and *Calendula officinalis*) obtained from Turkey was extracted with ethanol and methanol. Anti-QS activities were determined using disk diffusion method with 2 indicator strains *Agrobacterium tumefaciens* A136 and *Chromobacterium violaceum* 026. All extracts showed anti-QS effect. The highest effect was observed in the ethanol extract of *Prunella vulgaris* (13.75 ± 0.75 mm) followed by the ethanol extract of *Calendula officinalis* with a 13.25 mm zone.
As a result; it has been shown that plants with anti-QS may be an alternative to anti-QS-based antibacterial agents, particularly in bacterial pathogenicity control, by blocking communication signals between bacteria.

Keywords: Quorum sensing, Prunella vulgaris, Sambucus nigra, Calendula officinalis

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P2.8

Inactivation of *Salmonella* on peanuts by dry and oil roasting

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Salmonella is an enteropathogen responsible for innumerable foodborne outbreaks and hundreds of deaths annually worldwide. Some reports point out its ability to survive in hostile environments, including low moisture foods. The high heat resistance of *Salmonella* in these kinds of products raises particular issues for food safety. This study evaluated the effect of dry and oil roasting of *Salmonella* inactivation on peanuts. A pool of five *Salmonella* strains (Miami, Muenster, Yoruba, Javiana and Glostrup) previously isolated from the peanut supply chain was used as the inoculum. 500 g of in-shell and blanched peanuts were inoculated by spraying (0.56% *Salmonella* suspension) and the initial *Salmonella* count in the samples was 6 log MPN/g. Temperatures of 115, 125, 135 and 145 °C were used for the oil roasting for up to 4 min, while for dry roasting 130, 145 and 160 °C for up to 60 min were employed. The Most Probable Number (MPN) technique was used to determine the initial and final *Salmonella* count. During the dry roasting, the thermal resistance of *Salmonella* was greater on blanched peanuts when compared to in-shell peanuts at 130 and 145 ºC. For both temperatures, the D-value was 1.3 times higher for blanched peanuts than those calculated for in-shell peanuts. At 160 ºC, for the first period of time (0-30 min) the D-value was 5.43 min, whereas between 30-60 min it increased to 13.95 min. The time needed for one decimal reduction of *Salmonella* on blanched peanuts submitted to oil roasting was 0.78 min at 115 ºC, 0.41 min at 125 ºC, 0.21 min at 135 ºC and 0.14 min at 145 ºC. A reduction > 5.0 log MPN/g were obtained after dry roasting for 60 min at 145 ºC and 45 min at 160 °C, while in the oil roasting the time required to reach the same reduction ranged between 0.5 and 4.0 min. Therefore, the oil roasting process was more effective in inactivating *Salmonella* on peanuts than the dry roasting. Furthermore, the results demonstrated that the type of matrix influenced *Salmonella* resistance during dry roasting.

Keywords: oil roasting, dry roasting, peanuts, Salmonella, food safety

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P2.9

Glutathione system in lactic acid bacteria: Synthesis, import and metabolism

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Glutathione is an important antioxidant distributed widely in biological systems. *In-silico* analysis of whole genome sequences of lactic acid bacteria was first carried out to study the distribution of glutathione (GSH) system genes (*gshF, gshR, gpx, gst*) in this group. Bioinformatic analysis revealed the presence of glutathione biosynthesis bifunctional fusion gene (*gshF*) in *Streptococcus thermophilus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus, Lactobacillus plantarum, Lactobacillus ruminis* and *Lactobacillus sakei*. Biochemical evidence of GSH in cell lysates of cells grown in chemically defied medium (CDM) was only observed in *S. thermophilus* species. The highest amount of GSH synthesized was observed in *S. thermophilus* NCDC 659 (7.22nm/mg). *S. thermophilus* cells also exhibited the ability to import GSH from the medium when grown in CDM supplemented with 3.2 mM GSH with highest accumulation observed in *S. thermophilus* NCDC 295 (16.58 nm/mg). Bioinformatic analyses have revealed the presence of *gshR* homologs in genomes of all the selected LAB and *gpx* homologs in some species. Glutathione transferase homologs were found only in *S. thermophilus* and *Lb. casei* genomes. Glutathione reductase and peroxidase activity was widely observed in all the tested lactic acid bacteria but none were found to exhibit glutathione transferase activity. The study highlights the presence and activity of glutathione system genes in lactic acid bacteria. The glutathione synthesis and import ability could be a crucial parameter for enhanced stress resistance and technological performance of lactic acid bacterial strains.

Keywords: lactic acid bacteria, glutathione, antioxidant

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P2.10

Multidrug-resistant IncA/C plasmid 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 the minimum inhibitory concentration 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.

Keywords: Salmonella, antibiotic resistance, clone transmission, food animal

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P2.11

The genomic change in *Vibrio parahaemolyticus* via natural transformation-based horizontal gene transfer

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Vibrio parahaemolyticus is a foodborne pathogen, which mainly causes diarrhea and gastroenteritis. This pathogen often causes pandemic outbreaks worldwide, for example, a serotype O3:K6 strain originated from India caused the pandemic outbreak in 1990-2000's, and new serotype O4:K8 strain originated from China has caused a new pandemic outbreak in recent years. Horizontal gene transfer for promoting new phenotype acquisition is considered as one of the main factor of the outbreaks, however, the mechanisms are not explored completely.

It is required that to clarify mechanisms and impacts of horizontal gene transfer for prediction of new pandemic strain appearance and prevention of outbreak escalation. However, the genomic insertion site and its neighbor sites had been too complex for sequencing analysis. Recently, long read sequencing technology (over 30 kbp) has been made practicable, which allows whole genome sequencing including the insertion sites. Therefore, in this study, movement of the transferred genomic regions during natural transformation was analyzed using the new sequencing technology.

First, to make mutant, the genome extract from NFH-Vp16007 that showed high growth rate under stress condition was added to 4 other strains. After incubation, some isolates were obtained from the incubated culture by the pure isolation. The isolates were provided to growth rate examination to verify a natural transformation was happened. Finally, the isolates before or after transformation were provided to whole genome sequencing, and their ORFs were compared.

As a result of growth rate examination, an isolated from NFH-Vp16003 after transformation that increased growth rate under a stress condition was named NFH-Vp16003A. Then, ORFs of NFH-Vp16007, NFH-Vp16003 and NFH-Vp16003A were compared, and 18 ORFs of NFH-Vp16007 were detected from NFH-Vp16003A but not they were not detected from NFH-Vp16003. Although most of the transformed ORFs could not be defined their role, 3 ORFs were related to DNA recombination, which might have a role for horizontal gene transfer. Moreover, 5 ORFs were identified as a enzymes relating to metabolisms, which might be related to changes of growth rates. This study performed natural transformation of *V. parahaemolyticus* was reproduced in laboratory setting, and the knowledge can be expected to be helpful to prediction and prevention new outbreak in the future.

Keywords: Vibrio parahaemolyticus, whole genome sequencing, natural transformation

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P2.12

Effects of salts on heat resistance and recovery from injury of *Salmonella*

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Salt is necessary for food processing, which is used as a preservative, spice, agent for color maintenance and texture. However, with salt in food, some of pathogens including *Salmonella* aquire greater heat resistance. Mild heat treatment is used to minimize the degradation of nutrients, texture, and appearance in food, but has less effect on decontamination of the pathogens with increased heat resistance and leads to generation of injured but recoverable cells.
Sodium chloride (NaCl), potassium chloride (KCl) and magnesium sodium ($MgCl_2$) were tested in this study. *Salmonella* Typhimurium cultured at 37°C for 24 h in TSB containing with 2%, 4%, 6%, 8% and 10% chloride were subjected to heat treatment at 56°C for 10 min. Viable counts were determined by cultural method using TSA (measuring intact and injured but recoverable cells) and DHL agar (measuring intact cells). To monitor the recovery of cells during after heat treatment, cells (more than 99% were injured but recoverable) were observed on TSA under microscopy.
After the heat treatment, viable counts on TSA increased significantly in the cells grown in the medium including salts more than 2%, except 8% and 10% $MgCl_2$, compared to those grown in the medium including 0.5% NaCl. Counts of intact cells were the highest in the cells grown in the presence of salts at 6%, 8% and 2% for NaCl, KCl and $MgCl_2$, respectively. During the recovery in TSB, viable count on DHL increased and finally reach a similar level with viable count on TSA until 5 h. The total viable counts increased around 5 h after the heat treatment. The proliferation was confirmed by the single cell observation under microscopy.
S. Typhimurium improved heat resistance after cultivation in a media containing certain concentration of chloride. After heat treatment, the intact cell counts of *S.* Typhimurium was the highest in the medium including a certain molar concentration of chloride ion. The incorporation and distribution of chloride ion in *S.* Typhimurium cells will be investigated by using fluorescent microscopy. The increase of viable count on DHL until 5 h of the recovery culture in TSB seems due to the recovery of injured cells to intact cells.

Keywords: Salmonella; heat stress; chloride; injured cells; recovery

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P2.13

Mechanism for improvement of thermal resistance of *Salmonella* under high sucrose concentration

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Salmonella is the major cause of foodborne outbreaks related to low-moisture foods. It has been reported that *Salmonella* show high thermal resistance and prolonged survivability in low water activity (A_w) environment, but the mechanism is still unknown. We have investigated the effects of exposure period of low A_w formed by sucrose on thermal resistance of *Salmonella*. In addition, we have examined the changes in gene expression of *Salmonella* under high sucrose concentration by DNA microarray analysis. *Salmonella* Typhimurium cultured in TSB supplemented with 35% (w/v) sucrose was subjected to heat treatment. To know the effect of osmotic shock on thermal resistance of *S*. Typhimurium, the cells were directly transferred from TSB to TSB with 5%, 10%, 15%, 20%, 35%, 50%, 65% and 80% sucrose. For evaluating thermal resistance of the cells, viable counts were determined by the plating method using TSA before and after heat treatment. Temperature was monitored by using digital thermometer. Gene expression was compared between the cells cultured in in TSB and those in TSB with 35% sucrose by using DNA microarray analysis.

After heat treatment at 60°C for 5 min, viable counts of *S*. Typhimurium cultured in TSB with 35% sucrose were 3-log higher than in that cultured in TSB, and 1-log higher than that of the cells osmotically shocked by 35% sucrose. Temperature record suggested that supplement of high concentration sucrose decreased the heat transfer efficiency of medium, leading to delay in thermal stress to cells, resulting in longer treatment time to inactivate bacterial cells. DNA microarray analysis showed that the transcription of the genes involved in 1,2-propanediol metabolism and osmotic stress response significantly increased (fold changes≥4) but the genes involved in cytochrome decreased (fold changes≤4) after cultivation in TSB with 35% sucrose compared to those in TSB. *S.* Typhimurium increased thermal resistance after cultivation in the medium including 35% sucrose and osmotic shock by 35% sucrose. 1,2-propanediol metabolism seems important to improve thermal resistance in *Salmonella*.

Keywords: Salmonella, thermal resistance, low-moisture, sucrose

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P2.14

Effect of oxidizing agents in combination with temperature on *Listeria monocytogenes* survival

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Listeria monocytogenes is a foodborne pathogen frequently linked to the food processing environment (FPE). Usually, sanitizer agents used in the FPE are based on oxidizing agents able to disrupt cell membrane and inhibit nucleic acids and protein synthesis, but *L. monocytogenes* is capable of activating stress responses based on expression of sigma B factor or acquisition of plasmids or transposons from other microorganisms.

The aim of this study was to analyse the effect of the temperature and oxidative stress on the growth of *L. monocytogenes* strains isolated from different food industries.

Different genotypes (ST5, ST6, ST7, ST9, ST1, ST87, ST199 and ST321) of *L. monocytogenes* were submitted to oxidative stress with 13.8 mM cumene hydroperoxide (CHP) and 100 mM H_2O_2 diluted in RPMI broth. Inoculum concentration (10^9 cfu/ml) was prepared when cells grew to mid log phase. RPMI broth with each oxidizing agent was added to strains and incubated at 10° C and 37 °C for 3 hours. Aliquots from each condition were removed every hour to check the number of survival cells.

Results showed that the effect of oxidative stress is more notorious at higher temperature. When isolates were incubated at 37 °C in CHP, population cells were reduced in 6 - 7 log units during the first hour. Along incubation, ST9 and ST 321 were the most sensitive (counts were closely to the detection limit) and ST1, ST5 and ST87 (isolated from meat) were more resistant to CHP at the end of the essay. Instead, when treated with H_2O_2 at 37 °C only ST5 (dairy plant) and ST87 showed resistance after 1h of incubation (2 log units). CHP in combination with refrigeration temperatures reduced the population in 1-2 log between the first 2h in all the strains, but at the end, ST199 and ST 321 showed lower final counts (4.5 log) in comparison to others strains (6-7 log). Genotypes at 10°C were more sensitive to H_2O_2, especially ST9 and ST 321 that after 2h-3h were below the detection limit while ST1 and ST 5 represented an intermediate tolerance (4-5 log) and ST7 and ST87 showed higher counts (6-7 log) at the end.

In conclusion, high temperatures enhance oxidant effect of H_2O_2 exerting higher bactericidal effect in comparison to CHP. This study highlighted the relevance of compliance with the manufacturer recommendation of detergents and disinfectants during C&D to maintain the efficiency of chemical compounds used to eliminate microorganisms in food industries improving the food safety.

Keywords: Oxidative stress, cleaning and disinfection, resistance, stress responses

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P2.15

Role of autoinducer-2 and its detection mechanisms in *Campylobacter jejuni*

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Bacteria are able to recognize and adapt to changing environmental conditions by perceiving external environmental influences and self-produced signals. Autoinducer-2 (AI-2) is a signalling molecule synthesized by many bacterial species and assumed to be used also for interspecies-specific communication. AI-2 can be used to control the expression of virulence factors, the formation of biofilms and the motility of bacteria.

So far, two classes of AI-2 receptors have been identified. In *Vibrionaceae*, a signal cascade is induced by binding AI-2 to the receptor of a two-component signalling system (e. g. LuxPQ). *Salmonella* and *Escherichia coli*, on the other hand, internalise AI-2 via an ABC transporter (e. g. LsrABC), whereupon the intracellular phosphorylated AI-2 acts as a signalling molecule.

So far, the role of AI-2 in *Campylobacter* (*C.*) *jejuni* regulation processes has been controversially discussed, as *C. jejuni* synthesizes AI-2 but no AI-2 receptor has yet been identified. Therefore, all studies on AI-2-dependent phenotypes were conducted with mutants of AI-2 synthase (*luxS*). Since LuxS is responsible for the conversion of S-ribosylhomocysteine to homocysteine during the methionine cycle, it remains to be clarified whether phenotypes occur due to the loss of the metabolic function of LuxS or the AI-2 dependent regulation. The identification of an AI-2 receptor would significantly improve the investigation of AI-2-dependent phenotypes.

Our studies determined that the altered growth and motility of the *C. jejuni* NCTC 11168 delta*luxS* mutant could be complemented by the addition of synthetic AI-2 but not by the addition of homocysteine.

An AI-2-uptake assay was performed to investigate the type of AI-2 receptor in *C. jejuni*. We could demonstrate that AI-2 was only internalized by *E. coli* but not by *Vibrio harveyi* or *C. jejuni*. Therefore, the search for AI-2 receptors in *C. jejuni* should focus on two-component systems rather than transport systems.

Keywords: Campylobacter jejuni, Quorum sensing, AI-2

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P2.16

Inhibition of menaquinone biosynthesis reveals a new adaptation in membrane fluidity for *Listeria monocytogenes* strains at low temperatures

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Listeria monocytogenes is one of the major food-related pathogens and causes listeriosis, a potentially life-threatening illness. One of the most challenging task during growth at low temperatures is maintaining cytoplasmic membrane fluidity by modification of lipid membrane composition. For *L. monocytogenes* the dominating adaptation effect is shortening of fatty acid chain length. However, for some strains a weaker adaptive response in their fatty acid profiles to low growth temperature is known. For these strains we could demonstrate an increase in respiratory quinone concentration during growth at low temperatures. The strains showed a higher quinone content after growth at 6°C than after 37°C, which is contradictory to the supposed reduced respiratory activity at lower growth temperatures.

In this study quinone content for these *L. monocytogenes* strains was lowered by supplementation with aromatic amino acids. This supplement caused a feedback inhibition of the quinone synthesis pathway. For these quinone-reduced *L. monocytogenes* strains *in vivo* analyses of membrane fluidity by measuring generalized polarization and anisotropy revealed a change of the transition phase. Artificial reduction of the quinone content resulted in a narrower transition phase of the cytoplasmic membrane. In accord, strains with higher quinone content showed an expanded membrane transition phase. Experiments with vitamin K_1 supplemented dipalmitoyl phosphatidylcholine (DPPC) vesicles confirmed the transition phase modifying potential of naphtoquinones. The increase of the concentration of this neutral membrane lipid produced a fluidization of the membrane under low temperature conditions and therefor represents a fatty acid-independent adaptation mechanism to low temperatures.

Keywords: Listeria monocytogenes, low temperature, stress adaptation, membrane fluidity, menaquinone content

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P2.17

Competitiveness of *Staphylococcus xylosus* in meat

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Staphylococcus xylosus is commonly used as meat starter. *S. xylosus* genome analysis and its gene expression profiling in a meat model miming sausage batter, revealed that it can use diverse substrates as sources of carbon, nitrogen and iron.
S. xylosus possesses the genetic potential for transport of 18 carbohydrates. In meat model, *S. xylosus* imports the added glucose by a PTS-independent system and then glucose is catabolized through the EMP and the PP pathways. The lactate, naturally present in meat, is simultaneously imported by a lactate permease and catabolized to pyruvate.
Proteins, the main components of meat, are hydrolysed in peptides by endogenous proteinases during maturation. Peptides play a key role in bacterial nutrition. In *S. xylosus*, two gene clusters encoding peptide import and 20 genes encoding peptidases are present. In meat model, *S. xylosus* overexpresses genes encoding one peptide import and four peptidases. The free amino acids availability in the meat model leads to the down regulation of many genes involved the amino acid biosynthesis. In *S. xylosus*, arginine can be catabolised by arginase then by urease leading to NH3, a nitrogen source. Genes involved in glutamate transport and catabolism are overexpressed in meat model.
In meat, nucleosides such as xanthine and uracil can be released from ATP hydrolysis. *S. xylosus* overexpresses thirteen genes involved in purine and pyrimidine catabolism that generate ribose, which can fuel the EMP pathway resulting in energy production.
Meat is rich in hemic and non-hemic iron sources. *S. xylosus* has six systems to acquire iron. One of them is involved in the acquisition of iron from ferritin, an important substrate in meat. It can produce one siderophore to extract complexed iron.
This analysis establishes that *S. xylosus* is well equipped with all functions necessary for its adaptation to the meat substrates.

Keywords: Staphylococcus, starter, meat, competitiveness, metabolic activity

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P2.18

Effect of flavonoid enriched onion extracts on bacterial quorum sensing

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Quorum sensing (QS) is a bacterial communication system that influences gene expression. Studies indicate that plant organic extracts inhibit QS phenotypes and some works suggest that Quercetin, a flavonol present in high concentrations in onion (*Allium cepa*), presents anti-quorum sensing properties. However, there are no studies showing the anti-QS activity of plants containing quercetin in its native glycosylated form. The purpose of this study was to evaluate the anti-QS potential of organic extracts obtained from onion varieties against *C. violaceum* ATCC 12472*, P. aeruginosa* PAO1 and *S. liquefaciens* MG1. Additionally, we wanted to verify if structural changes of putative inhibitors could affect their anti-QS activity. Phenolic compounds of red and white onion varieties were extracted with organic solvents, then separated by solid-phase chromatography and identified by using HPLC-DAD and LC-*ESI-MS/MS.* The antimicrobial activity of the extracts was evaluated by MIC and growth curves. The QS inhibitory effect was tested by evaluating QS controlled phenotypes such as violacein production in *C. violaceum* ATCC 12472, as well as swarming motility and biofilm formation in *S. liquefaciens* MG1 and *P. aeruginosa* PA01. Furanone C30 was used as a positive control in QS inhibition assays. Quercetin aglycone and Queretin-3-beta-D-glicoside were also evaluated for their anti-QS activity. Our results showed that, Quercetin 3, 4'-diglucoside, Quercetin-3-glycoside and Isorhamnetin were the predominant compounds in both type of extracts. Cyanidin-3-glycoside and Quercetin aglycone were also identified in red onion extract. Inhibition of violacein production by *C. violaceum* was not observed due to the onion organic extracts. However, quercetin aglycone significantly inhibited violacein production in all concentrations. Inhibition of motility was seen with red onion extract in all concentrations, and white onion extract presented inhibition only at the highest concentration (125µg/ml). Even though both types of quercetin inhibited swarming motility, quercetin aglycone showed better inhibitory effect. Biofilm formation was not influenced by any extract and surprisingly we did not detect inhibition of biofilm formation by any type of quercetin either. Organic extracts of onion showed little to no effects on QS controlled phenotypes. It is likely that the glycosylated form of quercetin found in our extracts interfered with the antimicrobial activity of the extracts.

Keywords: Quorum sensing, antimicrobial activity, onion, anti-quorum sensing effect, quercetin, phenolic compo

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P2.19

Can *Campylobacter jejuni* be controlled by *Bacillus subtilis*?

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Campylobacter jejuni is one of the most prevalent causes of bacterial gastroenteritis worldwide, with a rising prevalence since 2005. It is a big health and economic concern. With its increasing antimicrobial resistance, the need for alternative options for the management of this pathogen rises. We here test the hypothesis that *Bacillus subtilis* has a potential to control *C. jejuni* growth. In microaerobic condition (either at 37°C or 42°C), where *C. jejuni* growth is favored, *B. subtilis* strains indeed showed a strong inhibitory effect on *C. jejuni*. We tested fifteen natural isolates of *B. subtilis* and they all inhibited the growth of this pathogen. We also tested fifteen *C. jejuni* strains (human, slaughtery, and water) with *B. subtilis* in co-culture. *C. jejuni* human feces strains were the most susceptible with a reduction of 4,2 log CFU/mL after 72 h, as compared to the control. In contrast, *C. jejuni* water strains were least susceptible (ΔlogCFU/mL=2,8). Next, we evaluated *C. jejuni* growth in co-cultures with different rations (10:1, 100:1, 10.000:1, and 1:10) of *C. jejuni* and *B. subtilis*. *B. subtilis* exhibited a strong inhibitory effect at all ratios except for the co-culture with initial frequency of *C. jejuni* 10.000 times higher than *B. subtilis* (average inhibition only ΔlogCFU/mL=1,5). In aerobic conditions, conditions toxic for *C. jejuni*, however, *B. subtilis* was less efficient and at 20°C *B. subtilis* facilitated the persistence of *C. jejuni* even after 48 h. To further test whether growth inhibition was due to *B. subtilis* competition or antagonism, we exposed *C. jejuni* to *B. subtilis* spent medium (SM). *C. jejuni* growth in SM was reduced only by 1-2 logCFU/mL after 24 h of incubation, as compared to the control. The low inhibition rate in SM is implying that the observed inhibitory effects of *B. subtilis* are not only due to the production of antibiotics.
We conclude that in environments that favor *C. jejuni* growth *B. subtilis* is a strong competitor of this pathogen, efficiently controlling its numbers even when the initial frequency of *C. jejuni* exceeds *B. subtilis* greatly. This inhibitory effect is not observed in environments where *C. jejuni* growth is limited by environmental factors (e.g. oxygen).

Keywords: Campylobacter jejuni, Bacillus subtilis, microbial interactions, biocontrol

Ecology and interactions in food-associated microbial communities

P2.20

Filamentation of *Campylobacter* in broth cultures

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The transition from rod to filamentous cell morphology has been identified as a response to stressful conditions in many bacterial species and has been ascribed to confer certain survival advantages. Filamentation of Campylobacter jejuni was demonstrated to occur spontaneously on entry in to stationary phase distinguishing it from many other bacteria where a reduction in size is more common. The aim of this study was to investigate the cues that give rise to filamentation of C. jejuni and C. coli and gain insights into the process. Using minimal medium, augmentation of filamentation occurred and it was observed that this morphological change was wide spread amongst C. jejuni strains tested but was not universal in C. coli strains. Filamentation did not appear to be due to release of diffusible molecules, toxic metabolites, or be in response to oxidative stress in the medium. Separated filaments exhibited greater intracellular ATP contents (2.66 to 17.4 fg) than spiral forms (0.99 to 1.7 fg) and showed enhanced survival in water at 4 and 37°C compared to spiral cells. These observations support the conclusion that the filaments are adapted to survive extra-intestinal environments. Differences in cell morphology and physiology need to be considered in the context of the design of experimental studies and the methods adopted for the isolation of campylobacters from food, clinical, and environmental sources.

Keywords: Campylobacter, filamentation, morphological changes, morphotypes, survival, intracellular

Ecology and interactions in food-associated microbial communities

P2.21

Evaluation of the thermal resistance of *Salmonella* Typhimurium ATCC 14028 after long-term peanut storage

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Traditionally cases of salmonellosis are linked to the consumption of products of animal origin with high water activity (a_w). However, outbreaks related to low a_w foods have been reported. The survival of *Salmonella* in this kind of product requires cell adaptation. The changes undergone by cells to adapt to one type of stress may result in resistance to others. The aim of this study was to evaluate the behaviour of *Salmonella* Typhimurium ATCC 14028 during the peanut dry roasting process (DRP) after the pre-adaptation to desiccation stress. To cause desiccation stress, portions of 400 g of blanched peanuts (a_w = 0.44) were inoculated by spraying with *S.* Typhimurium suspension to give a final concentration of approximately 5 log CFU/g. Then, the inoculated samples were stored at 28 °C for 0, 14, 30, 60, 120 and 180 days. After each storage time, the samples were submitted to dry roasting at 130 ºC for 10, 20 and 30 min. An inoculum without desiccation stress (control) was also submitted to DRP. The *Salmonella* population was determined by spread plating in xylose lysine deoxycholate agar (XLD). In the control sample, the pathogen was reduced to below the detection limit (< 1 log CFU/g) after 6 min at 130 ºC. At the same temperature after 30 min of DRP the samples submitted to the desiccation stress for 0, 14, 30, 60, 120 and 180 days reached reductions of 3.18, 2.16, 1.68, 1.20, 1.22 and 1.28 log CFU/g, respectively. The Weibull model provided a suitable fit to the data ($R^2 \geq 0.85$). The time of DRP required for the first reduction of *S.*Typhimurium inoculated in the peanut samples (delta) ranged between 14.25 and 28.76 min. These results indicate an increase in the thermal resistance of *S.* Typhimurium from 30 days on.

Keywords: peanuts, Salmonella, cross protection, roasting

Ecology and interactions in food-associated microbial communities

P2.22

Study of microbial dynamics during cassava retting for mash fermented cassava processing

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Cassava-based foods products are source of energy for nutrition of millions of peoples in the tropics. Due to the perishability and high cyanogen contents of cassava roots, several village processing techniques have led to the development of different cassava products for human consumption. *Bomboro* also called “baton de manioc” is one of the most consumed forms of cassava in Cameroon. This cassava-based indigenous food, is mainly manufactured using artisanal processing which is plagued with many problems including non reproducibility quality of products, lack of nutrients, non uniformity in the taste and flavour and poor hygienical quality. This problem seems to be linked to the steps of fermentation which occurs spontaneously in the manufacturing process. Unfortunately if literature on manies cassava based product abound, we have not found on the literature studies illustrating biochemical changes during the critical spontaneous fermentation phase for “bomboro” production. The aims of this paper were thus to study the microbiological and biochemical phenomena occurring during natural fermentation of cassava roots for “bomboro” production. The initial concentration of lactic acid bacteria was 4.9 log CFU g of dry dough_1. This concentration increased to 9.9 log CFU g of dry dough_1 after 24 h and was kept approximately constant for 72 h of fermentation. The initial number of ALAB was high (4.5 log CFU g of dry dough_1). This level increased during the first 24 h of fermentation to 8.4 log CFU g of dry dough_1 and remained constant until 72 h (8.7 log CFU g of dry dough_1). The pH value decreased from 7.4 to 4.8 in 24 h and to 4.4 in 72 h. A total of 257 strains of ALAB were isolated from both samples at different fermentation times. Rod and coccoid morphology were observed at all times. Strains isolated from sample B showed larger hydrolysis diameters (2 to 18 mm) on MRS-starch medium than those from sample A (1 to 9 mm). A starch hydrolysis zone of 7 mm in diameter was evident in a large number of strains. The largest starch hydrolysis zones (9 to 18 mm) were observed in strains isolated at 24 h in sample B. Similar results were obtained for sample A. The 40 most amylolytic strains (those with starch hydrolysis diameters larger than 9 mm) were further characterized by using a combination of phenotypic and molecular taxonomic approaches.

Keywords: Cassava, fermentation, Microbial

Ecology and interactions in food-associated microbial communities

P2.24

Variability in desiccation resistance and heat tolerance of a large set of strains within 24 serotypes of *Salmonella enterica*

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The occurrence of salmonellosis outbreaks associated with low water activity foods comprises a current public health problem. It is known that under desiccation conditions, foodborne pathogens such as *Salmonella* can become more tolerant to heat, for instance. This work aimed to evaluate the resistance of a large set of strains of 24 serotypes of *Salmonella* isolated from the soybean production chain. In addition, the survival of representative strains of 24 serotypes of *Salmonella enterica* exposed to a combination of desiccation conditions and heat at 90, 95 and 100 °C for 60 or 90 minutes was evaluated. For resistance to desiccation, 193 strains of *Salmonella enterica* from 24 different serotypes were studied. The results showed that the resistance to desiccation is related to the serotype studied and the time of desiccation. The longer the exposure time to stress (20 and 30 °C for 24 h and 7 days), the greater the number of decimal reductions observed. Strains *S*. Agona IOC 2305, *S*. Havana IOC 2310 and *S*. Schwarzengrund IOC 5691 showed a greater survival capacity under all conditions tested. In the heat tolerance assessment, the higher the temperature, the greater the reduction of *Salmonella enterica* counts. Despite this, the binomials tested failed to inactivate 4 to 5 log/CFU of *Salmonella enterica*. *S*. Idikan IOC 2350 strain presented a maximum reduction of 1.01 log CFU when exposed at 100 °C/ 60 min. The strain that presented the greatest reduction at 100 °C/ 60 min was *S*. Montevideo IOC 5607 (5.08 log CFU). The results presented in this work highlight the variability in desiccation resistance and heat tolerance of a large set of strains within 24 serotypes of *Salmonella enterica*. These findings are relevant for establishment of measures aiming to control the contamination by this bacterium during processing of soybean meal.

Keywords: survival; stress; foodborne pathogen

Ecology and interactions in food-associated microbial communities

P2.25

Biochemical changes occurring during fermentation of coconut milk by selected bacterial starter culture

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Plant-based analogues are fast becoming an emerging food product across the globe because of their functionality and specialty. Coconut was identified as a versatile fruit with high nutritional components and potential for producing milk. Fermentation process has been shown to enhance nutritional values of fermented foods. Hence, this study was designed to investigate the effect of starter culture on the biochemical properties and characterizes the quality of the fermented coconut milk.Coconut milk was prepared using the modified method of Olusola (2014) and fermented using a mixed culture at 37 for 72 h. Samples were taken at 12 h intervals for analysis. Changes in biochemical (lactic, acetic and formic acid) and nutritional properties (amino acid, vitamins soluble sugars) were monitored. Results showed that lactic and acetic acids increased as fermentation progressed. Essential amino acid; arginine, histidine, threonine, tryptophan, lysine and leucine increased significantly ($p< 0.05$), while valine, methionine, tyrosine, proline isoleucine and phenylalanine decreased at the end of fermentation. Significant ($p< 0.05$) reduction was observed in ascorbic acid, α-tocopherol, niacin and pantothenic acid contents of the fermented milk whereas, significant ($p< 0.05$) increases were observed in phylloquinone, thiamine, and pyridoxine. Soluble sugars decreased at the end of fermentation. This research characterizes the quality of fermented coconut milk by revealing changes that occur in amino acid, vitamins of coconut milk inoculated with mixed culture of *Lactobacillus delbrueckii sub bulgaricus, Lactobacillus acidophilus and Streptococcus thermophilus.* This study gives insight into the effect of fermentation technology on the quality of coconut milk.

Keywords: Fermentation, coconut milk, starter culture, biochemical changes

Ecology and interactions in food-associated microbial communities

P2.26

Gene profiling-based phenotyping for identification of cellular parameters that contribute to fitness, robustness and virulence of acid- resistant *Listeria monocytogenes* variants

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When bacterial populations are subjected to food relevant lethal stresses, such as heating or low pH, a small fraction of the initial population may survive. Such heterogeneous bacterial populations may lead to tailing of the inactivation curve, which can be problematic for the assessment of the efficacy and accurate modelling of inactivation procedures. Previous studies reported isolation of natural *Listeria monocytogenes* variants after a single exposure to food relevant acid stress. Whole genome sequencing of these variants, that proved to be multiple stress resistant, revealed various mutations in *rpsU*, encoding the ribosomal protein S21. Using a comparative gene profiling and phenotyping approach on two different *rpsU* variants, we elucidated features that play a role in fitness, robustness and host interaction of *L. monocytogenes*. Both variants showed increased expression of 116 genes, including a major contribution of Sigma-B controlled genes involved in stress defence such as the acid resistance-associated glutamate decarboxylase (GAD) system, glycerol metabolism (*glpF*, *glpK*, *glpD*), compatible solute uptake (*OpuA*, *OpuC*) and virulence (*lntA*, *lntB*).
Phenotyping results matched the gene profiling data including enhanced acid resistance, higher glycerol utilisation rates, enhanced freezing resistance conceivably mediated by higher intracellular levels of compatible solutes, and better adhesion to Caco 2 cells presumably linked to higher expression of internalins.
Our study provides further insights into cellular parameters that can affect robustness, fitness, and virulence of *L. monocytogenes* variants that might be selected for upon exposure to food relevant stress conditions.

Keywords: SigB, rpsU, Listeria monocytogenes, stress resistant variants

Ecology and interactions in food-associated microbial communities

P2.27

The role of the newly discovered bacterial second messenger (cyclic-di- AMP) in stress responses in lactic acid bacteria

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Lactic acid bacteria (LAB) are used in a wide range of food applications, including as starters and flavour forming adjuncts in food fermentations. In these situations they are exposed to a variety of stressors including heat, osmotic, nutrient starvation and acid. They must be able to sense and overcome these stressors in order to perform efficiently in food fermentations. Our work using a model LAB, *Lactococcus lactis*, which is used in cheese fermentation, has identified an important signalling nucleotide molecule called cyclic-di-AMP which controls a variety of stress adaptation processes in the cell. The level of c-di-AMP in most bacteria is modulated by one synthesis (CdaA) and one degradation (GdpP) enzyme. Our prior work and that of others has identified that c-di-AMP regulates osmoresistance: mutants with high c-di-AMP are hypersensitive to salt and mutants with low c-di-AMP are dependent on salt for growth. This appears to be due to c-di-AMP binding to and inhibiting K+ and compatible solute transporters. Other phenotypes in *L. lactis* regulated by c-di-AMP include heat resistance and cell lysis. Using a genetic screen, our group has identified new genes which affect the c-di-AMP level and related phenotypes in *L. lactis*, which include a potassium importer and a peptide export system. I will present the results of this screen and subsequent results identifying a signal which triggers c-di-AMP level changes in *L. lactis* and other Gram-positive bacteria. The c-di-AMP system, which is ubiquitous in lactic acid bacteria likely plays a significant role in responses to stressors encountered in fermentations.

Keywords: Stress, Lactococcus, osmoresistance, signalling

Ecology and interactions in food-associated microbial communities

P2.28

Shining light on *L. monocytogenes* – A transcriptome analysis

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Listeria monocytogenes is a non-phototrophic bacterium and the causative agent of food-borne listeriosis. Different strategies have been both suggested and applied to prevent food contamination with L. monocytogenes. However, L. monocytogenes is proven to be hard to control, and there is a need for increased knowledge on how this agent copes with a broad spectrum of stressors. It is recently discovered that L. monocytogenes is able to detect and respond to visible light as a stressor, and wavelengths within both the blue and the red area are documented to have an effect. This study aimed to explore the effects of broad spectrum visible light on L. monocytogenes.

The EGDe strain, one mutant for the blue light receptor and one mutant for the stressosome regulator Sigma B were grown on Brain Heart Infusion (BHI) motility agar, after exposure to simulated day-light at 12, 20 and 37 °C. A transcriptomic analysis using High Throughput Sequencing was further applied on the EGDe strain at 20 and 37 °C after exposure to simulated day-light in BHI broth. Altogether, 42 samples were library prepped for sequencing on Illumina Hiseq 3000 (150 bp PE reads). Bbduk was used for cleaning the raw data before alignment with Hisat2 using genome and annotation references from the ensemble bacteria. Counting aligned reads was done with FeatureCounts and finally, DESeq2 was used for analysis of differentially regulated genes.

In general, visible light inhibited growth of L. monocytogenes within all tested strains and temperatures. As expected, the colony diameter was also generally reduced when the strains grew in simulated day-light. However, in contrast to previous reports, light exposure caused a significantly increased cell density on 0.3 % BHI agar plates. This effect is in line with the significantly reduced motility observed when L. monocytogenes are exposed to visible light. Preliminary transcriptomic results confirm this light-dependent negative effect on motility; several genes in the flagellar motility pathway were significantly down regulated when the bacteria where exposed to simulated day light. These results challenge the assumption that temperature is the only critical factor for regulating flagellar motility in L. monocytogenes. Flagellar motility is further assumed to be critical in the initial phase of biofilm formation, which is relevant for L. monocytogenes persistence and resistance to stressors applied in the food processing environment.

Keywords: L. monocytogenes, transcriptome analysis, visible light

Ecology and interactions in food-associated microbial communities

P2.29

Isolation and characterisation of *Listeria monocytogenes* mutants that are hypersensitive to some plant essential oil compounds

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Plant essential oils and their constituents are of interest as potential natural food preservatives. Although some studies have addressed their mode of action, the cellular structures or pathways that determine the tolerance of bacteria to these compounds are poorly understood. We have conducted a genome-wide transposon mutant screening in *Listeria monocytogenes* to isolate mutants with altered sensitivity to t-cinnamaldehyde (CIN), allyl isothiocyanate (AIT) and hop β-acids (HBA). We previously reported that an *ilvE* mutant, which has strongly reduced membrane branched chain fatty acid levels, is hypersensitive to CIN (1). Using adaptive laboratory evolution and whole genome sequencing, we isolated and identified suppressor mutations that partially restored CIN tolerance to the *ilvE* mutant. Suppressor mutations were found in three genes. Suppressor mutations in two of these genes partly restored the levels of membrane branched chain fatty acids, but suppressor mutations in the third gene did not. Further analysis of these mutants will provide a detailed view on the mode of action of CIN.
Screening of the transposon mutant library with AIT yielded a set of hypersensitive mutants that overlapped significantly with the CIN-hypersensitive mutant set. This was expected because the antimicrobial activity of both compounds is believed to depend at least partly on their ability to react with thiol groups. However, we also isolated mutants which were only sensitive to one compound, suggesting that there are also differences in the mode of action.
Finally, to our surprise, no HBA-hypersensitive transposon mutants could be isolated, and no HBA resistance could be generated by adaptive laboratory evolution. The reasons for this are currently unclear.

References: Rogiers et al., 2017. Res. Microbiol. 168: 536-546.

Keywords: Listeria monocytogenes, natural preservatives, mode of action, mutant analysis

Ecology and interactions in food-associated microbial communities

P2.30

Transposon mutagenesis and adaptive laboratory evolution to investigate the mode of action of t-cinnamaldehyde against *Listeria monocytogenes*

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Plant essential oils and their constituents are of interest as potential natural food preservatives. Although some studies have addressed their mode of action, the cellular structures or pathways that determine the tolerance or sensitivity of bacteria to these compounds are poorly understood. We have previously isolated several transposon mutants in *L. monocytogenes* with altered sensitivity to t-cinnamaldehyde (CIN). A knock-out mutant in the *ilvE* gene had strongly reduced membrane branched chain fatty acid levels and was hypersensitive to CIN (1). Knock-outs in the genes *asnB* (encoding a glutamine transaminase), *gdpP* (encoding a cyclic-di-AMP phosphodiesterase) and *yvcK* (a conserved but poorly characterized gene in many *Firmicutes*) were also CIN hypersensitive. These three mutants were characterized phenotypically in more detail, revealing a variety of phenotypes including altered morphology and/or hypersensitivity to lysozyme and cell wall-targeting antibiotics, and/or loss of motility. For *asnB*, the evidence suggests that this gene probably mediates amidation of mesodiaminopimelic acid, as is the case in *B. subtilis*. To further investigate the causes underlying the CIN hypersensitivity of the three mutants, we isolated suppressor mutants which had (partially) regained CIN tolerance and performed whole genome sequencing to identify the suppressor mutations. The results of this analysis will be discussed.

(1) Rogiers et al., 2017. Res. Microbiol. 168: 536-546.

Keywords: Listeria monocytogenes, natural preservatives, mode of action, mutant analysis

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P2.31

Microbial and physico-chemical characterization of "Sanganel", typical blood sausages from Friuli, the Eastest region of Italy

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Sanganel is a typical blood sausage produced in Friuli, the Northeastest region of Italy by butchers in shops, farms and small factories. It is made with pork meat, boiled blood, lard, spices, and salt. *Sanganel* is stored at 4 ± 2 °C for maximum 14 days and eaten after boiling. Little is known about its microbial ecology, physic-chemical parameters and changing during shelf life. The aim of this study was to characterise the microbial population and the physic-chemical parameters of Sanganel in order to establish its quality and safety. Pseudomonads, psychotrophic enterobacteria and lactic acid bacteria (LAB) represent the main microbial population. Both enterobacteria and LAB grow during the sausage storage. Enterobacteria, in particular, seemed to predominate over LAB and consequently increased the pH, that reached approximately a value of 6.9, by the production of basic volatile nitrogen (TVB-N) and biogenic amines. At 14 days TVB-N concentration and the total biogenic amines content were 42.5 mg N/100 g and 34.5 ppm, respectively, increasing to 52.5 mg N/100 g and 44.5 ppm after 30 days. *Salmonella* spp., *Listeria monocy*togenes and pathogenic *Escherichia coli* were never detected. Considering the microbial population and the physico-chemical characteristics of *Sanganel*, a shelf-life of 14 days is suggested. In addition, *Sanganel* is boiled before its consumption and that can further increase its safety and healthiness.

Keywords: Sanganel

Ecology and interactions in food-associated microbial communities

P2.32

Multidrug resistant, extended spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolated from one of the UK dairy farms

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Antimicrobial resistance is a crucial problem that is now of great concern in public health, with food and food producing animals as a potential route for spread of these resistances, especially resistance to cephalosporins, which is increasing. Approximately 400 tonnes of antibiotics are used every year in treating infections in farm animals in the UK, and as prophylactics against infection. The main aim of my study was to determine the prevalence and range of multidrug resistance (MDR) and extended spectrum β-lactamase (ESBL) or ampicillin C (AmpC) β-lactamase producing *Escherichia coli* within a commercial dairy farm, to understand the diversity of resistance to β-lactam antibiotics, and to determine if co-carriage of other antimicrobial resistance (AMR) was associated with ESBL/AmpC producers. This would allow a better understanding of the contributions that farms and farm slurry may make to the presence of AMR in the environment, and the reservoir of resistance in agriculture. In this study, *E. coli* strains were isolated from a single dairy farm (East Midlands, England, United Kingdom) using different culture media. Antimicrobial sensitivity tests were performed using a disk diffusion test for all the strains against 17 antimicrobials representing seven different antimicrobial groups. Antimicrobial resistance profiling showed 92% of isolates showed resistance to at least 1 antimicrobial, of which 27.8% of the isolates were isolated without antibiotic selection, and 57.9% of the isolates were multidrug resistant to between 3 and 15 antimicrobials, of which 43.6% of the isolates were isolated using antibiotic supplemented media. Two strains showed resistance to imipenem, the finding was unexpected and of concern as imipenem is not used in veterinary medicine. bla_{CTX-M}, bla_{TEM} and bla_{OXA} genes were detected by PCR among the cephalosporin resistant strains. Four strains were fully sequenced and the genetic/genomic environment surrounding β-lactamase genes and analysis of some other AMR genes showed these genes are associated with transposable elements, such as IS*Ecp1,* IS*CR2*, IS*26*-IS*26*, Tn*2*, Tn*10* or within a class I integron. The association of AMR genes with these transposable elements might make the dissemination rate of these genes greater. The spread of such highly resistant strains to the environment and possibly to humans could present a real threat to human health especially if they are pathogenic.

Keywords: E.coli, ESBL, transposable element, AMR

Ecology and interactions in food-associated microbial communities

P2.33

Impact of grape seasonings on the invasive capacity of *Listeria monocytogenes* in Caco-2 cells

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The food industry tries to maintain food safety and preserve the health of consumers, which demand changes in the production methods and in the ingredients used. There is a special interest for those ingredients of natural origin that confer a preservative and beneficial effects for health.

The grape seasonings are constituted by the skins of grapes after fermentation and contain high concentrations of phenolic compounds with antioxidant and antimicrobial effect. On the other hand, the disease caused by *Listeria monocytogenes,* known as listeriosis, is a serious problem in the food industry. This microorganism can be present in a wide range of foods as well as survive various processing technologies. The virulence of *L. monocytogenes* is due to its ability to adhere, invade and multiply inside the cells. It is the adhesion to the intestinal epithelium the first step in the pathogenesis. In this sense, the use of phenolic antimicrobials and their potential anti-*Listeria* invasive effect in intestinal cells could be great interest.

The objective of this study was to evaluate the effect of grape seasonings on the ability of different strains of *Listeria monocytogenes* to invade Caco-2 cells. For this, phenolic composition and antioxidant capacity of the seasonings were also analyzed to establish possible implications of phenolic compounds in the anti-invasive effect.

Two seasonings from different geographic origin, obtained from grape skins according to the process described in the patent CCP:ES2524870, were used. Phenolic composition by HPLC and antioxidant capacity (Q-FC) were evaluated in the grape seasonings. Two *Listeria monocytogenes* strains isolated from food were used; E10625, from cheese and S11 from salmon. The virulence test was carried out on Caco-2 cells of colon adenocarcinoma.

The results showed that the treatment of the *Listeria monocytogenes* strains with the seasoning caused a reduction of the invasive capacity that is associated to the anti-adhesive capacity that the phenolic compounds present. Definitely, our preliminary study suggests that the treatment with the grape skin seasonings could be a promising antimicrobial alternative to food-borne outbreaks.

This study was financially supported by the research project BU056U16 from Junta Castilla y León (Regional government), Spain.

Keywords: Listeria monocytogenes, grape seasoning, Caco-2 cells, invasive capacity

Ecology and interactions in food-associated microbial communities

P2.34

Synthesis and *in vitro* evaluation of peracetyl and deacetyl glycosides of eugenol, isoeugenol and dihydroeugenol acting against foodcontaminating bacteria

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Essential oils, as well as their separate components, have shown promise as alternatives to synthetic preservatives. Therefore, it would be interesting to optimize the effect of these compounds and to evaluate their applicability as additives in food. To this end, six peracetyl and deacetyl glycosides were synthesized from eugenol, isoeugenol and dihydroeugenol. All of the glycosides were characterized by IR and NMR. The synthesized compounds and their aglycones were evaluated to determine their minimal bactericidal concentrations (MBC) against the spoilage food bacteria Escherichia coli, Listeria monocytogenes, Staphylococcus aureus and Salmonella enteritidis. All deacetyl glycosides were about twice as active as aglycones, and the peracetyl glycosides were, in most cases, equipotent with aglycones. The deacetyl glycoside of dihydroeugenol proved to be the most active compound against the bacteria tested, with a 0.37% MBC v/v for E. coli and 0.18% v/v for the other bacteria.

Keywords: NMR; Glycosylation; antibiosis

Ecology and interactions in food-associated microbial communities

P2.35

The changing epidemiology of *Salmonella enterica*: Distribution of serotypes among 2000 to 2016 in Brazil

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Although many studies reveal a high frequency of resistant *Salmonella* strains, little is known about the population dynamics of *Salmonella enterica* serotypes from different sources in the food chain. The objective of this study was to study the epidemiology of *Salmonella* serotypes, antimicrobial resistance and sequence types of *Salmonella* strains from different sources from 2000 to 2016. *Salmonella* strains (n=264) were characterized for serotype using classical serotyping methods and for antimicrobial resistance and sequence type using whole genome sequencing. Twenty six serotypes were identified, and in order of highest to lowest (36% to 0.7%) was Heidelberg, Typhimurium, Infantis, Enteritidis, Schwarzengrund, Minnesota, Ohio, Panama, Abony, 4,[5], 12:i:-, Newport, Senfteberg, Mbandaka, Isangi, Kentucky, Montevideo, Rochdale, Saphra, Derby, Havana, Muenchen, Orion Ouakan, Abaetetuba, Carrau and Grupensis. Overall, representative resistant (n=48) and susceptible (n=60) strains were sequenced (n=108) and 37 discrete resistance genes from different antibiotic classes were found. The majority of sequenced isolates exhibited resistance to the tetracycline gene *tetA* (23/108; 21.2%). Resistance to β-lactams: [bla_{TEM-1B}, $bla_{CTX-M-2}$, bla_{CMY-2}, bla_{TEM-1A}, $bla_{CTX-M-8}$]; quinolones: [*qnrB19*, *qnrE1*, *qnrS1*, *qnrB5*, *oqxA*, *oqxB*]; fosfomycin: [*fosA7*]; aminoglycoside: [*aadA1*, *aadA2*, *aac(3)-lla*, *aph(6)-ld*, *strA*, phenicol: [*floR*, *cmlA1*, *catA1*]; sulphonamide: [*sul1*, *sul2*, *sul3*] and trimethoprim: [*dfrA12*, *dfrA1*, *dfrA8*] were present in different serotypes. Fifteen sequence types were found and assigned by serotype such as ST15, ST19, ST3438, ST32, ST11, ST329, ST413, ST548, ST138, ST1524, ST2041, ST40, ST48, ST226 and ST1604. Our results indicate that from 2015 to present, the population dynamics changed as Heidelberg emerged, surpassing Typhimurium, Infantis and Enteritidis. This change may be explained by the efforts of biosecurity and management changes throughout the poultry production chain. Furthermore, adaptive genomic characteristics play crucial roles in the persistence and spread of these strains along the food chain. As such, we may regard *S.* Heidelberg , which exhibited multidrug resistance [bla_{CMY-2}, *fosA7*, *sul2*, *tet (A)*] and harbors at least two type of plasmids (IncA/C2, IncX1), as highly adaptable in the host environment and an important vector in the dissemination of resistance genes throughout the poultry production chain.

Keywords: Antimicrobial resistance, whole genome sequencing, Salmonella enterica

Ecology and interactions in food-associated microbial communities

P2.36

Influence of sublethal temperatures on the hygienic quality of 'folere' beverage

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Artisanal drinks play a special part in the daily lives of households in Africa. This is the case of "foléré", a homemade beverage made from *Hibiscus sabdariffa* leaves extract and sugar, much appreciated by the populations in northern regions of Cameroon. Despite its interesting organoleptic and nutritive properties, this beverage presents questionable hygienic quality. To overcome this, the hygienic quality of "foléré" beverage and the role of post-production sublethal temperatures on the hygienic quality of this local drink were investigated. We focus our investigation on the physico-chemical analysis and the microbiological profile. The main results indicated that, "foléré" beverage is of poor hygienic quality according to Cameroon standards based on the French Agency norms despite its low pH (2.01). Simulations of the artisanal production process coupled with an inoculation of the beverages at pH 2.01 by referenced bacterial strains (*Escherichia coli* ATCC 25922 and *Bacillus cereus* T) indicated that the beverages samples produced at pH 2.01, pasteurized and contaminated with thermally stressed bacteria (10°C, 45°C, 50°C and 60°C) for respectively 45 min, 90 min and 180 min, resulted in poor hygienic quality than the same traditional beverage samples contaminated with unstressed bacteria. This phenomenon was named "heat-induced bacterial acid resistance". This one shows that some chilling treatment cannot sometimes improve food preservation. Consequently, the control of this phenomenon which remains to be elucidated can lead to the production of a better hygienic quality homemade beverages.

Keywords: Cameroon, homemade drinks, bacteria, thermal stress, acid resistance.

State of the Art techniques for the analysis of biodiversity and microbial interactions of foodborne microbes

P3.1

Enhancing the use of microbiological, chemical and sensory data in food quality monitoring

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Recent advances in the development of intelligent packaging technologies have provoked increasing interest towards using the products of microbial metabolism in food quality monitoring. Fresh food products such as seafood are highly susceptible to microbial growth and metabolism, which lead to the production of volatile organic compounds (VOCs) that often contribute to consumer rejection via the generation of unacceptable off-odors. Identification of spoilage-indicating VOCs thus requires comprehensive knowledge of the relations between microbiological, chemical and sensory quality of the packaged food products. However, this typically results in the production of multidisciplinary, multivariate and time-dependent datasets that are characterized by complex interactions. Appropriate methods are thus needed for enhancing the acquisition, analysis and application of these datasets. In this presentation, we describe the characteristics and challenges associated with producing information for the development of VOC-based food quality monitoring for fresh food products. Consequently, we describe how different methods can be implemented as a part of a systematic three-stage procedure aiming at the identification and quantification of most potential spoilage indicators under different packaging and storage conditions. Changes in microbiological, chemical and sensory quality as a function of storage time are illustrated in seafood packaged under modified atmospheres (MAs).

Keywords: Data analysis; food quality monitoring; microbial metabolism; seafood; volatile organic compound

State of the Art techniques for the analysis of biodiversity and microbial interactions of foodborne microbes

P3.2

The fermented food microbiome

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With increasing evidence highlighting the microbiome as an important contributor to host health, attention has turned to positively modulating microbiota composition and function, including through enhancing its diversity. There has been a particular emphasis on the gastro-intestinal tract (GIT), which is home to the greatest proportion and diversity of the human microbiome. Importantly, diet has a major impact on this microbiome, and there is an ever-greater interest in the potential role of fermented foods in enhancing human health through GIT-mediated activities. While the mechanism for improved health via fermented foods is not fully understood in all instances, it is thought that the large numbers of bacteria with potential health-promoting characteristics, and associated bioactive molecules, are responsible and, thus, investigations that provide a greater understanding of the microbiota of fermented foods, and their potential functions, may be of great value.

For this reason, 62 fermented foods from artisanal producers in Ireland, the UK, Germany, Benin, China, Mexico, Russia and Japan were collected. Microbial enumerations were performed by plate counting and, simultaneously, DNA was extracted for shotgun metagenomic sequencing and analysis. Bray-Curtis dissimilarity analyses showed a clear clustering of samples according to type of fermentation (Brine, Dairy and Sugar). Alpha diversity was calculated using Shannons Index, Simpsons Diversity Index and Total Species Count, and it was apparent that brine and sugar-based fermented foods had significantly higher diversities than dairy food across all alpha diversity metrics. Heatmaps were used to visualise and compare the taxonomic, genetic and bacteriocin content of the foods, with Kruskal-Wallis analyses revealing several taxa and gene families that were significantly different between the foods.

Ultimately, this study presents a large body of new information on a broad range of fermented foods facilitating future investigations to uncover the full health-promoting potential of these foods.

Keywords: Fermented Food, Shotgun metagenomic sequencing, Microbiome

State of the Art techniques for the analysis of biodiversity and microbial interactions of foodborne microbes

P3.3

Molecular characterization of whole genome sequence of human norovirus strain GII.4 variant in South Korea

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Human Norovirus (HuNoV) is the leading cause of epidemic and sporadic gastroenteritis outbreaks worldwide affecting across all age groups, responsible for approximately 90% of all outbreak of viral gastroenteritis. HuNoV, a genus within the *Caliciviridae* family, is small non-enveloped virus with a positive single-stranded RNA genome of 7.5-7.7 kb organized into three open reading frames (ORFs). ORF1 encodes six non-structural proteins, including RNA dependent RNA polymerase (RdRP). ORF2, ORF3 encode VP1 and VP2 capsid proteins. Generally, the genome of RNA virus has been known to change constantly from mutational event resulting in emergence of novel variant. In the previous reports, the NoV GII.4 strains had been known to evolve at a rate of 4.3-9.0 × 10^{-4} mutations per site per year and to share a most recent common ancestor in the early 1980s. The goal of this study was to identify NoV complete sequence and characterize variants using Next Generation Sequencing (NGS) assay. Reverse transcriptional PCR (RT-PCR) was performed with viral genome detected in stools associated with an outbreak. The DNA library was constructed using Ion Torrent library kit and was sequenced by the Ion Torrent S5. Sequence reads of NoV were assembled with reference genome using CLC genome workbench software version 11. In phylogenetic analysis, the whole genome sequence showed high similarity with those of GII.4 strains and was clustered with those of 2006b variants. The amino acid sequence of VP1 (i.e. capsid protein) was altered at three epitope residues in hypervariable domains, and it led to changes in protein structure. Continued molecular studies of NoV using NGS may provide epidemiological support for the utility of monitoring changed in epitopes in emergent strain surveillance.

Keywords: Norovirus, Next Generation Sequencing, GII.4 strains

State of the Art techniques for the analysis of biodiversity and microbial interactions of foodborne microbes

P3.4

A model smart quality assurance and safety system for fresh poultry products (QAPP)

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The aim of "QAPP" project is the development of an "Intelligent" Management System for food-specific Quality and Safety, based on an integrated Information and Communication Technology (ICT) services platform, that will incorporate methods, data and decision-support tools for all target market participants. Poultry products (minced meat/burgers etc.) will be used as model systems to validate the approach.

The "Intelligent" Management System for food-specific Quality and Safety, will include a novel cloud-enabled data and model storage system, which will allow the integration of heterogeneous information derived from food microbial ecosystem throughout its production and distribution chain. These heterogeneous data will be derived from (i) conventional microbiological analyses, (ii) advanced signal analysis: spectroscopic profiling data (vibrational spectroscopy) and surface chemistry spectra will be compiled using non-invasive analytical sensor devices, (iii) data mining methods and prediction of microbial population or quality.

The targeted scientific breakthrough is the joined utilization of conventional microbial data, 'omics' (through spectroscopic signals) and measurements in the context of an ICT-based tracking system. The ultimate goal of the holistic approach of the project is to establish a next-generation monitoring reference system for food safety & quality (open software architectures/web services, Machine2Machine /Internet of Things, Data Analytics/Machine Learning), through the integration and analysis of complex structured or non-information data (Big data), that enables real time predictions for safety and freshness profiling. This integration will provide comprehensive maps of important food traits, as well as predictive models of the contribution of individual microorganisms to aid in decision-making on food quality and safety. In other words, product profiling will be based on massive and systematic collection of information on storage times and temperatures, and sensor-based fingerprints of food products at any given time point. The "Intelligent" Management System for Product-specific Quality and Safety of QAPP will ensure poultry quality and safety promptly, with a longer-term vision of expanding to other food sectors.

Keywords: Internet of things, food safety, omics

State of the Art techniques for the analysis of biodiversity and microbial interactions of foodborne microbes

P3.5

Yeast diversity in traditional Portuguese soft cheese by culture dependent and independent DNA approaches

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Serpa is an artisanal ripened Portuguese cheese granted the Protected Designation of Origin (PDO) label. It is produced from raw ewe's milk, using flowers of *Cynara cardunculus* L. as rennet and without the addition of a starter culture, therefore, the complex microbial community present during the ripening strongly contribute to the final quality and safety of the cheese. Most of the microbiota present in raw milk cheese are lactic acid bacteria and their importance is well known. Additionally, yeasts are considered member of the secondary microbiota, where they play an important role during the ripening. Thus, considering their importance, yeast species must be identified to determine their influence on the maturation and deterioration of cheese. In this context, the aim of the present study was evaluated the yeast community present in traditional Portuguese Serpa cheese by different DNA approaches.

Cheese samples were taken at the end of the ripening from five different dairy industries. Three industries belonged to PDO "Queijo Serpa", while the other two were non-PDO registered. The yeast community was investigated by culture dependent techniques using PCR-RFLP analysis of ITS region and sequencing of the 26S rRNA and culture independent approach by HTS of the ITS rRNA.

The results obtained showed that yeast counts ranged between 4.2-5.66 log CFU/g. The species identified by culture dependent methods, mainly corresponded to *Debaryomyces hansenii* and *Kluyveromyces marxianus*, with *Candida* spp. and *Pichia* spp. present to a lesser extent. The culture-independent results confirmed the prevalence of *Debaryomyces* spp. and *Kluyveromyces* spp. but, also, that *Galactomyces* spp. was relevant for three of the five producers, which indicates its importance during the early stages of the cheese ripening process, considering it was not found among the dominant viable yeast species. In addition, the differences between yeast species from PDO and non-PDO registered industries, showed that the lack of regulation of the cheese-making practices may influence the final yeast microbiota. In conclusion, the prevalent of yeast isolates from *D. hansenii* and *Kluyveromyces* spp., indicate that may have an important role during cheese ripening and in the final sensorial characteristics. Thus, the study of their technological and functional properties could be relevant, in the development of an autochthonous starter culture, to ensure final quality and safety of the cheese.

Keywords: Cheese, PCR-RFLP, High-Throughput Sequencing, Yeast community

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P3.6

Quantification of viable Salmonella on roasted peanuts during long-term storage using Real-Time PCR and propidium monoazide (PMA)

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Epidemiological surveys of salmonellosis outbreaks involving low moisture foods such as nuts, peanuts and chocolate indicate that the infectious dose of *Salmonella* in these products can be extremely low (0.04 MPN/g). The stress condition provided by the environment of low water activity can result in viable but non-cultivable cells (VBNC). Traditional methodology can recover only cultivable cells. Nevertheless, new molecular methodologies can be used for rapid and reliable quantitative detection of *Salmonella* VBNC, such as the use of propidium monoazide (PMA). The aim of this study was to evaluate the use of PMA-qPCR for the enumeration of *Salmonella* on roasted peanuts during long-term storage. For this study, portions of 400 g of roasted peanuts were inoculated by spraying with a suspension of *S.* Typhimurim ATCC 14028 to achieve an initial concentration of approximately 5 log cfu/g. Then, the samples were stored at 28 ° C for 30, 60, 120 and 180 days. After each period, the *Salmonella* population was simultaneously quantified by qPCR (with and without PMA sample treatment) and by conventional culture in XLD agar (xylose lysine desoxicholate). The reactions of qPCR were carried out by using a specific pair of primers and a specific probe for *S.* Typhimurim. To evaluate the qPCR methodology, a standard curve was produced by a linear relationship between the CT (cycle threshold) and the bacterial count expressed in log cfu/ml of a known cell concentration. After 30 days of inoculation, PMA-qPCR was capable of detecting 2.45 ± 0.04 log cfu/ml, whereas in the conventional culture method the count was 3.95 ± 0.07 log cfu/ml. After 60, 120 and 180 days, no significant difference ($p>0.05$) between the methods was observed. For all time periods, qPCR without treatment with PMA quantified 5.0 log cfu/ml of total cells (viable and dead), corroborating with the initial inoculum count. PMA-qPCR assay underestimated the contamination of *Salmonella* on long-term storage of roasted peanuts. More studies are needed to optimize the use of this technique.

Keywords: Low water activity, Salmonella, PMA.

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P3.7

Application of metagenomics for food surveillance: From sequencing technique to bioinformatic analysis

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The detection of food and water contaminations with pathogenic microorganisms is a race against time. Therefore fast and reliable techniques and procedures are required to identify the source of infection. The usage of next-generation sequencing for metagenomic food samples is a powerful method for the food surveillance since it allows the detection of a broad range of pathogens without pre-cultivation in a single experiment within a couple of days. Nevertheless, sample handling, sequencing and data analysis is challenging and can introduce errors and biases into the analysis.
Two prevalent approaches for species identification in metagenomic samples are currently widely used. Whereas in shotgun metagenomics the whole nucleic acids in a sample are sequenced and analyzed, rRNA amplicon sequencing relies on differences between species and genera within only a single gene that is amplified and sequenced.
In order to evaluate both approaches in food metagenomics, we generated a mock community containing DNA of foodborne bacteria. With this strategy we were able to create a workflow for data generation and a well-adapted bioinformatic analysis pipeline that avoids the introduction of bias in the analysis of food and food-related samples. At the species level, the shotgun approach revealed a substantially higher recovery rate. Furthermore we validated our workflow in a controlled real-world scenario: Various animal food products were spiked with virus, bacteria and parasite DNA. From different matrices we sought an optimal protocol for DNA extraction and performed shotgun sequencing with deep coverage. Our data analysis pipeline revealed a high recovery rate of the spiked-in pathogens as well as a quantitative abundance estimation and sub-species characterization.

Keywords: metagenomics, shotgun, NGS, bioinformatics, microbial community analysis, food surveillance

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P3.8

Bacterial community succession and metabolite changes during sufu fermentation

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Sufu, a Chinese traditional fermented soybean food product, is obtained by solid-state fermentation of tofu followed by ripening in a dressing mixture. Dynamic of bacterial community and metabolites during sufu fermentation was investigated and their relationships were elucidated here with the aim to explore the functionality important bacteria for sufu industrial production.
The bacterial community was analyzed by both culture-dependent approach and high-throughput sequencing. Flavor compounds and non-volatile polar metabolites were identified and quantified by HS-SPME-GC-MS and 1H NMR, respectively. Furthermore, the correlation between metabolites and bacteria was analyzed. O2PLS model was constructed to determine the association between flavor compounds and bacterial genera.
The results showed that 36,527 high-quality sequences were obtained for each sample. Enterobacteriaceae, Enterobacter, Acinetobacter, Lactococcus were predominant taxa during sufu fermentation. 33 non-volatile metabolites and 72 flavor compounds were detected. Correlation analysis revealed that Enterobacter and Lactococcus strongly influenced the final characteristics of sufu. Unclassified genera of Enterobacteriaceae and Enterobacter were correlated to sugars, such as glucose and fructose, and most of the amino acids. Enterococcus was linked to eight amino acids. Lactococcus is one of the major flavor producers, especially for ester and acids. Pseudomonas was correlated with biogenic amines which were undesirable in sufu, such as histamine and cadaverine.
Except for the starter culture-Actinomucor elegans, bacteria also played important roles throughout sufu fermentation. The results could contribute to the prediction of metabolically active bacteria that have significant effects on sufu fermentation and facilitate the isolation and screening of indigenous strains for safe and high-quality sufu.

Keywords: Sufu, Bacterial community, Metabolite, Volatile compounds

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P3.9

Species classifier choice is a key consideration when analysing low complexity food microbiome data

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The use of shotgun metagenomics to analyse low complexity microbial communities in foods has the potential to be of considerable fundamental and applied value. However, there is currently no consensus with respect to choice of species classification tool, platform or sequencing depth. Here, we benchmarked the performances of three high-throughput short-read sequencing platforms, the Illumina MiSeq, NextSeq 500, and Ion Proton, for shotgun metagenomics of food microbiota. Briefly, we sequenced six kefir DNA samples and a mock community DNA sample, the latter constructed by evenly mixing genomic DNA from 13 food-related bacterial species. A variety of bioinformatics tools were used to analyse the data generated, and the effects of sequencing depth on these analyses was tested by randomly subsampling reads. Compositional analysis results were consistent between the platforms at divergent sequencing depths. However, we observed pronounced differences in the predictions from species classification tools. Indeed, PERMANOVA indicated that there was no significant differences between the compositional results generated by the different sequencers ($p=0.693$, $R^2=0.011$), but there was a significant difference between the results predicted by the species classifiers ($p=0.01$, $R^2=0.127$). The relative abundances predicted by the classifiers, apart from MetaPhlAn2, were apparently biased by reference genome sizes. Additionally, we observed varying false-positive rates among the classifiers. Strain-level analysis results were also similar across platforms. Each platform correctly identified the strains present in the mock community, but accuracy was improved slightly with greater sequencing depth. Notably, PanPhlAn detected the dominant strains in each kefir sample above 500,000 reads per sample. Again, the outputs from functional profiling analysis using SUPER-FOCUS were generally accordant between the platforms at different sequencing depths. Finally, and expectedly, metagenome assembly completeness was significantly lower on the MiSeq than either the NextSeq ($p=0.03$) or the Proton ($p=0.011$), and it improved with increased sequencing depth. Our results demonstrate a remarkable similarity in the results generated by the three sequencing platforms at different sequencing depths, and, in fact, the choice of bioinformatics methodology had a more evident impact on results than the choice of sequencer did.

Keywords: shotgun metagenomics, bioinformatics, high-throughput sequencing

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P3.10

Establishment and research of FT-IR as a new method for the identification and differentiation of microorganisms in food microbiology

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The monitoring of all steps in the food industry is essential today to produce safe products. If a specific microorganism is detected in different stages of the production process, it is important to know where it entered the factory. For manufacturers, it is hence of interest to know if a certain microorganism present in the raw material, for example, and a microorganism present in the final product are identical. Since food products are increasingly more complex in their composition nowadays, it is even more important to find the problem and fix it quickly in order to reduce or avoid high follow-up costs.
With Fourier-Transform Infrared (FT-IR) spectrometry it is possible to compare microorganisms from different production stages and to make a statement as to which of the strains found matches the strain in the final product. Therefore, the authors decided to establish this technology. The method must be validated with regard to its robustness, precision and accuracy. Different microorganisms from official as well as in-house strain collections are examined.
Within the framework of this project, the species *Bacillus subtilis* from different areas of production of a food producer was examined. Strains were isolated from the finished product, the intermediate and the raw material. A total of 17 isolates were divided into two groups for comparison. The strains were first identified by MALDI-TOF MS and classified according to their morphology. This was followed by further identification by FT-IR spectrometry because of the difficulties of MALDI-TOF MS to identify some taxonomic groups of Bacilli. Both methods were able to correctly identify all of the strains. After this confirmation, comparison was carried out by cluster analysis. To check the reproducibility of each strain on several independent days measurements were made. Thus, outlier or indistinguishable strains can be detected.
The results were convincing. Based on the cluster analyses, the authors were able to determine which strains occurred repeatedly in the production chain and which occurred as an isolated case.
In addition, the clearly distinguishable macroscopic strains could be clearly distinguished in the analysis. This is a solid basis for further experiments and a successful step into the analytics of FT-IR spectrometry.

Keywords: FT-IR, MALDI-TOF

State of the Art techniques for the analysis of biodiversity and microbial interactions of foodborne microbes

P3.12

Identification of coagulase negative staphylococci – Comparison of biochemical and mass spectrometry method

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The purpose of the study was the comparison of two methods of identification of microorganisms - biochemical method and mass spectrometry which is based on analysis of intracellular proteins profile characteristic for particular genus and species.
The identification of 150 staphylococci isolates from raw goat milk was performed by biochemical method (Vitek 2) and mass spectrometry (MALDI-TOF MS). Isolation of *Staphylococcus* spp. was performed using Baird-Parker agar with rabbit plasma fibrinogen (BP-RPF) (bioMerieux, France). The isolates were identified as coagulase negative staphylococci (CNS) on the basis of their colony morphology. *Staphylococcus* spp. strains were stored in a cryobank. Before identification by both methods *Staphylococcus* spp. isolates were cultured only on nutrient agar or were grown in brain heart infusion broth and then were cultured on nutrient agar.
Identification of CNS using VITEK 2 automated microbial identification system
The GP test card is used in the identification of Gram positive organisms, including Staphylococcus species. The VITEK 2 GP card is based on 43 biochemical tests measuring carbon source utilization, enzymatic activities, inhibition and resistance. The results were interpreted by the ID-GPC database, and the final results were obtained automatically.
Identification of CNS using MALDI-TOF MS
Samples were processed in a MALDI Autoflex Speed mass spectrometer (Bruker Daltonics, Bremen, Germany) with MALDI Biotyper software (Bruker Daltonics). To prepare the MALDI target plate a direct transfer method was used. Single colonies were smeared as a thin layer directly onto spots on a plate and covered with solution (HCCA). Afterwards, formic acid extraction method was used.
The percentage of isolates confirmed as *Staphylococcus* spp. using the biochemical method and mass spectrometry method was 63.3%.
The number of 69 (72.6%) of CNS strains were identified to species level with the same results by both methods, whereas 83 (87.4%) of them were accordingly identified to genus level. Higher efficiency of identification results of *Staphylococcus* spp. was found using the extraction method than the direct method of MALDI-TOF MS.
It was also found that the identification of *Staphylococcus* spp. was better by both methods if the isolates before testing were grown in brain heart infusion broth and then were cultured on nutrient agar.

Keywords: MALDI-TOF MS, VITEK 2, coagulase negative staphylococci

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P3.13

Evaluation of *gyrB* amplicon sequencing in comparison to 16S rDNA amplicon sequencing to evaluate intra-species bacterial diversity in food microbiota

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Advanced Next Generation Sequencing (NGS) methods greatly improved the sensitivity and efficiency of the microbial diversity evaluation. Notably, complex microbial community characterization has been transformed through the partial sequencing of the small subunit 16S ribosomal RNA encoding gene. However, there are several shortcomings with 16S-based taxonomic methodologies, particularly at the shallowest taxonomic levels. The extremely slow rate of evolution hinders the resolution of closely related bacteria individual 16S phylotypes. Particularly, the practice of clustering OTUs at 97% sequence identity will group together functionally diverse lineages, concealing significant amounts of species and strain level variation. To solve this problem, many studies have ventured beyond the analysis of 16S sequences by targeting coding regions with conserved primers or by extracting coding-gene orthologs from shotgun metagenomics surveys. Among them, *gyrB* which is a single-copied housekeeping gene encoding the subunit B protein of DNA gyrase, a type II DNA topoisomerase, could be a suitable molecular marker substitute to 16S rRNA. This gene is essential and ubiquitous throughout microorganisms and is sufficiently large in size for use in analysis of microbial communities. The main objective of this work was thus to validate the usefulness of *gyrB* as an alternative phylogenetic marker to accurately and precisely discriminate closely related species within various food microbiota. Understanding the complexity of microbial ecology specifically associated with food chains is of major concern for elucidating contamination routes, controlling microbial food spoilage, better predicting shelf life and improving food safety. We thus carried-out a comparison between 16S rDNA-based amplicon sequencing and *gyrB*-based amplicon sequencing on five types of meat and seafood products (pork sausage, poultry sausage, cod filet, salmon filet, and ground beef), packaged under modified atmosphere. These products were specifically chosen both because of their well-studied microbiota and because of the broad spectrum of bacterial species assemblage they cover between Firmicutes and Proteobacteria. In order to assess the added value brought by *gyrB* sequencing compared to 16S rDNA sequencing, 5 mock communities considered as quality controls were elaborated.

Keywords: Food metagenetic, 16S-amplicon sequencing, gyrB-amplicon sequencing, FROGS pipeline

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P3.14

A pandemic theoretical and practical review on metagenomics for basic research and applied industrial biotechnology

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Interacting microbial communities flourishing in a healthy matrix or surviving in a monopolistic environment, as red meat. Colorectal cancer is just one of the issues tacked in this collection of international papers, but I wan't to spoiler more with this digest and I let you undecode the following PCR-DGGE, HD-SPME-GS/MS, HTS Illumina sequencer, hence, awake of them, but not of the review.

Keywords: metagenomics, foodomics, MD, microbiome, atherosclerosis, CVD, cancer, starters, biotech, bioinfo

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Microbial diversity in dairy products consumed in the north-east of India

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Diet plays an important role in shaping the gut microbiome. Bovine milk and its products are widely consumed due to their therapeutic and nutritional values. Consumption of milk has been shown to increase the abundance of *Lactobacillaceae* in the gut of mice. In Assam, people mostly prefer consuming curd prepared from raw milk (RMC) over curd prepared from boiled milk (BMC). RMC is prepared directly from raw milk while BMC is formed only after addition of starter culture. In this study, we aimed to explore and compare the microbial diversity present in raw milk (RM), boiled milk (BM), RMC and BMC using culture dependent as well as culture independent methods. Metabolite differences between the dairy products were studied by using GC-MS/MS. A complex microbial community was observed in raw milk in comparison to boiled milk. The most abundant bacterial genus present in the dairy products belong to *Lactobacillus*, *Leuconostoc, Lactococcus*, *Acetobacter* and *Staphylococcus*. There was a higher dominance of *Lactobacillus* spp. in BMC compared to RMC. However, the diversity of *Lactobacillus* spp. was more in RMC compared to BMC. A significant difference was also observed in the metabolites among dairy samples. These microbes were further tested for potential probiotic properties. Microbial isolates present in raw milk have desirable probiotic traits which include the ability to survive bile juice, to tolerate gastric acid conditions and to adhere to the intestinal cells. The consortia of these bacterial isolates might have probable health implications.

Keywords: Diet, Probiotics, Dairy products, NGS, Metabolomics

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Genomics for the risk assessment of microbial cultures intentionally introduced in the food chain

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The risk assessment of microbial strains used in the food chain, traditionally based on phenotypic testing to assess virulence and antimicrobial susceptibility, has now been reshaped by whole genome sequencing (WGS). The effectiveness of WGS has been recognized by the European Food Safety Authority, which has just issued a new guidance for the characterization and the safety assessment of microorganisms intentionally introduced in the food, primarily based on WGS. Key aspects of this new guidance are the use of WGS for: species identification, unique recognition of the strain, safety assessment, by detecting genes coding for virulence factors and/or antimicrobial resistances (AMR) and the analysis of intended effects of genetic modifications in microbial GMOs. The WGS-induced revolution in the study of bacteria raises three main questions: (i) what we can really learn by interrogating the whole genome sequence? (ii) Can genome analyses substitute the phenotypic testing for safety assessment and physiological characterization? (iii) how can WGS-derived information modify our knowledge on a bacterial species?
To answer these questions, we have analyzed the genomic data of bacteria intentionally introduced in the food chain, with particular attention to species frequently used as food starters or probiotics: *Lactobacillus plantarum, L. rhamnosus, L. sakei, Staphylococcus xylosus and Bifidobacterium animalis*. The deposited genomes of these species were analyzed mainly using web-based tools: detection of AMR genes was performed using CARD, the Comprehensive Antibiotic Resistance Database and an in-house developed database which contains 7963 AMR genes. Genomic studies were made to identify mobile genetic elements eventually harboring AMR genes. Virulence was predicted using the tools of the Center for Genomic Epidemiology while pan genome and unique genes were examined using PanX and JGI-IMG. The advantage and limits of bacterial risk assessment based on WGS will be discussed.

Keywords: risk assessment, WGS, GMO, antimicrobial resistance

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Third generation sequencing of lactic acid bacteria

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Next generation sequencing has revolutionized microbial genomics in providing fast and inexpensive access to many genome sequences. The short read technology providing these possibilities does however in most cases not allow for assembly of the complete genome in one fragment but results in few to many contigs. The number and length of contigs is greatly dependent on the occurrence and distribution of repetitive sequences in the respective genomes. The sum of these contigs is in general called ”draft genome”. Some lactic acid bacteria are unfortunately characterized by an especially high number of repetitively inserted sequences resulting in high contig numbers. An additional challenge with draft genomes is the difficulty to unambiguously identify plasmid sequences. Plasmids are of special importance in lactic acid bacteria, as they often harbour industrially relevant traits and can also be associated with probiotic properties. In the end, only circularized genome sequences including the circularized plasmid complement give a full picture of the genetic potential of a bacterium. With Oxford Nanopore sequencing technology it is now possible to obtain long reads of up to several 100 kb in an increasingly cost-efficient manner. Using a combination of assembly of long read pools obtained with this third generation sequencing technology and short paired read pools from Illumina, we succesfully produced circularized genome sequences including plasmid complement. Results obtained with third generation sequencing and their significance in an industrial context will be presented.

Keywords: long read sequencing, plasmid, circularized genomes

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Microbiota and metabolomics analysis to evaluate the quality of cocoa beans fermentation

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Cocoa quality depends strictly from beans fermentation and is related to plant variety, agricultural practices and the post-harvesting processes. The aromatic compounds produced during fermentation depends on the activity of different microbial communities that colonize beans pulp or surface. The chemistry of cocoa beans fermentation is still to discover in deep since it's mostly an artisanal and not-standardised process driven by indigenous species of lactic acid bacteria, acetic bacteria and yeasts. A correlation between aroma and taste development in the final product and biochemical pathways during fermentation would be of help to drive the fermentation process itself. In this work, an efficient method to extract DNA from dried cocoa beans, a PCR-DGGE-analysis together with next-generation Illumina-based sequencing of 16S rRNA gene were used to study microbial populations in seven sub-varieties of Criollo dried cocoa beans; moreover, a metabolomics approach was applied to analyse volatile compounds. Two main genera were found to be dominant in all the samples: *Acetobacter* and *Lactobacillus,* with *Acetobacter nitrogenifigens, Gluconacetobacter diazotrophicus* and *L. fermentum* as the dominant species. Metabolomics analysis differentiates a total of 52 compounds, with flavonoids and phenolic acids being the more abundant. Cluster analysis was performed to correlate metabolomics results with bacterial composition in each sample in order to distinguish between good or bad fermentation. The combined use of NGS and metabolomics techniques could give useful information to evaluate the quality of cocoa beans after fermentation and to select the best variety for chocolate production.

Keywords: cocoa beans, fermentation, NGS, metabolomics, microbial communities

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Whole genome sequence of *Bacillus oleronius* confirm its closest phylogenetic neighbour

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The genus *Bacillus* are Gram-positive, rod shaped bacteria that are ubiquitous in nature. Their ability to produce endospores during harsh conditions make them significant to the clinical and food industries. In the food industry, *Bacillus oleronius* has been isolated from pasteurized milk, and related milk products. Bacillus oleronius is a non-motile endospore forming bacterium, originally isolated from the hindgut of the termite *Reticulitermes santonensis*, where it plays a symbiotic role in aiding digestion. Albeit staining Gram-negative, *B. oleronius* has Gram-positive cell wall components shared amongst all *Bacillus* species. Consequently, this means *B. oleronius* is closely related to other Bacillus species that contaminate food. Previous studies using fatty acid, quinone cell wall (polar lipid) analysis confirmed *Bacillus sporothermodurans* (a highly heat resistant spore-forming *Bacillus* species) as its closest relative. The present study, involving whole genome sequencing of *B. oleronius* was undertaken to enhance understanding of the target organism in relation to other *Bacillus* species of importance to the dairy industry. Genome sequencing was carried out on an Illumina MiSeq system. The assembly contains 587 contig sequences of longer than 500 bp, covers 5,083,966 bp with G + C content of 35.00%. The total number of 5,168 genes predicted by PGAP includes 4,899 protein coding genes, 130 pseudo genes, and 139 RNA genes. Phylogenetic analysis using the whole genome of *B. oleronius*, identified *B. sporothermodurans* as its closest relative, and the subsequently clustered outside both the *B. subtilis* and *B. cereus* groups. Whole genome information of related *Bacillus* species will enable the selection of alternative organisms to produce industrial enzymes and of the identification of candidate genes for genetic modification studies. Most important to this study is the identification of similarities between other bacterial food contaminants, with the aim of bacterial inactivation to enhance food safety and quality.

Keywords: Whole genome, Bacillus oleronius, Bacillus sporothermodurans, food safety

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Metaproteomic study of pozol

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Food fermentation is traditionally made in different regions, represent a valuable cultural tradition and play a decisive role in nutrition. They are produced in artisanal way, therefore the microorganisms inoculation occur during the process, resulting in a complex microbial diversity.

Despite limitations of the techniques used to study fermented foods, the physiological, morphological and metabolic characteristics of their microbial community is becoming increasingly clear. Tremendous efforts have been made to change the perspective from taxonomy to function and provide deeper comprehensions into the molecular mechanisms in fermented foods through omics approaches. Metaproteomic is powerful tool to study microbial ecosystem functions, providing fundamentally insights in the role of microbial diversity through the study of proteins.

In pozol, a traditional fermented beverage, culture-dependent and independent methods have allowed to determine that during pozol fermentation an abundant a diverse microbiota develops. Amylolytic lactic acid bacteria (ALAB) are an important group since the starch is the main carbohydrate for lactic fermentation. However, there are few isolated amylolytic bacteria from this spontaneous fermentation and those that have been studied result weakly amylolytic.

To elucidate how a low free sugar content might determine such microbial diversity, we decided to study the enzymes related to the degradation of polysaccharides through a metaproteomic approach.

Both bacterial and fungal proteins could be identified. Of the total identified bacteria proteins, 48% belong to the genus *Streptococcus*, widely described in this fermentation. The genus *Lactobacillus* has 7% representation, also proteins of *Leuconostoc* and *Enterococcus* were identified. In the case of fungi, the major proteins belong to the genera *Neurospora*, *Schizosaccharomyces*, *Saccharomyces* and *Aspergillus*.

Among the proteins identified, those that belonged to the dominant genera were categorized according to the COG nomenclature (Clusters of Orthologous Groups). In all cases the prevalent proteins are those related to the metabolic process, that are mostly represented by carbohydrate metabolism. The results clearly indicate that the microorganisms are metabolically active since the beginning of fermentation. Necessary condition for improve the nutritional quality and organoleptic characteristics in pozol. Currently, enzymes related to these processes are being identified.

Keywords: Pozol, metaproteomic, traditional fermented food

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P3.21

Metabolic change of hazelnuts by harming processes and quality control by 1H-NMR-spectroscopy

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The study of metabolic changes (metabolomics) is a rapidly growing field of research. Starting with the detection of potential disease markers in the 1970´s, applications reach from toxicological and / or pharmacological studies to the field of food analysis.[1-3] The metabolome is the last step in the so-called omics cascade where it reacts most sensitive to changes in genome, transcriptome, proteome or metabolome level of an organism. A major problem in food processing is the contamination or infestation with mold. Molds or their metabolites can lead to allergic reactions in the human organism. Furthermore, they can produce mycotoxins, which are toxic and carcinogenic. These mycotoxins are problematic because they are stable to heat and acids and they can remain stable even when processing the affected food. It is estimated that about 50% of all grains are contaminated with detectable mycotoxin concentrations.[4] Their detection in food usually applies LC/MS and is dependent on comparatively large sample amounts.

We used the metabolomics approach and associated advantages, e.g. small sample amount and high sample throughput, to detect significant changes in metabolite level due to mold growth. In this study hazelnuts were infected with eight different strains of fungi to observe changes in the metabolome over a period of two weeks. To visualize these changes, we developed a signal pattern plot showing alteration (trend) of individual signals over the observation time. The sign and intensity of alteration is color coded and allows simple interpretation of the signal pattern plot. Each species investigated generates an individual color pattern and therefore exhibits specific metabolic changes. Signals that change upon infection were compared a to reference sample. They are easily identified and allow the assignment of chemical/biological markers.

Keywords: 1H-NMR-spectroscopy

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P4.1

Inhibition of diverse *Alicyclobacillus acidoterrestris* by chlorhexidine disinfectant

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Alicyclobacillus acidoterrestris, a spoilage-causing bacterium that survives food pasteurization, contaminates fruit juice processing when it attaches to the surfaces of the fruits used as raw materials or production area. In this study, diverse *A. acidoterrestris* strains (DSM 3922^T, ULAG14 and ULAG15) were treated with different concentrations of chlorhexidine disinfectant. Chlorhexidine at 0.5% inhibited the cells and spores of the test strains on acidified yeast starch glucose (YSG) agar at 44 °C for 72 hours. However, it was observed that for five minutes contact time, a minimum concentration of 5% chlorhexidine was required to inhibit 10^6 cells and spores of the *A. acidoterrestris* strains under optimum growth conditions (aerobic incubation at 43 °C) in YSG broth (pH 3.7) for 144 hours. Lastly, scanning electron micrographs showed clear differences between chlorhexidine-treated and untreated *A. acidoterrestris* cells. These results show that chlorhexidine maybe potentially useful for inactivating *A. acidoterrestris* on fruits and production surfaces for improved quality control during the fruit juice processing.

Keywords: Alicyclobacillus acidoterrestris, fruit juices, spoilage, control

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P4.2

Inactivation kinetics of *Escherichia coli* in cranberry juice during multi stage treatment by electric fields

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The inactivation of *Escherichia Coli (E.coli)* inoculated in cranberry juice by processing with radio frequency electric fields (RFEF) was studied. *E. Coli* ATCC 35218 was chosen among three non-pathogenic strains based on its ability to survive in low pH cranberry juice. Studies were conducted by measuring the survival population when changing the electric field strength between 2.2 and 13.2 kV cm^{-1}, number of treatment stages from 1 to 6 and flow rates between 13 and 25 L h^{-1} at moderate temperatures of 20, 30 and 40 °C. A minimum inactivation of 5-log reduction, as requested by the Food and Drugs Administration (FDA), can be achieved by increasing the number of treatment stages, temperature or both. At 40 °C and 6 treatment stages, 6.57±0.02 log CFU ml^{-1} reduction in the initial population of *E.coli* (ATCC 35218) was obtained. At a constant electric field, increasing the temperature produced higher microbial inactivation, consuming lower radio frequency energy input, than increasing the number of treatment stages. Furthermore, a primary model that accounts for the combined effect of time and electric field is proposed. The model represented the sigmoidal curve composed of shoulder, log-linear and tailing sections as observed when changing electric fields. A secondary model that accounts for the effect of temperature and flow rate on the primary model constants is also proposed. The combined primary and secondary models were found to fit the data well with a high coefficient of determination (R2=0.965) . The proposed model can be extended to kinetic models for pulsed electric fields.

Keywords: Predictive microbiology; radio frequency electric fields; pulsed electric fields; E.coli, Steinmetz

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P4.4

Effects of *Lactobacillus acidophilus* (LA-03) metabolism on phenolic acids and flavonoids in acai- and mango-based smoothies

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Probiotic lactobacilli have been added to fruit beverages with the purpose of improving health promoting properties. Phenolic compounds (PC) are abundant in fruit such as mango and açaí and can affect positively the growth and metabolism of lactobacilli. Through decarboxylation, dehydroxylation or cleavage probiotic lactobacilli transform PC into more bio-active and/or available forms. This study assessed the effects of metabolism of *Lactobacillus acidophilus* (LA-03) on phenolic acids and flavonoids in açaí and mango-based smoothies. The açaí based-smoothie (AS) was prepared in a proportion 80:20 (w/w) of açaí and banana, respectively, while the mango based-smoothie (MS) was formulated in a proportion of 60:40 (w/w) with mango and passion fruit, *respectively. Both,* AS and MS were *pasteurized at 80 ºC for 10 minutes, cooled to room temperature and added of the inoculum of L. acidophilus (final viable counts of* 9 log CFU/g). Smoothies of each fruit without *L. acidophilus* were assayed as controls. PC *were determined just after the manufacture and after 24 h of fermentation (4ºC)* using a chromatograph coupled with a diode arrangement detector and a refractive index detector. RP-C18 column (100×4.6 mm, 3.5µm) and a C18 pre-column (12.6×4.6 mm, 5µm) were used to separate the PC using water and methanol acidified as mobile phases at a flow rate of 0.8 mL/min. The peaks were identified by comparing the retention times obtained with those of standards and the average area was used for PC quantification. A total of 16 PC (4 phenolic acids and 12 flavonoids) and 10 PC (1 phenolic acid and 9 flavonoids) were identified in AS and MS, respectively, after the manufacture. In AS, *L. acidophilus* increased ($p < 0.05$) the contents of the flavonoids *procyanidin B1 and B2,* catechin, and decreased ($p < 0.05$) the contents of *p*-coumaric acid, as well as *of the* flavoids quercitin 3-glucoside, malvidin 3-glucoside, epicatechin galate and cyanidin 3-glucoside*. In MS, L. acidophilus increased* ($p < 0.05$) *the contents of the flavonoids h*esperidin, catechin, cis-resveratrol and procyanidins A2, B1 and B2, and decreased ($p < 0.05$) the contents of gallic acid. These results show that *L. acidophilus* LA-03 is capable of changing the PC contents in fruit-smoothies and could be a strategy to produce high-added value products from PC-rich fruit.

Keywords: probiotics, Lactoabacillus, functional beverages, phenolics

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P4.5

The aerobic microbial population and Pseudomonas spp. on meat marinated with cinnamon *(Cinnamomum zeylanicum)* extract under isothermal storage temperature

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Meat spoilage caused by temperature abused is a major problem for the producer, retailers, and consumers that can generate substantial economic losses. Pseudomonas spp. is one of the specific spoilage organisms responsible for meat spoilage. The natural preservative use as meat marination has the potential to delay the spoilage. Therefore, this study aimed to evaluate the antimicrobial properties of the cinnamon extract against Pseudomonas aeruginosa and to determine the effect of cinnamon extract marination on meat at various isothermal storage temperatures. The antimicrobial analysis consists of disc diffusion, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and time-kill analysis were performed to reveal the antimicrobial potential of the cinnamon extract against Pseudomonas aeruginosa ATCC 27853. The microbiological and physico-chemical analysis was carried out for marinated meat stored at 25°C for 48 h, 15°C for 72 h, 10°C for 96 h and 5°C for 168 h. The antimicrobial results of cinnamon extract showed a zone of inhibition at 100% concentration (0.0523 ppm) is 13.50 mm with MIC of 25% and MBC of 50%. The time-kill showed total inhibition at 6 hours, and Pseudomonas aeruginosa ATCC 27853 decreased from 8 to 5 log CFU/g at 1 X MIC concentration. For meat marinated with cinnamon extract at various isothermal storage temperature (25, 15, 10 and 5°C), a significant ($p< 0.05$) growth increase is observed at higher temperature and short storage time for aerobic microbial population at 25°C (5.63 to 9.23 log10 CFU g-1) after 48 h; 15°C (5.63 to 8.56 log10 CFU g-1) after 72 h; 10°C (5.63 to 7.95 log10 CFU g-1) after 96 h; and 5°C (5.63 to 8.76 log10 CFU g-1) after 168 h and Pseudomonas spp. count at 25°C (4.66 to 9.00 log10 CFU g-1) after 48 h; 15°C (4.66 to 7.99 log10 CFU g-1) after 72 h; 10°C (4.66 to 7.76 log10 CFU g-1) after 96 h; and 5°C (4.66 to 8.41 log10 CFU g-1) after 168 h. The pH of meat at 25 °C ranging from pH 5.74-6.48; 15 °C pH 5.90-6.34; 10 °C pH 5.95-6.20 and 5°C pH 5.86-6.35, showed no significant difference ($p>0.05$) observed in the meat samples stored at 25, 15 and 10 °C while at 5 °C showed significant difference ($p< 0.05$). The water activity of marinated meat is ranging from 0.95-0.99 in all temperature. The applications of cinnamon extract as the marination agent for meat slower the growth of aerobic microbial and Pseudomonas spp. Therefore could assist in extending the shelf life of meat.

Keywords: Pseudomonas, cinnamon extract, meat, isothermal temperature

Impact of interventions during food production on microbial biodiversity

P4.6

Fermented milks containing propionibacteria-: Physicochemical, rheological, microbiological and sensory properties

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The aim of the study was to investigate using *Propionibacterium freudenreichii* for the production of a probiotic dairy drink. *Propionibacterium freudenreichii* which belongs to the Propionibacteriaceae family is usually mesophilic (optimum growth temperatures are between 25 - 37°C), G(+) and immobilized. *Propionibacterium freudenreichii* increases the stability of foods by producing antimicrobial compounds including acetic acid, bacteriocin and propionic acid from sugars. *Propionibacterium ssp.* have a role as both a support culture and a protective culture in dairy industry, and it is mostly used in cheese production. Although *Propionibacterium* ssp. is used for many purposes including biocontrol, culture and support culture, in probiotic dairy drinks which contain *Propionibacterium freudenreichii* was evaluated in terms of physicochemical, rheological, microbiological and sensory properties in this research. The samples in our study were as follows, AP: dairy drink produced using *Lactobacillus acidophilus* and *Propionibacterium freudenreichii ,* BD: dairy drink produced using *Bifidobacterium animalis* subsp. *lactis* and *Propionibacterium freudenreichii*, CD: dairy drink produced using *Lactobacillus casei* and *Propionibacterium freudenreichii*, and RD: dairy drink produced using *Lactobacillus rhamnosus* and *Propionibacterium freudenreichii*. Standardized UHT cow milk (% 3 fat and % 8.5 non-fat dry matter) was used in production and starter cultures were inoculated into the milk. When the pH value reached 4.7-4.8, fermentation was ended. The probiotic dairy drinks were stored at 4°C for 21 days. Physicochemical, rheological, microbiological and sensorial analyzes were carried out on the 1st, 7th, 14th and the 21th day of the storage period. *Propionibacterium freudenreichii is* important due to its many beneficial effects and fermentative feature. Nowadays, *Propionibacterium freudenreichii* preferred for cheese production is also suitable for the production of probiotic drinks. The results of the study showed that there were no adverse effects on the product characteristics which was produced using starter cultures combined with *Propionibacterium freudenreichii.*

Keywords: probiotic dairy products, fermented milks, Propionibacterium freudenreichi

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P4.7

Improvement of the production of artisan game meat sausages: The power of indigenous lactic acid bacteria as starter cultures

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During the last years, the application of starter or bioprotective cultures in food production has gained increasing relevance regarding the safety and standardization of the product. This trend has also provoked the need for having new starters containing powerful microbial strains with defined properties. Against this background, this study exemplarily addresses the development procedure of starter cultures for standardization of game meat sausages production, covering the search for suitable strains, assessing their safety and technological characterization. In details, starting with 1326 presumptive lactic acid bacterial isolates of game meat sausages origin, a step-by-step selection of candidate strains meeting special criteria was thoroughly examined, finally leading to the designed starter cultures consisting of three individual LAB strains (two *Lb. sakei* and *Le. mesenteroides*). Those strains were encapsulated using alginate-starch mixture and applied in encapsulated and non-encapsulated form in game meat dough. In total, 8 batches were prepared. Viable cell count of beneficial, pathogenic and spoilage microbiota, survival rate of applied starter cultures (rep-PCR and DGGE), pH and a_w were analyzed during the fermentation and ripening of sausages. Final products were tested for aroma compounds, biogenic amines and sensory properties. Generally, although all starters survived throughout the whole production process it seems that two native *Lb. sakei* genotypes were the most effective in suppressing the growth of pathogenic and spoilage microbiota and in reducing the content of biogenic amines. Such results corresponding to the strong pH decline observed in respective batch during the first week; ensuring unfavorable environment for undesirable microbiota and reducing the ability of biogenic amine formation. Additionally, frequency distribution results assigned to the expressed likeability of traits (hedonic test using 80 untrained consumers) was the highest for two native *Lb. sakei* while the control batch without starter culture had the lowest score. Those results correspond to the analysis of volatile compounds where little or no differences between batches were established. As such the typical sensory quality of traditional sausages has been preserved or even improved by the indigenous *Lb. sakei* application. The clear and positive effect of encapsulation on sausages safety and quality was not noticed.

Keywords: game meat sausages, lactic acid bacteria, starter cultures, Lb. sakei, Le. mesenteroides

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P4.8

Selection of antagonist yeasts isolated from *Ficus carica* L. as biocontrol agent

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In recent years, the consumption of horticultural products is increasing due to consumers are more aware of the link between diet and health. Furthermore, food safety is major global public health issues being necessary ensure food products free of pathogens, toxins and phytosanitary residues. However, fruit and vegetables are highly perishable products. Nowadays, the losses of horticultural products are quite high. The main cause of postharvest losses is infection caused by different species of fungal pathogens. Synthetic fungicides are widely applied to control postharvest deterioration of fruit, however, their application is being limited due to their negative impact in environment and human health, and the appearance of fungal resistance. It is because of that biological control is one of the most promising alternatives to synthetic fungicides. Then, the aim of the present work was to assess the *in-vitro* antagonistic capacity of yeasts isolates against two phytopathogenic molds: *Monillia laxa* and *Botrytis cinerea*. A total of 41 yeasts isolated from different cultivars of *Ficus carica* L. were identified at species level by sequencing of the ITS1/ITS2-5.8S rRNA region. Antagonist ability of the yeast isolates were evaluated against *Monillia laxa* and *Botrytis cinerea* by two different antifungal assays. Firstly, it was measured the ability to produce volatile organic compounds through a double Petri dish test and secondly, the capacity to parasite hyphae through a direct confrontation of yeast- molds on PDA agar plates. Among the 41 yeast isolates, thirteen different species were identified, although *Aureobasidium pullulans* was clearly the dominant species, comprising the 60% of the total of yeasts. The antagonist assays showed that two isolates of *Hanseniaspora uvarum*, 789L and 793L, decreased significantly the fungal growth of two molds tested. However, the second test does not show relevant results regarding parasitism of hyphae. In conclusion, although more *in-vivo* studies are necessary, these two *H. uvarum* yeasts are a promising alternative in the control of postharvest disease as antagonistic microorganisms.

Keywords: antagonist yeasts, biocontrol, horticultural products

Impact of interventions during food production on microbial biodiversity

P4.9

Assessment of the good manufacturing practice in mass catering by the use of indicator organisms

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Many cases of food poisoning are caused by contaminated food produced in mass catering and poor manufacturing practices can lead to contamination of food
Indicator organisms are most often used to assess both, food safety and good manufacturing practice (GMP).
The aim of this work is to evaluate the microbiological quality of surfaces in direct or indirect contact with foods to evaluate good manufacturing practices in mass catering. Total Viable Count (TVC) and Coliforms (C) were used as indicators to evaluate the disinfection and cleaning protocols .
A total of 55 samples were collected by swabbing surfaces which are in direct or indirect contact with food. The surface samples was carried out according to the SO 18593: 2004 method recommendations
All samples were analyzed using a conventional cultivation method NF EN ISO 4833 (2003) and NF08-060 (2009). The satisfaction 's thresholds have been set at 1 X 10^2 CFU/cm^2 to TCV and at undetectable /cm^2) to coliforms.
Out of the 55 samples, 11 (20%) showed a satisfying bacterial quality (TVC < 1 X 10^2 CFU/cm^2 and C undetectable /cm^2). 44 (80%) samples showed a unsatisfying bacterial quality (either the CTV exceeded the thresholds or the coliforms or both
This study revealed that Good Manufacturing Practice (GMP) are poorly implemented in this mass catering, particularly disinfection and cleaning protocols which are not efficient.

Keywords: Indicator organism; TVC; Coliforms ; GMP ; Mass catering

Impact of interventions during food production on microbial biodiversity

P4.10

Evolution of the superficial contamination of broiler carcasses in refrigerated state

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The aim of this work is the study of the superficial contamination of fresh and refrigerated state (+4°C) broiler carcasses.
Our sampling consists of 100 samples of chicken necks with their skin. Fifty samples were collected before the cooling off phase, just after evisceration and another 50 sampling were collected just after this step, in the packing workshop.
At the first day (J1), each sample is subjected to a bacteriological analysis which concerns the enumeration of total mesophilic aerobic flora (TAMF) at 30 °C (ISO 4833) and enumeration of *Pseudomonas* spp. (NF V04-504 modified), then a measurement of the pH of the meat. The reading and interpretation of the results of the counts are carried out according to the French Standard XP V08-102
All the samples were kept at +4°C, in order to repeat the same analyzes on the 7th day (J7) following the slaughter. The statistical test used is the chi-square comparison test (χ 2) with a risk α of 5%. The difference is considered significant if the probability (p) is less than or equal to α risk ($p \leq 0.05$).
At J1, before cooling, the maximum number of TAMF colonies is 2.96 x 10^7 CFU/g whereas after cooling, it was 1.29 x 10^7 CFU/g (NS: P > 0,05).
At J7, before cooling, the maximum number of TAMF colonies is 2.40 x 10^9 CFU/g whereas after cooling, it was 5.90 x 10^8 CFU/g (S: (P < 0,05).
Results obtained after cooling show a range meat pH between 6.35 and 7.82. The results of the TAMF enumeration are between 1.28 10^7 UFC/g and 5.90 10^8 UFC/g. The values of the enumeration of Pseudomonas spp. are between 1.11 10^6 UFC/g à 1.44 10^7 UFC/g.
The number of Pseudomonas spp. has increased between the first and seventh sampling days, and the difference between these values is statistically significant (P < 0.05). Since this germ is psychrotrophic, low temperatures contribute significantly to its proliferation.

Keywords: Poultry carcasses, superficial contamination, refrigeration, TAMF, Pseudomonas spp.

Impact of interventions during food production on microbial biodiversity

P4.11

Isolation and characterisation of bacteriocins from *Escherichia coli* isolates

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Introduction: Increasing bacterial resistance rates against antibiotics and decontaminants lead to an enhanced search for antimicrobial alternatives. Bacteriocins are antimicrobial proteins produced by bacteria to inhibit the growth of related strains. Bacteriocin production, the presence of bacteriocin genes and the phylogroup of 122 *E. coli* isolates was analysed. The colicins of two bacteriocin producing strains were examined individually on their efficiency to reduce *E. coli* cell numbers.

Methods: Overnight cultures and extracts of 80 *E. coli* isolates from food and 42 from poultry samples were examined by spot assay for the lysis of six indicator strains. The presence of 29 bacteriocin genes and the phylogroup were determined by PCR. The colicins of two bacteriocin producing strains were concentrated by tangential flow filtration, precipitation and dialysis. To determine cell number reductions, the overnight culture of *E. coli* DH5α was added to the concentrates of the two bacteriocin producing isolates and stored at 4°C. After certain time points, the CFU were analysed.

Results: In a spot assay, the overnight culture of 53 % of the food and 86 % of the poultry isolates lysed at least one of six indicator strains. Bacteriocin genes were detected in 87 % and 75 % of the food and poultry isolates, respectively. In food isolates, the predominant bacteriocin genes were E7, Ia, B, V and E8 and in poultry U, B17 and Y. Bacteriocin producing food isolates belonged mostly to phylogroup A, B1 and B2 and poultry isolates to F, A and B1. Initial experiments showed a reduction of *E. coli* DH5α cell numbers below detection limit after one hour at 4°C.

Discussion: We have found a similar number of bacteriocinogenic strains as described previously in literature. The detected bacteriocin genes could not be associated with a specific phylogroup. Use of bacteriocins for the reduction of the *E. coli* cell number seems very suitable at 4°C. In following experiments, the efficiency of the bacteriocin concentrates to reduce *E. coli* cell numbers will be determined in meat and on surfaces.

Keywords: bacteriocins, colicins, Escherichia coli

Impact of interventions during food production on microbial biodiversity

P4.12

Isolation and application of broad-host range bacteriophages to control *Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Escherichia coli* O157:H7 simultaneously in *in vitro* and food matrices

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Salmonella Enteritidis, *Salmonella* Typhimurium and *Escherichia coli* O157:H7 are the most important foodborne pathogens causing serious food poisoning outbreaks worldwide. Bacteriophages are increasingly considered as novel antibacterial agents to control foodborne pathogens. In this study, 18 phages specific to *Salmonella* or/and *E. coli* O157:H7 were isolated from raw chicken samples. Phage PS5 capable of infecting *S.* Enteritidis, *S.* Typhimurium and *E. coli* O157:H7 was characterized and its efficacy in reducing these three foodborne pathogens was evaluated at 4˚C and 24˚C in both *in vitro* and food matrix. One-step growth analysis of phage PS5 in *S.* Enteritidis, *S.* Typhimurium and *E. coli* O157:H7 host showed relatively short latent periods and large burst sizes. Stability test indicated that phage PS5 was stable under various stress conditions (temperature: 40-60˚C, pH: 4-10, NaCl: 2-11%). *In vitro*, phage PS5 significantly decreased the viable counts of *S.* Enteritidis, *S.* Typhimurium by at least 1.3 log CFU/ml compared to untreated control after 2 h of incubation at 4˚C and 24˚C, while it reduced *E. coli* O157:H7 viable counts to lower than detectable level. In food matrix (chicken skin, beef, lettuce, pasteurized milk, liquid egg), phage PS5 also significantly reduced viable counts of *S.* Enteritidis, *S.* Typhimurium and *E. coli* O157:H7 compared to phage free control at both 4˚C and 24˚C. The greatest efficacy of phage was observed in the treatments against *E. coli* O157:H7, followed by *S.* Typhimurium and *S.* Enteritidis. In the experiment using the same bacterial host, the efficacy of phage varied between different foods. This is the first report of single bacteriophage capable of controlling *S.* Enteritidis, *S.* Typhimurium and *E. coli* O157:H7 simultaneously in both *in vitro* and food matrix.

Keywords: Salmonella; E. coli O157:H7; Bacteriophages; Food safety

Impact of interventions during food production on microbial biodiversity

P4.13

Development of interspecific interaction models between *Listeria monocytogenes* and lactic acid bacteria in cooked meat products

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Microbial predictive models in meat products can effectively monitor the contamination of microorganisms in products, so as to ensure meat safety. Thus it is very important to consider the microbial ecosystems of meat products during the construction of the growth kinetics models of foodborne pathogens. Therefore, this study was to construct microbial interspecific interaction models of *Listeria monocytogenes* and lactic acid bacteria in cooked ham, which was then validated and evaluated, so as to provide theoretical foundations for the use of microbial predictive technology to control *L.monocytogenes* in meat products. Results have shown that there was the Jameson effect between lactic acid bacteria and *L.monocytogenes* in 4°C stored cooked hams. After many trials using different microbial interaction models (Lotka-Volterra model and different types of Jameson-effect models), the fitness and prediction accuracy of a modified Jameson-effect model based on f(t)=1- (Na(t)+Nb(t))/Nmax was best, and was suitable for the analysis of the growth kinetics of *L.monocytogenes* and lactic acid bacteria in 4°C stored cooked ham, where the Jameson effect occurred. At 8 °C, 12 °C, 16 °C and 20 °C of storage, there were no effects between the growth of *L.monocytogenes* and lactic acid bacteria, and so the Baranyi model was used to fit the growth of these two bacteria. Therefore, the selection of predictive models was based on the growth characteristics of target bacteria in meat products when the background bacteria were present.

Keywords: predictive models; microbial interaction; cooked ham; Listeria monocytogenes; lactic acid bacteria

Impact of interventions during food production on microbial biodiversity

P4.14

Fine-tuning of predictive microbiology models through microlocal food characterization by nuclear magnetic resonance (NMR)

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Numerous mathematical models have been proposed to describe bacterial population behaviors in foods. These models generally depict the growth kinetics of particular bacterial strains based on their cardinal values. Recently, these traditional / macro-environmental approaches have been complemented for a food-borne pathogen *Listeria monocytogenes* by an individual-based modelling (IBM) focusing only on a few cells and their surrounding microenvironment. It takes into account the single-cell growth probability according to key physicochemical parameters of the food matrix and its storage temperature[1].
In this context, there is an increasingly prominent issue to accurately characterize the physicochemical properties of foodstuffs. While robust methods are usually used to individually investigate pH, a_w, as well as NaCl, organic acid and total phenol concentrations, one sample per analysis and per parameter is required. Besides for pH and a_w, there are few devices for microlocal scale analysis[2].
The present work describes an NMR-based multiparametric approach to characterize the microenvironment of foods. It shows how NMR can simultaneously measure the physicochemical parameters of interest for predictive microbiology purposes using a single 10-mg sample. The approach was designed and validated on four food matrices: a smear soft cheese, cooked peeled shrimps, smoked salmon and smoked ham. This proof of concept and application opens new doors for the improvement and fine-tuning of predictive microbiology models through systematic spatial characterization of foodstuffs at microlocal scale.

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[2]Ferrier, R., Hezard, B., Lintz, A., Stahl, V. and Augustin, J. (2013). Combining individual-based modeling and food microenvironment descriptions to predict the growth of *Listeria monocytogenes* on smear soft cheese. *Applied and Environmental Microbiology*, 79(19), pp.5870-5881.

Keywords: Predictive microbiology, individual-based modelling, microenvironment, multiparametric analysis, NMR

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P4.15

Estimation of growth parameters and *in vivo* validations of pear postharvest fungal isolates at different temperatures

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The effect of temperature on the growth kinetics of *Penicillium expansum*, *Alternaria alternata*, *Botrytis cinerea* and *Rhizopus stolonifer* was assessed. Cardinal model with inflection (CMI) was used to describe the effect of the temperature on the growth rate (μ) and the lag time (λ) of each isolate. Values of T_{min}, T_{max} and T_{opt} were estimated and isolates were sorted according to their aggressiveness in terms of growth rate. Additionally, model validation was performed on a medium prepared from fresh pear pulp and on artificially wound-inoculated pear fruits. Results showed that *R. stolonifer* was the most aggressive fungus since it had the highest μ_{opt}= 1.22±0.02 [mm x h^{-1}], while *P. expansum* could be the most psychrophilic fungus since it had lowest estimated T_{min}. Model validation on pear pulp agar showed growth rate over-prediction in the case of *R. stolonifer* and *B. cinerea* but a good correlation in the case of *P. expansum* and *A. alternata*. *In vivo* experiments on pear fruits showed discrepancies from the synthetic and the simulated counterparts for all the fungi with the only exception of *P. expansum*. It can be concluded that nutrient substrate has big impact on fungal growth rate and this must always be considered in predictive mycology.

Keywords: fungi, predictive mycology, parameter estimation, pears, post-harvest

Impact of interventions during food production on microbial biodiversity

P4.16

Using spectral signatures to identify bacterial species

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Methods currently applied to detect and identify bacterial species tend to be slow and/or expensive. The most commonly used methods for bacterial identification are microscopy, biochemical testing, selective and differential growth media, mass spectrometry, antigen testing, nucleic acid amplification tests, and DNA sequencing. Some of these are capable of returning results quickly, while others are low cost but identification methods that are both rapid and affordable remain scarce. Rapid identification of microorganisms in a sample is essential to provide important information for downstream decision making but the cost of these detection methods prevents their widespread use.

SYTO9 is a commercially available fluorescent nucleic acid stain commonly used to detect "live" in the LIVE/DEAD BacLight Bacterial Viability Kit, but is also capable of staining "dead" cells. Upon binding to nucleic acids SYTO9 has a large fluorescent signal with an excitation/emission of 485/498 nm when bound to DNA and 486/501 nm for RNA [1]. SYTO9 fluorescence can be detected using any laser excitation or broadband illumination fluorescence-based instrument with an appropriate spectral range that overlaps with SYTO9 absorbance [1]. The 'optrode' is a time resolved fibre-optic device capable of measuring fluorescence from SYTO9 stained cells through the use of a 473 nm laser coupled to a spectrometer. We hypothesis that SYTO9 stained bacteria emit a species specific spectrum that can be identified using the optrode for near real time speciation.

Using six different bacterial organisms; *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Staphylococcus aureus* at different growth phases; lag, log, and stationary, we measured the emission spectrum from the SYTO9 stained cells using the optrode. A machine learning algorithm was applied to this spectral dataset to differentiate bacterial species on the basis of respective SYTO9 emission spectra. Our results demonstrate an ability to do so. We are planning on building a database of spectra collected from a wider range of bacterial species and bacterial endospores to further explore the use of SYTO9 emission spectrum to identify bacterial species of interest.

References: [1] Life Technologies, "User Guide: SYTO Green-Fluorescent Nucleic Acid Stains," (Thermo Fisher Scientific, 2014). https://www.thermofisher.com/order/catalog/product/S34854 Retrieved: 31/08/2017

Keywords: Bacterial detection, Bacterial identification, Fluorescent staining, Optics, Bacterial spectra

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P4.17

Inactivation of *Salmonella* Thompson, *Salmonella* Senftenberg and *Escherichia coli* P1 during air drying of onions

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Dried onions are commonly used in the food industry as ingredients for culinary products. Most of these vegetables undergo only mild thermal treatments, such as air drying and there are gaps to be filled in the industry to better understand the level of food safety achieved towards vegetative pathogenic bacteria by this process.

The objective of this study was to understand the level of inactivation reached during the first steps of dehydration during air drying, on bacterial pathogens *S.* Thompson, *S.* Senftenberg and the surrogate *E. coli* P1 (ATCC BAA 1427), commonly used for validation studies. To achieve this, inactivation data were produced at two water activity (a_w) values: 0.99 and 0.95. These data sets will allow better assessment of risks and better management of the microbial safety during the drying of onions.

Fresh onions (a_w=0.99) were sliced and divided into two portions. One was wet inoculated using fresh cultures of *S.* Thompson, *S.* Senftenberg and *E. coli* P1. The other was partially dehydrated using a bench scale ventilated oven (Heratherm OMH, Thermo Scientific) to reduce the a_w to 0.95. After a moisture equilibration time of 24 hours, onions were wet inoculated using the same microorganisms as for the inoculation of the fresh onions. Both portions of inoculated onions were left to dry for 30 minutes to allow microorganisms to attach to the surface. After this time, both fresh onions and onions at a_w of 0.95 were placed into plastic bags, vacuum sealed and heat treated in a water bath in static conditions at a constant product temperature of 60°C for different times. Afterwards, samples were enumerated for survivors.

On fresh onions, >4 $\log_{10}$ CFU/g reductions were achieved after 10 minutes for *S.* Thompson and *S.* Senftenberg and 15 minutes for *E. coli* P1. On onions at reduced a_w (0.95), >4 $\log_{10}$ CFU/g reductions were achieved after 15 minutes for *S.* Thompson and *S.* Senftenberg and after 35 minutes for *E. coli* P1.

Air drying of onions at 60°C for the times applied showed to be an effective control measure towards *S.* Thompson and *S.* Senftenberg. *E. coli* P1 can be considered a suitable surrogate for the validation of air drying of onion because it showed higher heat resistance than pathogens and a very similar inactivation kinetic. Additional inactivation data from a dynamic air drying process are needed to validate the present results.

Keywords: Inactivation, process validation, surrogate, air drying

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P4.18

How decontamination of seeds for sprout production by cold atmospheric plasma and low-energy electron beam treatment impact germination and seedling quality

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The consumption of fresh fruit and vegetable products has dramatically increased during the past few decades and contributes substantially to consumers' health due to the nutritional values and health benefits of fresh produce. However, inherent to all minimally processed products is the short shelf life, and the risk of foodborne diseases, which have been increasingly related to such products in many parts of the world. In particular, sprouts were frequently identified as a source for pathogenic bacteria, because of the potential for pathogen growth during the sprouting process. The current lack of a consistent and effective but sustainable seed disinfection technology demonstrates the urgent need for research on relevant prevention and intervention technologies in the production process of sprouts, which guarantee food safety and prolonged shelf life of the product. Cold atmospheric pressure plasma and low-energy electron beam treatment have the potential to address the current lack of suitable methods for decontamination of seeds. In order to investigate the decontamination efficiency and the impact of these treatment types on seed germination and seedling growth, artificially inoculated lentils were treated in a diffuse coplanar surface barrier discharge or an electron beam device. The microbial inactivation mechanisms on the bacteria were further characterized by determination of inactivation kinetics and confocal laser scanning microscopy analysis of treated bacteria. Furthermore, the influence of treatment on the germination of seeds was investigated by analysis of seed topology, seed wettability and other seed quality attributes. Cold atmospheric pressure plasma yielded higher reduction rates than low-energy electron beam treatment and maintained the normal germination capacity and morphology of seedlings. In contrast, electron beam treatment resulted in a high percentage of abnormal seedlings. In conclusion, cold atmospheric pressure plasma and low-energy electron beam treatment resulted in 3 to 5 log reduction of microorganisms on the seed surface, and an acceleration of seed germination, at least after short treatment times. The addressed technologies revealed promising potential for decontamination of sprout seeds while maintaining or even improving the germination capacity of the seeds. However, further research will be required to investigate whether these technologies may be considered as future routine applications in the food industry.

Keywords: seeds, sprouts, germination, cold atmospheric pressure plasma, low-energy electron beam treatment

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P4.19

Antilisterial activity of lactic acid bacteria in a Brazilian fresh cheese model

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Lactic acid bacteria (LAB) produce antimicrobial substances with potential to be used as natural food preservatives, to inhibit spoilage and pathogenic microorganisms in dairy products. *Lactococcus lactis* QMF 11 was isolated from fresh Minas cheese (FMC), a very popular dairy product in Brazil, and previous studies indicated that this strain produces bacteriocin like inhibitory substances (bac$^+$). Thus, in this study, the bioprotective activity of *L. lactis* QMF 11 (bac$^+$) against *Listeria monocytogenes* was evaluated in a FMC model developed using commercially pasteurized bovine milk. *Lactobacillus sakei* ATCC 15521 was used as negative control for bacteriocin production (bac$^-$). FMC samples were co-inoculated with different combinations of *L. lactis* QMF 11, *L. sakei* ATCC 15521 and *L. monocytogenes* ATCC 7644 and stored at 8° C for up to 26 days. A sample of FMC without inoculum was used as control. Bacterial populations were enumerated at different times on selective suitable media. At the end of the experiment, an increase of 4.0 log CFU/g was observed for the pathogen in monoculture, compared to an increase of 2.0 log CFU/g and 1.6 log CFU/g in the presence of the LAB bac$^+$ and bac$^-$, respectively. The pH variations in the FMC were similar in all evaluated conditions during the studied period. Using a bac$^+$ strain of LAB did not offer an additional barrier to listerial growth in the studied FMC model. As both LAB interfered with the pathogen growth, it is possible that inhibition was due LAB´s competitive advantage in colonization of the product.

Keywords: dairy products, Lactic acid bacteria, bacteriocins, Listeria monocytogenes

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P4.20

Expanded cardinal parameter model with terms for phosphate salts to predict growth of Listeria monocytogenes in spredable cheeses

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Absence of *L. monocytogenes* growth in ready-to-eat foods at the consumer phase is estimated to reduce listeriosis cases per year by 37% (EFSA, 16, 1-173, 2018). Spreadable cheeses are ready-to-eat foods with products characteristics including pH of 5.6-6.4 and NaCl of 0.7-1.6% w/w that may support growth of *L. monocytogenes* if contaminated by consumers after opening of packed products that are typically hot filled. To reduce growth of *L. monocytogenes* in spreadable cheeses at the consumer phase recipes and the content of phosphate salts is important. These salts are known to inhibit growth of some microorganisms; however, their anti-listerial effect remains little studied. Product reformulation and recipe optimization can be assisted by challenge testing but the use of validated predictive models is faster and more cost effective. The objective was to develop and validate an extensive model to predict growth of *L. monocytogenes* in spreadable cheese containing phosphate salts.
The new model was developed by expanding an existing cardinal parameter-type model that included 12 environmental parameters (IJFM, 141, 137-150, 2010). MIC-values for orthophosphate, pyrophosphate and triphosphate salts were determined in broth and terms modelling their antimicrobial and interactive effects were added to the existing model. The new model has been evaluated; so far, under constant temperature using a total of 48 growth/no growth responses in well characterized spreadable cheeses. Average bias and accuracy factor values were 1.02 and 1.20 for 24 growth curves. The model predicted growth/no growth correctly for 87.5% of the responses with 12.5% being fail-safe. The new model can be used to facilitate product reformulation as shown here for spreadable cheese at 8 °C, pH 6.3, a_w 0.972 and water phase organic acid concentrations of 0.8 % (lactic), 0.3 % (citric), 0.1% (acetic) and phosphate salt of 1.9 % (orthophosphate). If this product is contaminated with 1-10 cfu/g by the consumer it will take 3-6 days for *L. monocytogenes* to reach the critical limit of 2 log cfu/g. However, by substituting the orthophosphate with 1.9% triphosphate the reformulated product prevents growth of *L. monocytogenes* from exceeding the critical limit for 11-22 days.
The high number of environmental factors included in the new model makes it a flexible tool to support product development where the anti-listerial effect of phosphate salt can be taken into account when recipes are optimized.

Keywords: Dairy products, mathematical modelling, validation

Impact of interventions during food production on microbial biodiversity

P4.21

Influence of the establishment of a quality assurance system based on HACCP principles on the hygienic quality of products made from meat

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Following the health crises that shook the world, food security has become a global requirement; These crises have increased consumer awareness, which is becoming more demanding in terms of quality and safety. Meat preparations are most often incriminated in TIAC; With this in mind, our work consists in studying the influence of the implementation of the HACCP system on the hygienic quality of these products.
The implementation of the HACCP system was carried out according to the international code of practice of the codex alimentarius (2003).
The hazard analysis was conducted according to 03 methods: The codex alimentarius decision tree, The intuitive method, and the determination of the CCP from the manufacturing diagram.
The implementation of the HACCP system has led to an improvement in the hygienic quality of the products (according to the three-class plan used for the interpretation of the results).
The determination of CCPs from the manufacturing chart was the most efficient method.
The establishment of the HACCP system makes it possible to define, evaluate and control the hazards that ensure hygiene and product safety.

Keywords: HACCP, hygiene, contamination, ccp, risk analysis

Impact of interventions during food production on microbial biodiversity

P4.23

Effectiveness of immersion treatments with lactic acid and potassium sorbate and modified atmosphere packaging against *Listeria monocytogenes* in poultry

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Raw poultry is a well-recognized source of Listeria monocytogenes and many surveys have confirmed the presence of this pathogen on fresh poultry. Some authors have associated cases of listeriosis with the consumption of undercooked chicken. There is a great interest in reducing surface microbial contamination of poultry, with particular regard to reducing the levels of pathogens. The aim of this study was to evaluate the combined effect of lactic acid and potassium sorbate washing and packaging in modified atmospheres on the growth of Listeria monocytogenes on poultry legs stored at 4ºC.Fresh chickens legs were inoculated with Listeria monocytogenes. After the inoculation, the chicken legs were decontaminated by immersion during 75 seconds on either a mixture containing 1.25% lactic acid (v/v) and 1.25% potassium sorbate (w/v), or 1.25% lactic acid and 3.75% potassium sorbate, or 3.75% lactic acid and 3.75% potassium sorbate. Control legs were treated with distilled water Inoculated samples were packaged under a gas mixture: 20%CO_2 / 80%N_2,Surface pH values, sensorial characteristics and Listeria monocytogenes, mesophiles and psychrotrophs counts were evaluated after treatment (day 0) and after 1, 3, 5, 8, 11 and 14 days of storage at 4º C.Significant differences ($p< 0.05$) in mesophiles and psychotrophs counts were found between the legs treated with lactic acid and potassium sorbate mixtures and the control legs after treatment. The lowest mesophiles counts were observed in those samples treated with 3.75% lactic acid and 3.75% potassium sorbate and packaged in 20%CO_2/ 80% N_2.Legs washed with 3.75% lactic acid and 3.75% potassium sorbate and packaged in 20%CO_2/ 80% N_2 showed a significant ($p< 0.05$) inhibitory effect on Listeria monocytogenes compared to control legs, being about 2.78 log units lower in the first ones than in control legs after 3 days of storage. Significant reductions on Listeria monocytogenes were observed between samples treated with 3.75% lactic acid and 3.75% potassium sorbate and those treated with 1.25% potassium sorbate and 1.25% lactic acid.In conclusion, the combined effect of 3.75% lactic acid and 3.75% potassium sorbate and packaging under 20%CO_2/80%N_2 can reduce Listeria monocytogenes populations on fresh poultry.

Keywords: Food safety, pathogens, poultry, modified atmosphere packaging, Listeria monocytogenes

Impact of interventions during food production on microbial biodiversity

P4.24

Antimicrobial effect of cold atmospheric plasma jet treatment on cutting blade surfaces

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Cutting tools can highly contribute to bacterial recontamination of product surfaces during industrial slicing of different food. The introduction of spoilage or pathogenic microorganisms can result in significant reduction of product shelf life or in potential health risks for consumers. Therefore, high hygienic standards are required including effective and sustainable decontamination technologies.

In this context the objective of a recent study* is to investigate the suitability of cold atmospheric plasma for the decontamination of industrial cutting blade surfaces. Based on the achieved results the method finally be developed should be capable to achieve at least an equivalent or better bactericidal effect compared to currently used wet disinfection procedures.

The results show the potential of a microwave-excited atmospheric pressure plasma jet (He (2.0 L min^{-1})/ N_2 (0.3 L min^{-1}) mixture) to significantly inactivate *Listeria monocytogenes* (*L*.), *Lactobacillus* (*Lb*.) *sakei* and *Serratia* (*S*.) *liquefaciens* artificially inoculated on stainless steel cutting blade surfaces at negligible temperature increase ($\Delta T < 10$ °C). Thereby, the inactivation rate increases proportionally to the treatment time and energy as well as inversely proportional to the contamination dose (10^3 - 10^7 CFU/ blade surface). Additionally, differences in sensitivity to plasma treatment are observed between the various bacteria and even between the three isolates of each tested species. However, all isolates of *S*. *liquefaciens* are highly sensitive to plasma treatment while those of *Lb*. *sakei* are the most resistant. This is clearly demonstrated by the almost complete inactivation (4.0 log units) of *S*. *liquefaciens* by plasma treatment (180 s, 6 W, 6 mm, contamination dose: 10^3 - 10^4 CFU/ blade surface) whereas with the same parameter settings the mean reductions of *L*. *monocytogenes* and *Lb*. *sakei* are only 2.6 and 1.7 log units, respectively.

In summary, results show that cold atmospheric plasma jet treatment is generally suitable for bacterial decontamination of industrial cutting tools, but different inactivation rates achieved for the tested strains and species have to be considered. Further studies are needed to improve inactivation rates for *L*. *sakei*.

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Keywords: cold atmospheric plasma, cutting blade, antimicrobial effect

Impact of interventions during food production on microbial biodiversity

P4.25

Synergistic antibacterial and antibiofi lm efficacy of pterostilbene in combination with silver nanoparticles against food born bacteria *Staphylococcus aureus* and *Bacillus cereus*

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Contamination of foods with pathogenic microbial such Staphylococcus aureus and Bacillus cereus could induce food-borne diseases. Various food preservation methods and technologies have been developed to counteract the action of such food related pathogens and to extend the shelf life of the foods. The synthetic preservatives like nitrates, benzoates, and formaldehyde are known for their adverse healthy effects. Therefore, application of natural antimicrobials as an alternative to chemical preservatives, and in the same time for ensuring body safety. Pterostilbene is a phytoalexin compound found in several foods including blueberries and grapevines. Pterostilbene has various beneficial effects against human diseases including cardiovascular diseases, diabetes and cancer. Pterostilbene is also shown to possess antibacterial and anti-fungal effects, however, some bacteria are resistant to pterostilbene. Silver nanoparticles (AgNPs) have attracted much attention on their antibacterial effects against both Gram-positive and Gram-negative bacteria. Therefore, we investigate the antibacterial and antibiofilm effects of pterostilbene in combination with AgNPs against food born bacteria Staphylococcus aureus and Bacillus cereus. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are determined using microdilution technique. The synergistic antibacterial activities of pterostilbene in combination with AgNPs are assessed using checkerboard assay and time-kill kinetic study. In addition, biofilm thickness is assessed by confocal microscopy. The results show that MIC and MBC values of pterostilbene are about 100 μg/ml, and AgNPs is effective in the range of 50-100 μg/ml against Staphylococcus aureus. Bacillus cereus is shown more resistant to pterostilbene, whereas AgNPs are effective against Bacillus cereus in the range of 25-100 μg/ml. The results of the synergistic antibacterial effects of pterostilbene combined with AgNPs are determined by calculation of FIC values. FIC values are 0.25 and 0.4 against Staphylococcus aureus and Bacillus cereus, respectively, which indicates a synergistic interaction. The results of time kill-kinetic and antibiofilm studies also show the synergistic bactericidal activities against Staphylococcus aureus and Bacillus cereus. Our findings demonstrate the potential implications in the development of pterostilbene in combination with AgNPs as alternative preservatives against food-born pathogens.

Keywords: Staphylococcus aureus, Bacillus cereus, Pterostilbene, Silver nanoparticles

Impact of interventions during food production on microbial biodiversity

P4.26

Effectiveness of immersion treatments with citric and acetic acids against *Campylobacter jejuni* in poultry

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Raw poultry is a well-recognized source of Campylobacter jejuni and many surveys have confirmed the presence of this pathogen on fresh poultry. There is a great interest in reducing surface microbial contamination of poultry, with particular regard to reducing the levels of pathogens.
The aim of this study was to evaluate the combined effect of different mixtures of citric and acetic acids on the growth of Campylobacter jejuni in poultry legs stored at 4ºC.
Fresh chicken legs were inoculated with Campylobacter jejuni. After the inoculation, the chicken legs were dipped into a mixture containing either 1% citric acid and 1% acetic acid or 2% citric acid and 2% acetic acid. Control legs were treated with distilled water. Surface pH values, sensorial characteristics and Campylobacter jejuni, mesophiles and psychrotrophs counts were evaluated after treatment (day 0) and after 1, 3, 6, 8, 10, 13, and 15 days of storage at 4º C.
Significant differences ($p < 0.05$) in mesophiles and psychotrophs counts were found between the legs treated with a mixture of citric and acetic acid and the control legs after treatment. The control legs had the fastest increase in mesophiles counts. The lowest mesophiles counts were observed in those samples treated with 2% citric acid and 2% acetic acid. Legs washed with a mixture of 2% citric and 2% acetic acid solution showed a significant ($p < 0.05$) inhibitory effect on Campylobacter jejuni compared to control legs, being about 1.09 log units lower after treatment. After 3 days of storage, Campylobacter jejuni counts in samples treated with 2% citric acid and 2% acetic acid were 1.55 log units lower compared to control samples.
In conclusion, immersion of chicken legs in a mixture of 2% citric acid and 2% acetic acid solution can reduce Campylobacter jejuni populations on fresh poultry.

Keywords: Food safety, pathogens, poultry, organic acids, Campylobacter

Impact of interventions during food production on microbial biodiversity

P4.27

Monitoring the microbial status of raw and pasteurized milk from milk filling stations in Brandenburg, Germany

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In Germany, ready-to-consume milk may only be offered as certified raw milk ("Vorzugsmilch") or heat-treated milk. On the other hand, raw milk can be delivered directly from dairy farms´ milk filling stations to the final consumer. However, this raw milk is not intended for direct consumption. Milk filling stations are an increasingly popular form of milk marketing for direct sellers offering raw milk or pasteurized milk. The number of milk filling stations in Brandenburg has almost tripled in 2017.
Since September 2017, more than 130 raw milk and pasteurized milk samples from 19 milk filling stations have been examined. It was shown that in about one third of all samples selected reference values for the total bacterial count were clearly exceeded. Increased bacterial counts of *Enterobacteriaceae* were detected in about 35% of the samples. In order to assess a health risk under the assumption of direct consumption, the raw milk samples were additionally examined for *Salmonella*, VTEC, *Campylobacter* and *Listeria monocytogenes*. Pathogens were detected in more than 25% of these samples.
Furthermore, the dominant microorganism flora from the total bacterial count as well as technical details of the machines and the cleaning and disinfection processes were surveyed, in order to identify sources of contamination and to give advice to the farms with regard to their milk and milk vending machines. From this data, problems with milking hygiene, interruptions of the cold chain or faults in the cleaning and disinfection processes of the milk vending machines were deduced.
As part of a multiple step quality control regimen, microbiological weak points in the milking and production processes were exposed on-site for three dairy farms. Based on these results, countermeasures were developed and then quality assurance concepts proposed for monitoring hygiene conditions.

Keywords: milk filling stations, raw milk, process hygiene, milking hygiene, cleaning and disinfection

Impact of interventions during food production on microbial biodiversity

P4.28

Indications of biopesticidal *Bacillus thuringiensis* residues in vegetable food

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The *Bacillus (B.) cereus* group (also called *B. cereus* sensu lato or presumptive *B. cereus*) currently comprises eight closely related species which are difficult to differentiate and are thus not distinguished in routine diagnostics. However, the potential to cause foodborne disease differs between these species. Especially, *B. cereus* and *B. cytotoxicus* are considered foodborne pathogens whereas discussions on the pathogenic potential of *B. thuringiensis* are ongoing.
Presumptive *B. cereus* are common contaminants of vegetable food, while the individual *B. cereus* group species with its toxinogenic potential is mostly unknown. A possible unnatural source of contamination is the application of *B. thuringiensis* based biopesticides. Still, evidence for biopesticidal residues on food is scarce.
In order to address these issues we analyzed samples of spices and dried herbs (8), fresh herbs (20) and bell pepper (100) for presumptive *B. cereus*. Additionally, 426 tomato and 339 sprout samples were analyzed by food inspection laboratories of the federal states in Germany.
Using cultural, microscopic and PCR-based approaches, obtained isolates were further characterized in terms of species affiliation, toxinogenic potential and partially their multilocus sequence type (MLST). The same methods were used to characterize commercially applied biopesticidal *B. thuringiensis* strains.
The presumptive *B. cereus* prevalence was 75 % in spices and dried herbs, 95% on fresh herbs, 42 % on bell pepper, 8% on sprouts and 28 % on tomatoes. Most strains were able to produce toxins. The proportion of *B. thuringiensis* ranging from 8 to 21 % in spices and dry/fresh herbs as well as sprouts was comparable to previous studies. In contrast, the presumptive *B. cereus* populations from bell pepper and tomatoes were strikingly dominated by *B. thuringiensis* (93 % and 99 %, respectively). These strains were indistinguishable from the biopesticidal strains *B. thuringiensis* subsp. *aizawai* ABTS 1857 or *B. thuringiensis* subsp. *kurstaki* ABTS-351, respectively, based on the following parameters: present toxin genes, toxin production, *cry1* gene and parasporal crystal content as well as MLST profiles (exclusively ST 8 and 15, in a subpopulation of 71 isolates).
These findings indicate that the *B. thuringiensis* burden in the analyzed bell pepper and tomato samples may originate from residues of *B. thuringiensis* based biopesticides.

Keywords: B. cereus; spore; biopesticide; MLST; toxin

Impact of interventions during food production on microbial biodiversity

P4.29

Inactivation of yeasts by ultra violet light (UVC) on moist and dry surfaces

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Due to its mutagenic properties, ultraviolet light has been used for many years to inactivate microorganisms on surfaces in many industries. Short wavelengths (250-260 nm) of UV light (UVC) are considered the most germicidal. However, the antimicrobial effectiveness of UV light varies among microorganisms, surfaces exposed and method of exposure. The aim of this investigation was to evaluate the effect of ultraviolet light at 254 nm (UVC) on five yeast species on both moist and dry surfaces. Yeast species investigated include *Candida zeylanoides*, *Debaryomyces hansenii, Pichia anomala, Rhodotorula glutinis,* and *Saccharomyces cerevisiae.* For the moist surface method, yeast cell suspensions were spread on MEA plates and exposed to UV treatments (254 nm) for 0, 5, 10, 15, 30, 60 and 120 seconds. For the dry surface method, a suspension of yeast cells were dried onto a membrane filter and then treated with UV (254 nm) for 0, 30, 60, 120, 180 and 300 seconds. The membranes were placed in 0.1% sterile peptone water, mixed in a stomacher, diluted and plated onto MEA plates. All plates were incubated at 25°C for 3 days and colonies were counted. All the experiments were done in triplicate. On moist surface, after 30 seconds exposure, a significant reduction (70-99%) was observed for most of the species tested. On the dried filter membranes, a reduction of viable cells of up to 100% was observed for *D. hansenii* and *S. cerevisiae* after 30 to 120 seconds exposure. A reduction of 2.0 $\log_{10}$ was achieved for *C. zeylanoides* after 300 seconds exposure. *R. glutinis* and *P. anomala* were found to be more resistant to UV, especially on dry surfaces. They were significantly ($P < 0.001$) more resistant to UVC than the other yeast species. This study indicates that UV light is an effective process to inactivate yeast cells on moist and dry surfaces. Although, longer exposure periods should be used for dry surfaces.

Keywords: Ultraviolet light, UVC, Yeasts, Inactivation

Impact of interventions during food production on microbial biodiversity

P4.30

Dealing with variability for a more realistic estimation of non-thermal inactivation dynamics of *Listeria monocytogenes*

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Providing an easy way to access prediction, the deterministic approach to the description of microbial populations as a whole, in response to the environment, has long been successful in managing food safety. Process and formulation of fermented dried sausages benefit from non-thermal inactivation (NTI) models, which represent valuable tools to control pathogens. However, deterministic models do not take into account variability, which clearly appears when cell numbers are small (Koutsoumanis & Aspridou, 2017). In consideration of the low numbers of pathogens usually occurring in food, it is of importance to estimate variability of the response of a pathogen, such as *L. monocytogenes*, during a NTI process. The aim of the present study was to evaluate the responses of multiple replications of various initial concentrations (N_0) of *L. monocytogenes* Scott A populations subjected to NTI conditions, and to describe the observations in a modelling approach that incorporated variability. The applied stress was osmotic, resembling the contamination of a product, such as an Italian dry sausage, with low a_w to which individual *Listeria* cells could respond and adapt with different mechanisms to address the challenges imposed on them by food preservation technologies (Burgess et al., 2016). Each inactivation data set of the pathogen was fitted to a deterministic first order kinetic model [$N_t = \exp(N_0 + b^*t)$] by using the Conway-Maxwell-Poisson (COM-Poisson) generalized linear model, which is suitable for over- and under-dispersed count data (Francis et al., 2012). The distribution of N_t values was characterized by various levels of dispersion and could be described by a COM-Poisson distribution while the coefficients of variation (CV) of N_t and b reflected the variability in the initial levels of *Listeria* population. Based on these observations, the NTI model for predicting the behavior of *L. monocytogenes* at different N_0 was conceived by introducing the COM-Poisson distribution for N_t values and the normal distribution for b values. From the Monte Carlo simulation results with various initial cell concentrations it was assumed that variability in the pathogen number during non-thermal inactivation could be mainly associated with natural random processes for small populations and with biological variability for larger cell numbers.

References:

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Francis et al, 2012. Risk Anal, 32,167.
Koutsoumanis & Aspridou, 2017. IJFM, 240,3.

Keywords: Non-thermal inactivation model, variability, Listeria monocytogenes, low populations

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P4.31

A meta-regression model of the growth rate of *Listeria monocytogenes* as affected by temperature

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The presence of *L. monocytogenes* in naturally-contaminated foods, its ability to endure various environmental stresses and grow at low temperatures and during the shelf life of some foods are great challenges for the food industry. To overcome this issue, predictive models can be used on the decision-making process in case of presumed contamination and possible growth of pathogens as they can assess bacterial levels before a control step is applied and evaluate if the process allows the pathogen's inactivation or reduction to an acceptable level.

In this sense, Cardinal Parameters Models (CPM) have been widely used to describe the effect of environmental factors on microbial growth rates. To be used, the determination of the parameters, known as cardinal values, is needed, but since experimental estimation is a laborious task, it is proposed here that meta-analysis of literature data could be useful to perform such assessments. This statistical analysis of results from published studies aims to integrate and interpret the findings to achieve an enlarged vision about the topic's results.

Suitable scientific articles were collected through search in several databases. Following study quality checking, 88 studies remained from which 3079 growth rates were extracted.

To evaluate temperature's effect on growth rates and estimate comprehensive cardinal values, meta-analysis was performed on a set of growth rates assessed at optimal conditions of pH (6.5-8) and a_w (≥0.98). To appraise the share of the possible sources of variability, the CPM was also fitted on subsets of growth rates estimated using (i) distinct reading methods, (ii) distinct broth types and (iii) sub-optimal conditions of pH and a_w.

The pooled parameters from the optimal set were T_{min}=-1.15±2.43 °C, T_{opt}=37.42±2.00 °C, T_{max}=45.20±0.37 °C and μ_{opt}=1.06±0.13 h^{-1}. Regarding the possible sources of variability, it was concluded that the reading method (R^2=24.8%) and the broth type (R^2=60.1%) used to estimate growth rates largely affect the estimation of cardinal values. Moreover, data at sub-optimal conditions, especially in food products, were found inadequate to assess cardinal values, unlike optimal conditions, as mean estimates changed and standard errors increased.

The meta-analysis performed allowed the fitting of the CPM to growth rate data retrieved from scientific articles, showing that literature can be useful to assess cardinal values and to provide an insight on sources of variability.

Keywords: Meta-analysis, Cardinal Parameters Model, optimal conditions, broth

Impact of interventions during food production on microbial biodiversity

P4.32

Effects of potassium lactate and modified atmosphere packaging on fresh poultry sausage spoilage

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The spoilage of meat products is mostly considered as a result of unavoidable bacterial contamination during food production. From a consumer point of view, spoilage is associated with organoleptic defects (colour, odour, aspect). Spoilage can be delayed by the use of preservation techniques such as packaging under modified atmosphere (MAP) or addition of preservatives like lactate. The aim of this work was to follow the spoilage of meat products over time.

Experimental data were obtained from 10 batches of fresh poultry sausages. For each batch, sausages were made with three initial potassium lactate contents (0g, 0.9g, and 1.8g/kg). They were afterward conditioned under three different MAP (air, 30%CO2-70%O2 and 50%CO2-50%N2), stored at 4°C during 5 days and then 8°C until the 22nd day after production. Different analyses were performed during storage: characterization of visual defects (colour changes, exudate, inflated packaging), measurement of off-odour intensity, pH and gas composition of the packaging headspace, enumeration of total aerobic mesophilic and lactic acid bacteria (LAB).

The results revealed an increase in the two studied bacterial groups and a decrease in pH during storage. These evolutions were stabilised from the 15th day of storage, at which all batches presented visual defects. The total viable count was neither affected by the packaging atmosphere nor by the lactate content. The growth of LAB was particularly favoured in the anaerobic atmosphere containing 50% of CO2, regardless of the lactate content. For this atmosphere, although at least 3 out of 10 batches presented visual defects very early from the 4th day, the pH remained constant during the first 8 days for the sausages with lactate, suggesting a delayed acidification in the presence of lactate. The average off-odour intensity was the highest in sausages conditioned under air, and the lowest under the atmosphere with 30%CO2-70%O2. Under the atmosphere containing 50% of CO2, high concentration of lactate seemed to reduce the average off-odour intensity.

Lactate addition and MAP influenced several measured responses such as the growth of LAB, pH dynamic and off-odour intensity, which could be then potentially considered as spoilage indicators. The variability in these responses beyond the similar overall bacterial population level reveals impacts of these preservation techniques on the bacterial metabolism and ecosystem composition.

Keywords: Fresh Poultry Sausage Spoilage, Modified Atmosphere Packaging, Potassium Lactate

Impact of interventions during food production on microbial biodiversity

P4.34

Quantifi cation of viable *Campylobacter* in raw milk by real-time PCR

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With 246,307 reported human cases in 2016 campylobacteriosis continues to be the most frequent bacterial food-borne disease in the European Union. The main causative agent is *C. jejuni*, which is mostly transmitted via contaminated poultry meat. However, *Campylobacter* outbreaks may be caused by the consumption of raw cow´s milk implying an increasing problem after the European milk quota regime came to an end and farmers intensified the sale of raw milk via milk filling stations. With regard to the detection and quantification of pathogens, milk displays a particularly challenging food matrix due to its high protein and lipid content. Additionally, it has been repeatedly shown that cfu determination underestimates not only cell viability but also the pathogen´s potential to cause infections.

We have developed a procedure that allows real-time PCR based quantification of viable *Campylobacter* in raw cow´s milk. Various DNA-intercalating dyes were evaluated for their ability to reduce the non-viable signal during real-time PCR. Only PMA and PMAxx qualified for diagnostic use. Entry of these dyes into H_2O_2-killed bacteria depended on cell membrane integrity and passive exclusion was observed with bacteria, transiently inactivated in the presence of the protonophore CCCP but able to regain their colony-forming ability after stress release. The procedure displays a further preferential exclusion of non-viable *Campylobacter* due to different sedimentation properties of live and dead bacteria. Application of the method showed that while various strains of *Campylobacter* significantly lost their colony-forming capacity, the number of viable bacteria detected by real-time PCR remained high, even after 6 days of aerobic incubation at 4 °C. Subsequently, the biological relevance of qPCR-confirmed *Campylobacter* survival in raw milk was supported by partial recovery of CFU upon modification of growth conditions.

Keywords: Campylobacter, raw milk, live/dead discrimination, quantitative real-time PCR, PMA, PMAxx

Impact of interventions during food production on microbial biodiversity

P4.35

Effect of irradiation and thermal inactivation on microorganisms in spices and dried herbs

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Spices and dried aromatic herbs are products of low water activity which is inhibitory to microbiological growth. As products they are often found to be naturally contaminated with various microorganisms, including pathogenic bacteria and toxigenic moulds. Spices and dried herbs sold in the EU market are generally decontaminated either by steam or irradiation. This study aimed to bring together available microbiological resistance data, and to obtain quantitative estimates of microbial decontamination efficacy by thermal and irradiation decontamination treatments. A meta-analysis was performed to identify the most influential factors that affect microbial inactivation in spices and herbs, and to estimate global parameters for inactivation and corresponding variability. To confirm confidence of the estimated parameters, the meta-analysis results were compared to our experimental studies on spices and herbs, and to existing data within databases on other food types. Fifty $D_{(T)}$ values of thermal resistance and 329 D_{10} values of irradiation for various microorganisms in spices and dried herbs were calculated from published studies. Additional $D_{(T)}$ and D_{10} values were calculated from experiments which fitted within the meta-analysis variability. Results from both the thermal and irradiation meta-analysis indicated that microbial identity was a significant factor. Resistance patterns showed spore-forming bacteria had the highest resistance, followed by total plate counts, fungi and *Enterobacteriaceae*. There was no statistical difference between the means of the latter two groups. Meta-analysis of irradiation D_{10}values indicated that factors such as product type (spices or herbs), particle size and irradiation technique (Gamma or Electron Beam) were non-significant. A previous meta-analysis on irradiation D_{10} for bacteria and spores in various products, other than spices and herbs was used. When compared to our meta-analysis and experimental results for spices and herbs, the data indicated that the mean logD_{10} values from spores in spices and dried herbs were significantly higher than those from other food types; however, the prediction intervals were clearly overlapping. This comparison between spices and herbs and other products suggests that the role of food matrix in irradiation resistance is much lower than that for microbial identity. The impact of these observations is that consumption spices and herbs can be a risk for consumers.

Keywords: decontamination, variability, meta-analysis, spices, herbs

Impact of interventions during food production on microbial biodiversity

P4.36

Survival of selected pathogens during the production and storage of iru (fermented vegetable condiment)

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Pathogens have been isolated from some fermented foods indicating that they survived the fermentation process, which is a pointer to one of the major public health problems worldwide. This indicates that there is a food safety risk associated with the survival of pathogens in fermented foods. Therefore, there is a need to investigate the survival of some bacterial pathogens during the fermentation and storage of *iru*; a traditional fermented condiment obtained from African locust beans (*Parkia biglobosa*) which is commonly consumed in West Africa. The pathogens used were *Pseudomonas aeruginosa*, *Salmonella* Typhi, *Staphylococcus aureus* and *Escherichia coli*. They were obtained from the culture collection center of the Food Microbiology Laboratory, University of Ibadan, Nigeria. The pathogens were inoculated into the fermenting locust beans during production for 96 h and during storage at different temperatures (4, 25, 37 and 42 °C) and different salt concentrations (2.5, 5, 7.5 and 10 %) for 5 days. During the production, the growth rate of all the pathogens were repressed as the pH, temperature and days of fermentation increases. *E.coli* growth was inhibited after 72 h, *S.* Typhi, after 48 h, *P. aeruginosa* after 24 h, while *S. aereus* survived the 96 h of fermentation but with a significant reduction in growth rate. During storage of *iru* for 5 days, *Salmonella* Typhi growth was inhibited after 48 h; *E. coli* was repressed after 72 h, *P. aeruginosa* after 24 h while *S. aureus* survived until the 5th day of storage. Most of the pathogens survived salt concentrations of 2.5% and 7.5% but their growths were significantly inhibited at 10%. Temperature of 42 °C inhibited the growth of *S*. Typhi and *E. coli* over a storage period of 120 h while *P. aeruginosa* growth was inhibited at 37 °C and *S*. *aureus* survived all the temperature range with a significant decrease in growth rate. The combination of the alkaline pH, low water activity and free ammonia along with rapid growth of the essential microorganisms such as *Bacillus subtilis* at temperatures above 40 made it very difficult for the pathogens to grow during fermentation. The increase in pH was due to proteolytic activity, which lead to the release of amines and amino acids from the leguminous seed thus limiting the growth rate of the introduced pathogens. Storage of fresh iru with 10% salt concentration could be a safe way of keeping the condiment since the growth of all the pathogens at this concentration was inhibited.

Keywords: Iru, pathogens, survival rate, production, storage.

Impact of interventions during food production on microbial biodiversity

P4.37

Evaluation of the potential of an enzymatic product for the removal of biofilms from wild strains of *Listeria monocytogenes* on stainless steel surfaces

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In food industry environments, the majority of microbial populations grow as adhered communities with subsequent biofilm formation to food contact surfaces. Biofilms have significant implications within the food industry, where they confer protection to microbial populations from a range of harmful conditions and suppose a persistent source of contamination.
Cleaning and disinfection programs are an essential part of the HACCP system. These programs consist on a series of operations using cleaning products and disinfecting agents to maintain the food contact surfaces in an adequate hygienic condition. Conventional procedures are not able to detach effectively biofilms from surfaces as they do not break up or dissolve the matrix associated with the biofilms completely so that disinfectants, in most cases, do not gain access to the viable cells, and therefore may contribute to inefficient biofilm control.
The aim of this study was to evaluate the impact that a developed cleaning product based on enzymatic technology have on the removal of mature biofilms of wild *Listeria monocytogenes* strains isolated from meat industry on stainless steel surfaces in comparison with a conventional cleaning and disinfection treatment.
Biofilm production assays were carried out by culturing *L. monocytogenes* in a rich undefined medium ($TSYEB_{gluc1\%+NaCl2\%}$). From this suspension, 30 µL were inoculated on stainless steel coupons. The inoculated surfaces were incubated at 30 ºC for one week, in static conditions and with renewal of the culture medium at 48 h + 24 h + 24 h + 72 h. Quantification of cells conforming the biofilms before and after the treatment was done by shaken rinses and cultivating them by using TEMPO system.
Results showed that enzymatic treatment was able to detach between 10-15% more *L. monocytogenes* cells than conventional treatment. The maximum detachment percentage obtained was 90.97%, with a release of 5.70 log CFU cm^{-2}, and the minimum obtained was 56.82% with a cell release of 3.48 log CFU cm^{-2}, however, in all cases higher than the conventional treatment.
This study demonstrates that enzymatic treatments have great potential to be implemented for cleaning procedures, as they have been proved to break down biofilm matrix and therefore disinfectants can penetrate to cells and eliminate them. Nowadays food industry needs to make an effort in looking for efficient biofilm control strategies, and enzymatic technology is a promising one.

Keywords: Listeria monocytogenes, Biofilm Control, Enzymatic Technology, Conventional Treatments, Food Hygiene

Impact of interventions during food production on microbial biodiversity

P4.38

Toxin binding capacities and antioxidant activities of *Lactobacillus plantarum* and *Pichia kudriavzevii* against cadmium and lead toxicities

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Cadmium and lead are lethal heavy metals, which causes adverse health conditions in humans and animals. Twenty-four lactic acid bacteria (LAB) and 24 yeast isolates were obtained from fermenting cassava mash (*gari*, *lafun*, *fufu*) and *ogi* (fermenting maize slurry) and were screened for their ability to tolerate cadmium and lead between 100 mg/mL-1050 mg/mL. The tolerant isolates were screened for probiotic potentials, safety assessments and antioxidant activities such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP). Molecular characterization was carried out on the LAB and yeast, which possessed the best potentials. These microorganisms were used for *in vivo* studies in male Wistar rats. Antioxidative capacities such as reduced glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) and histopathology studies were carried out on the liver and kidney of the animals. Twelve LAB and 10 yeast isolates were resistant to cadmium and lead between 500 mg/mL-1050 mg/mL. *Lactobacillus* sp. ML05 and *Candida* sp. FY05 had the highest growth of 92% and 71% to 0.3% bile salts concentration and 79% and 51% to 1% bile salts concentrations respectively. *Lactobacillus* sp. ML05 and *Candida* sp. FY05 had the highest survival rates in simulated gastric juice (85.6% and 90.3%) and in intestinal juices (90.3% and 97.2%) respectively. Ten LAB and 7 yeasts isolates showed ɣ-haemolysis, and were negative to DNAse and gelatinase tests. *Lactobacillus* sp. ML05 and *Candida* sp. FY05 showed the highest antioxidant activities of 74.24% and 88.5% to DPPH and 84.9% and 87.9% to FRAP respectively. They were genotypically identified as *Lactobacillus plantarum* ML05 and *Pichia kudriavzevii* FY05. The *in-vivo* studies of the antioxidant capacities showed positive impacts in the GSH, MDA, SOD and CAT in the liver and kidney of the rats treated with *Lactobacillus plantarum* ML05 and *Pichia kudriavzevii* FY05 in comparison to the untreated. The histopathology results showed mild inflammation in the kidney and liver of the treated rats administered with *Lactobacillus plantarum* ML05 and *Pichia kudriavzevii* FY05 while the not treated rats showed cell necrosis and cell vacuolization induced by lead and cadmium toxicities. This study suggests that *Lactobacillus plantarum* ML05 and *Pichia kudriavzevii* FY05 were effective against acute cadmium and lead toxicities and can be considered as dietary therapeutic against cadmium and lead toxicities.

Keywords: Lactobacillus plantarum, Pichia kudriavzevii, Cadmium toxicity, Lead toxicity

Impact of interventions during food production on microbial biodiversity

P4.40

Modeling the growth and survival of *Salmonella* in whole and cut cucumbers as a function of temperature and relative humidity

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Recent multistate outbreaks of salmonellosis associated with fresh cucumbers underscore the importance of understanding *Salmonella* behavior in cucumbers at different storage conditions. However, no validated models currently exist which describe the impact of environmental factors on growth and survival of *Salmonella* in cucumbers. This study developed mathematical models that predict the growth and survival of *Salmonella* on whole and cut cucumbers at different temperature and relative humidity (RH) storage conditions.

Fresh cucumbers were purchased from a local supermarket and used in whole or cut cucumber experiments. Whole or cut cucumbers were spot inoculated with a four-strain cocktail of *Salmonella enterica*. All strains were originally linked to fresh produce outbreaks and were made resistant to nalidixic acid. Inoculated cucumbers were placed in desiccators containing saturated salt (lithium chloride, potassium carbonate, and potassium sulfate) used to create controlled RH environments (~15, 50, 100 % RH) at 7, 14, and 21 °C. Samples were enumerated at appropriate time intervals ranging from 0 to 240 h. Predictive models were developed using Baranyi and Roberts equation as a primary model and estimated kinetic parameters were fitted into a polynomial equation or square root (or Ratkowsky) equation for secondary models.

Salmonella on whole and cut cucumbers showed better survival and slower growth at lower temperatures. RH had no impact on *Salmonella* growth on cut cucumber. RH did affect *Salmonella* survival on whole fresh cucumber, with the greatest decline in *Salmonella* populations observed at 15% RH. When a polynomial equation was used to describe maximum death rate and the degree of decline of *Salmonella* on whole fresh cucumber as a function of temperature and RH, a linear trend with high R^2 values (>0.98) was observed. The maximum growth rates for *Salmonella* on cut cucumber depended only on temperature and ranged from 0 to 0.18 log CFU/h. The square root model for *Salmonella* was SQRT(μ) = 0.0297 * (T - 6.52), with high R^2 value (0.98).

The models in this study will be useful for future microbial risk assessments and predictions of *Salmonella* behavior in the cucumbers to manage the risk of *Salmonella* with respect to cucumbers.

Keywords: Cucumber, Salmonella, Temperature, Relative humidity, Modeling, Risk assessment and management

Impact of interventions during food production on microbial biodiversity

P4.41

Could ferulic acid be an effi cient natural antimicrobial against *Listeria monocytogenes* in model food systems?

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Natural phenolic compounds can be found in large quantities in plant extracts and by-products from agro-industries. They are described to have interesting dual antioxidant and antimicrobial properties which could be used for ensuring quality and safety of several perishable products.[1,2] For example, ferulic acid (FA), which can be found in sugar beet pulp and wheat and maize bran[3], could be a good candidate. However, its mechanism of action is not very well understood and there is few literature about its antimicrobial activity in multiphasic food systems.

In this study, we first aimed to understand the complex mechanism of action of ferulic acid by decomposing different antimicrobial effects of i) the impact on extracellular pH (γ(pH)), ii) the undissociated acid form (γ(Au)) and iii) the dissociated form of FA (γ(Ad)). Different models were tested to decipher the relative part of each of them in growth inhibition. Experimentally, *Listeria monocytogenes* was cultivated in presence of different concentrations of FA at different pHs. The dataset of growth rates for each condition was challenged through three models and the Minimum Inhibitory Concentrations (MICs) of the two chemical forms of FA were calculated with the model that best fits with the experimental dataset. Results show that antimicrobial activity of FA is mainly due to the effect of the undissociated form but interestingly, its dissociated form also has a significant antimicrobial activity.

Afterwards, we evaluated the ability of FA to inhibit *L. monocytogenes* growth in a complex food system: an oil-in-water emulsion, prepared with fish oil, an aqueous phase composed of TS broth and Tween 80 as surfactant. Bacterial growth was followed over 72 h by plate counting in the emulsion and in the referent aqueous phase TS Broth-Tween. Both systems contained the same concentrations of FA: 5.5 mmol/L, which is above the MIC or 1 mmol/L, a sub-inhibitory concentration. Control cultures were conducted with both systems without FA. Results point out that FA inhibits the growth of *L. monocytogenes* in the emulsion as well as in the referent aqueous phase. This is probably due to its hydrophilic properties: FA seems to stay in the aqueous phase of the emulsion, therefore retaining most of its antibacterial activity.

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Keywords: phenolic compound, ferulic acid, antimicrobial, Listeria monocytogenes, emulsion, CMI

Impact of interventions during food production on microbial biodiversity

P4.42

Microbiological safety indicators of canastra cheese in Brazil

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Canastra Artisanal Minas Cheese is the leading traditional product in Brazil. It is made from raw milk and manufactured by rural producers in the *Serra da Canastra* region in the state of Minas Gerais. The producers are usually farmers who may or may not be registered as Artisanal Minas Cheese producers in the Agricultural Institute of Minas Gerais. The production process employs back-sloping inoculation driving fermentation by an endogenous culture called "*pingo*", originated from the whey collected from the previous day's production. The use of raw milk is a risk factor for food safety. Therefore, it is important that foodborne pathogens are controlled after the ripening period of 22 days required by legislation. This work analyzed the microbiological safety indicators of Cheese from 61 rural properties in the region of *Canastra*. Total coliform, *Escherichia coli* and *Staphylococcus* coagulase positive counts were performed on Petrifilm® plates (3M). The detection of *Salmonella* spp. was carried out by using ISO 6579:2002 method and *Listeria monocytogenes* was investigated according to ISO 11290-1:1996/(A)1:2004 method. The *Enterobacteraceae* counts was determined by the APHA method 9.62:2015. The pH analyzes were performed on 10 g of cheese dissolved in 100 ml of distilled water (according to IAL 463-IV method) and the Water Activity (a_w) in an Aqua Lab analyzer.
The results show that 54% of samples from registered producers and 65% of samples from non-registered producers were unsatisfactory according to at least one requirement of the Brazilian legislation. Due to the probable influence of pH and a_w values on cheese safety, we stratified the data of these parameters according to the microbiological analyzes in two groups (satisfactory and unsatisfactory). However, the results suggest that there is no significant difference ($p < 0.05$) in pH and a_w values between the two groups. No sample was contaminated with *Salmonella* and *L. monocytogenes* was found in one sample analyzed so far. High counts of *E. coli* or total coliforms or *Staphylococcus* coagulase positive were observed in several samples indicating a need to implement or improve hygienic-sanitary conditions in the production of Canastra cheese. The high number of non-compliances with the legislation indicates that even with registration of the producers in the state regulatory agency, quality has not improved appropriately.

Keywords: Canastra cheese; food safety; microbial indicators; pathogens

Impact of interventions during food production on microbial biodiversity

P4.43

Development of a predictive model describing the growth of *Staphylococcus aureus* in breads and whipped cream of bread products

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In this study, predictive mathematical models were developed to estimate the kinetics of *Staphylococcus aureus* (*S. aureus*) growth in breads and whipped cream of bread products. Three kinds of bread (White bread, soft Castella cheese cake and corn dogs) and whipped cream spread on bread products were inoculated with *S. aureus* strain mixture to final concentration of approximately 2 log cfu/g and stored at 7°C, 10°C, 30°C and 37°C. Baranyi model as the primary growth model was used to calculate lag-phase duration (*LPD*) values and maximum specific growth rate (μ_{max}), which were used to develop the secondary models using polynomial equation for LPD and square root model for μ_{max} as a function of storage temperature. While the LPD decreased, the μ_{max} increased as storage temperature increased from 7°C to 37°C. Developed models were confirmed by calculating RMSEs (Root Mean Square Errors) as statistic parameters. RMSEs were 0.51, 0.65, 0.45 and 0.55 for White bread, soft Castella cheese cake, corn dogs and whipped cream, respectively. The developed models are useful to predict the growth of *S. aureus* and to assess its risk in breads as an input model.

Keywords: Staphylococcus aureus, predictive model, White bread, soft Castella cheese cake, corn dogs, whipped

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P4.44

Adaptation and cross protection against temperature of acid stress *Listeria monocytogenes*

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Listeria monocytogenes is recognized as gram positive foodborne pathogen. This mesophilic organism does not grow only at 30-37°C, optimal temperature, but also at 2-4°C, so called psychotropic bacteria. Moreover, *L. monocytogenes* demonstrated the resistant to highly acidic condition. *L. monocytogenes* was contaminated on fresh food especially in milk, meat and vegetables. In case of Ready-to-Eat products (RTE), it was also isolated. The bacteria can cause Listeriosis, resulting to the lethal of infected patients. There are several factors affect to the survival of *L. monocytogenes* such as temperature and pH of foods. In this study, the survival, adaptation and cross protection against temperature of *L. monocytogenes* were examined in both microbiological media and food models at pH 7.0 and 3.8. *L. monocytogenes* was inoculated in Tryptic Soy Broth+Yeast Extract (TSB-YE) and TSB-YE acidified by 1 N HCl to obtain the acid-adapted cell. In food models, milk (pH 7.0) and apple juice (pH 3.8) were representatively selected. The results indicated that, *L. monocytogenes* survived better in normal conditions than in acidified conditions. The investigation of effective of temperature on the cross protection of both normal and acid stress *L. monocytogenes* were studied at 4, 37 and 60°C. The results presented that both normal and acid stress *L. monocytogenes* survived at 4°C during 24 h of storage. At 37°C, the reproduce activity of normal cell was detected, while decreasing of acid stress cell population was presented. The cross-protection phenomenon against temperature at 60°C was detected when normal *L. monocytogenes* was subjected to acid stress condition.

Keywords: Listeria monocytogenes , acid stress, cross protection, milk, apple juice

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P4.45

Mathematical description of variability in bacterial growth using individual cell division time *via* stochastic analysis and computer simulation

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Conventional bacterial growth models describe changes in the average bacterial cells. However, because of the variability in individual cell heterogeneity, conventional kinetic model does not give a precise estimate variability in growth behavior. It is difficult to evaluate the risk of achieving enough number of bacteria for foodborne illness or food spoilage. We attempt to describe the time required for reaching a level of population via mathematical formula and computer simulation. We assumed that bacteria grow discretely and exponentially over time. Our target model is exponential growth model without lag time. The variation of the dividing timing of individual cell as exponential distribution. Then, we aggregate the dividing timing of individual cell to describe time required for reaching a level of population. In addition, we simulated stochastic growth model based on our mathematical formula. We set 3 different growth speed parameters for comparison between stochastic bacterial growth. Then, stochastic bacterial growth is simulated with initial cell number 1, 10, and 100 cells until reaching 10^4 cells. The required time for reaching a level of population can be calculated as convolution of exponential distribution. Our computer simulation enables to reveal the trend of stochastic bacterial growth. For example, as the number of initial cells decreases, the variance of the time required for reaching 10^4 cells increase. As the increase speed slower, the variance of the time required for increase to a level of population 10^4 increase. Our stochastic analysis enables to calculate and visualize time required for reaching a level of population and number of bacteria as probability distribution, which has been described as point estimation. Stochastic calculation of variability in bacterial growth would be useful for determining the risk of the pathogenic and spoilage bacteria.

Keywords: Stochastic model

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P4.46

Growth of foodborne pathogens at refrigeration temperatures

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Some foodborne pathogen growth studies and predictive models claim that besides established psychrotrophs (e.g. *Listeria* and *Yersinia*) also *Salmonella* and *Escherichia coli* occasionally grow at temperatures below 8°C, in lab media or foods. This could be of concern for food safety management often relying on keeping the cold chain (< 8°C). Critical analyses showed that these findings seem to be often derived from poorly executed studies, incorrectly extrapolated models, or non-peer reviewed data.
This study aimed to re-evaluate the available data and models. Twelve strains were inoculated (ca. 10 cfu/mL) in 3 lab media (nutrient broth, spinach juice, beef juice). Growth was monitored at 4°C, 7°C and 12°C by plate counts at day 0, 5, 9 and 14. Three *Salmonella* strains (S. Thompson, S. Typhimurium and monophasic S. Typhimurium) were enumerated on XLD (24h at 37°C). Three *Listeria monocytogenes* were counted on ALOA (48h at 37°C). Numbers of two *Yersinia enterocolitca* 4/O:3 and one *Yersinia pseudotuberculosis* O:1 were determined on CIN (24h at 30°C). Counts of one *E. coli* and one ESBL/AmpC-producing *E. coli* were assessed on Rapid'*E.coli* 2 (24h at 37°C). One *E. coli* O157:H7 (*Stx* negative) was enumerated on CT-SMAC (24h at 37°C). Similar results were seen in all media, unless mentioned. The *Salmonella* and *E. coli* strains grew at 12°C with increases of 3 to 7 $\log_{10}$ cfu/mL after 5 days. At 4°C and 7°C, bacterial counts were stable or decreased. *Yersinia* strains reached stationary phase on day 5 at 12°C and day 9 at 7°C. The *Y. enterocolitica* strains showed bacterial counts of 5 to 8 $\log_{10}$ cfu/mL on day 14 at 4°C. The *Y. pseudotuberculosis* reached 6 to 7 $\log_{10}$ cfu/mL on day 9 at 4 ° C. All *Listeria* strains grew to 6 $\log_{10}$ cfu/mL in beef juice and 8 $\log_{10}$ cfu/ mL in nutrient broth and spinach juice after 5 days at 12°C. Levels ranging from 2 to 5 $\log_{10}$ cfu/mL (beef juice) and 7 to 8 $\log_{10}$ cfu/mL (spinach juice and nutrient broth) were achieved at 7°C on day 14. At 4°C, only two *Listeria monocytogenes* strains grew in spinach juice and nutrient broth with levels from 2 to 5 $\log_{10}$ cfu/mL at day 14.
Listeria and *Yersinia* grew as expected based on available data and predictive growth models. No growth was seen at 4°C and 7°C for *Salmonella* or *E. coli*. Consequently, it is recommended to analyse growth studies and predictive models at low temperatures for the latter 2 pathogens with precaution, especially in conditions near the limits of growth (e.g. low inoculum).

Keywords: Salmonella; Escherichia coli; Listeria; Yersinia; low temperature; growth; foodborne pathogen; model

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P4.47

Non-destructive real time monitoring of meat spoilage by fluorescence Spectroscopy

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Research in the field of rapid methods to determine bacterial contaminations in food is steadily increasing. The detection of bacteria in simple matrices is possible, but to perform rapid quantitative microbial analyses in a complex food matrix like fresh meat is still challenging. Also, non-destructive methods to analyse the bacterial load directly on-line during processing are still missing. The aim of the study was to determine the microbial count on different kinds of fresh meat in real time using a handheld fluorescence spectrometer and to validate the accuracy and applicability of the system.
Storage tests with cuts of porcine *logissimus dorsi* and chicken breast filets were conducted. The samples were stored for 10 days under controlled temperature conditions (pork: 7°C, chicken: 4 °C). Spectroscopic measurements were performed with a handheld fluorescence spectrometer and the total viable count (TVC) and the typical spoilage organisms were analysed by classical microbial cultural enumeration technique. Other quality parameters (i.e. colour, sensory attributes, pH) were analysed to assess their impact on the spectroscopic values. After collection of spectral data and TVC, chemometric methods were used to establish the calibration and prediction models. The accuracy, robustness, and reliability were assessed by cross validation, calculation of statistical validation indices and correlation with the other quality parameters.
The results show a strong correlation between the spectral data and the TVC for both, chicken breast and pork loin. A linear correlation is shown between 2.0 - 8.0 log CFU/cm². For pork, a prediction error (RMSE) of 1.1 log CFU/cm² is assessed. The accuracy of the results is not affected by intrinsic factors or meat colour. For chicken, a similar trend can be observed. The results illustrate that fluorescence spectroscopy is able to determine the TVC on different kinds of fresh meat within seconds. As a rapid and non-destructive technology, fluorescence spectroscopy has a promising potential for applications in the meat industry, i.e. for incoming and outgoing inspection as well as a process control instrument. By knowing the microbial status of a product in real time, the prediction of the real remaining shelf life is possible using predictive food models. The integration of rapid methods into industrial quality control combined with predictive models allows companies to optimise their logistic processes and storage management.

Keywords: rapid methods, predictive microbiology, fluorescence spectroscopy, meat spoilage, total viable count

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P4.48

Modelling the growth kinetics of *Pseudomonas* spp. on *Pleurotus ostreatus* mushrooms under non-isothermal conditions

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The objective of this work was the quantitative description of the growth behaviour of *Pseudomonas* spp. on *Pleurotus ostreatus* mushrooms at different temperatures. First, the primary model of Baranyi and Roberts was fitted to microbiological growth data obtained during aerobic storage of mushrooms at different isothermal conditions (4, 10 and 16°C). Then, the estimated maximum specific growth rate (μ_{max}) was modelled as a function of temperature using a square-root-type model. The estimated μ_{max} at 4, 10 and 16°C was 0.054, 0.079 and 0.106 h^{-1}, respectively. In any case, the highest mean squared error and the lowest adjusted-R^2 value were estimated to be 0.064 and 0.904, respectively, demonstrating that the models satisfactorily described the *Pseudomonas* spp. growth at isothermal conditions. Finally, the differential form of the primary model, merged with the square-root-type secondary model, was solved numerically using the fourth-order Runge-Kutta method, in order to estimate the *Pseudomonas* spp. concentration on mushrooms under dynamic temperature conditions. The performance of the dynamic model was evaluated using the bias (B_f) and accuracy (A_f) factors which were estimated to be 1.018 and 1.044, respectively. As indicated by its satisfactory performance, the dynamic modelling procedure described in this study can be efficiently used for the quantitative estimation of *Pseudomonas* spp. on mushrooms and, thus, for the reliable appraisal of this commodity's shelf-life.
This work has been supported by the project "PhasmaFOOD".

Keywords: Microbiological spoilage; Modelling; Pleurotus ostreatus; Pseudomonas spp.

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P4.49

Purification and characterization of the listeriophage vB_LmoS_293 endolysin and tail fibre proteins and their potential applications against biofilms of *Listeria monocytogenes*

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Listeria monocytogenes is a ubiquitous Gram positive bacterium that is a major concern for food business operators because of its pathogenicity and ability to form biofilms in food production environments. To date, a number of bacteriophages against *L. monocytogenes* have been isolated and some have been approved for use in food processing environments (ListShield and Listex). Listeriophage vB_LmoS_293, a *Siphoviridae* infecting *L. monocytogenes* serotypes 4b and 4e, was previously isolated from mushroom compost. Its lysogenic nature excluded it as a biocontrol candidate for *in situ* applications, however its proteins can be studied for biocontrol purposes. In particular, endolysins are proteins produced by bacteriophages in the host cell, capable of cleaving one of the five bonds of the peptidoglycan cell wall, thus allowing release of progeny phage into the environment. Gram-positive endolysins generally contain two or more domains: one or more catalytic domains, often including an amidase domain and a cell wall binding domain. Tail fibre proteins are components of the phage tail, and contain a cell binding domain (CBD) in addition to other hydrolases that facilitate the injection of the nucleic acids into the host cell. These tail fibre hydrolases could also potentially display antimicrobial activity. In this study, the full length vB_LmoS_293 endolysin (339AA), the Amidase domain of the endolysin (225AA), the full-length tail protein (377 AA), plus a fragment of the tail protein similar to *Lactococcus* phage TP901-1 tripod base (239 AA) were produced recombinantly for the purposes of purification and characterisation. The genes encoding these peptides were cloned into the expression vector, pCri8A and transformed in *E. coli*, expressed and purified with affinity chromatography. Some inactivation against *L. monocytogenes* cells was shown. This study shows the potential of using recombinant proteins for the reduction of *L. monocytogenes*. Future work could lead to the enhancement of hygiene applications and the development of novel procedures for the reduction of *L. monocytogenes* and its biofilm, especially in the food industry.

Keywords: Listeria; monocytogenes; bacteriophage; endolysin; biofilm; food safety

Impact of interventions during food production on microbial biodiversity

P4.50

Modeling bacterial growth of *Lactobacillus plantarum* as a function of temperature

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Lactobacillus plantarum is often the dominating *Lactobacillus spp.* in traditional lactic acid fermented foods based on plant material and is one of the most frequent species related with cheese production that plays an important role in ripening. Furthermore, it has several applications in the food industry and has been used as a starter culture in various food fermentation processes contributing to the organoleptic properties of final products. Studying the growth kinetics of *Lb. plantarum* in a real and synthetic media, considering temperature changes, allows different possibilities to control the development of undesirable microorganisms in food. Further, obtained data may contribute to a better control of fermentation process and may help to clarify in which manner and to which degree will the food environment interfere with the functionality of the strains. For this reason, this work deals with the quantification of temperature effect on the growth of *Lb. plantarum* HM1 in a real and model growth media further with applications of several models in relation to temperature in whole range.

Based on the experiments in a temperature range from 8 to 40 °C, growth dynamics of the studied isolate was positively determined by the cultivation temperature that led to increasing intensity of growth in the exponential phase (except for marginal temperatures). The following equations resulted from fitting the growth rates with Ratkowsky square root model in the temperature range from 12 °C to 37 ° in MRS broth, UHT and lactose-free milk, respectively: GRMRS =0.0171 T+0.1218 (R^2 = 0.971), GRUHT =0.0177 T+0.0335 (R^2 = 0.997), GRlacfree =0.0140 T+0.0719 (R^2 = 0.970). Using Gibson model, optimal temperatures were calculated (T_{optMRS} = 34 °C; $T_{optmilk}$ =33 °C; $T_{optlacfree}$ =32 °C). Verification of data was provided by CTMI model followed with determination other cardinal temperatures (minimal T_{min} and maximal T_{max} temperature) in MRS broth (T_{opt} = 36.6 °C, T_{min} = 2.0 °C, T_{max} = 41.0 °C), UHT milk (T_{opt} = 34.7 °C, T_{min} = 7.8 °C, T_{max} = 41.0 °C) and lactose-free milk (T_{opt} = 34.2 °C, T_{min} = 5.4 °C, T_{max} = 41.4 °C). In a detailed comparison of predictive models, all applied models are suitable for estimation of *Lb. plantarum* growth, while CTMI was most accurate in prediction ($\%D_f$ = 0.8 - 3.9).

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-15-0006

Keywords: Lactobacillus plantarum, growth kinetics, prediction, mathematical modelling

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P4.51

Effectiveness of cook-out on *Listeria monocytogenes* survival in the mushroom production industry

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Cook-out is the hygiene process that is undertaken at the end of mushroom harvest and prior to the beginning of a new crop. The growing tunnel is usually steamed (often to 65°C for 12 h) and subsequently sanitised and re-steamed, in order to inactivate all the microbes present after harvesting. *Listeria monocytogenes* is one of the organisms of concern that should be inactivated during cook-out. In this study, the presence of *L. monocytogenes* was investigated in the growth substrates and in the processing environment before and after cook-out, at two mushroom growing facilities. The results showed that the required temperature of 65°C was achieved in the growth substrates, with 100% inactivation of *L. monocytogenes*. However, at both facilities, *L. monocytogenes* was isolated from floor swabs after cook-out, which led to investigation of the floor temperature. It was subsequently determined that the target floor temperature (65°C) was not achieved. Before cook-out, 88% of the floor swabs were positive and after cook-out 16% were positive. These results highlight the effectiveness of the cook-out procedure on the growth substrates, although more attention is required on the post cook-out treatments of the floors, in order to reduce the possibility of survival of *L. monocytogenes*.

Keywords: Listeria; monocytogenes; mushroom; agaricus; bisporus; hygiene; coockout

Impact of interventions during food production on microbial biodiversity

P4.52

Microbiological quality and safety of raw goat milk

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In recent years, the consumption of goat milk and goat milk products has been increasing due to its enhanced nutritional properties in comparison to cow milk. Currently, there is limited information on the microbiological quality and safety of raw goat milk in Poland.

The aim of the study was to assess the hygienic quality of raw goat milk and microbiological hazards to human health.

A total of 100 samples of raw goat bulk-tank milk from 30 different dairy farms in Poland were collected in 2017. Hygienic quality of the samples was determined in relation to the number of *Escherichia coli*, *Enterobacteriaceae*, coagulase-positive staphylococci and total bacteria count. The milk samples were also screened for the presence of selected foodborne pathogens such as *Listeria monocytogenes, Salmonella* spp*., Escherichia coli* O157*, Campylobacter* spp., *Bacillus cereus* and *Yersinia* spp. The quantitative analysis was performed according to the standard ISO methods, whereas qualitative study using ELFA method and/or standard ISO methods.

The number of *E. coli* in raw milk samples was determined at an average level of $2.3x10^2$ cfu/ml, *Enterobacteriaceae* - $1.2x10^5$ cfu/ml, coagulase-positive staphylococci - $8.9x10^2$ cfu/ml. The average number of microorganisms in raw milk was $5.6x10^6$ cfu/ml, and the percentage of sample contamination >1500000 cfu/ml accounted for 20.4%. The maximum number of these bacteria was up to $1.7x10^4$, $5,3x10^6$, $2.7x10^5$ cfu/ml and $2.2x10^8$ cfu/ml, respectively. *L. monocytogenes* was detected in 8.0% of samples of raw goat milk. *E. coli* O157 (3.0%), *B. cereus* (11.0%), *Yersinia* genus (65.0%) and two isolates (2.0%) of *Campylobacter* spp. were also identified. Most of the analyzed samples (87.8%) were contaminated with *S. aureus*. *Salmonella* spp. was not isolated from any of the tested samples.

In conclusion, the number of *E. coli*, *Enterobacteriaceae* and coagulase-positive staphylococci in goat milk products have met the microbiological criteria listed in Commission Regulation (EC) 2073/2005, however some results of total plate count have exceed the hygiene criterion included in Commission Regulation 853/2004. The results obtained in this study indicate also the presence of foodborne pathogens in raw goat milk which may pose a risk to health of consumers. Therefore, the most important pathogenic microorganisms responsible for causing human zoonotic diseases as well as the hygienic quality should be controlled.

Keywords: goat milk, microbiological quality, bacterial pathogens

Impact of interventions during food production on microbial biodiversity

P4.53

Meat quality and spoilage characteristics of two different rearing lines in industrial poultry production

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Animal specific factors, such as breeding, fattening and stress resistance, are known to have a remarkable influence on meat quality parameters. The meat industry increasingly benefits from the knowledge about these relationships for the development of high quality meat production lines. Differing meat composition, nutritional values as well as meat pH and water holding capacity can have a striking influence on the microbial ecosystem of the food. But the effect of different production lines and husbandry conditions on the microbial diversity, the spoilage process or consumer acceptance is hardly investigated.
The aim of the study was the investigation of a special slow growing corn-fed chicken line in comparison to conventional industrial poultry production and the effect on microbiology, meat quality and spoilage.
The corn-fed production line is characterised by slow-growing poultry race, a diet based on 50% corn, enhanced roaming and the strict avoidance of antibiotics. Both production lines were received from a German poultry meat producer, where slaughter and processing took place in the same processing facility. At the laboratory, the poultry filets were packed aerobically and stored at 4°C under temperature controlled conditions for 12 days. The analysis of meat quality and microbiological parameters started 24h after slaughter and was conducted subsequently every two days during storage. Total viable count and typical spoilage microorganisms (Enterobacteriaceae, *Brochotrix thermosphacta, Pseudomonas spp.)* were investigated. The research comprised physicochemical parameters such as pH-value, meat colour, TBARS and cooking and thawing loss. The nutritional analysis focused on protein, water, intramuscular fat, collagen content as well as the amount of glucose and lactate. Additionally, sensory investigations to determine the freshness of the samples and purchase decision were conducted.
There were no differences in the diversity of microflora on the filets. Additionally, no differences in initial bacterial counts or microbial shelf life were observed. In contrast, significant differences in colour were measured, meaning that the corn-fed production line showed more yellowish filets. Other meat quality parameters as well as nutritional content showed no differences. Thus, different husbandry conditions did not lead to a changed microbial spoilage or meat quality. But consumer acceptance can be influenced due to the changes of meat colour.

Keywords: predictive microbiology, fresh poultry, meat quality, microbial spoilage

Impact of interventions during food production on microbial biodiversity

P4.54

Application and validation of an alternative approach to quantification of live and viable *Campylobacter* spp. in chicken carcasses

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Thermophilic Campylobacter are currently the most frequently isolated bacterial species in contaminated poultry worldwide. Recent estimates published by the Robert Koch Institute (RKI) showed a national prevalence of more than 70 000 cases of campylobacteriosis alone in 2016. Poultry meat constitutes the major reservoir of the species, with C. jejuni representing the predominantly isolated species. Generally, campylobacteriosis manifests as a gastrointestinal disease with diarrhoea, which in seldom cases can lead to an autoimmune condition.

As a result of the epidemiological significance of the organism, a reliable and rapid method for the quantification of live bacteria in various food matrices is important. A real-time PCR approach employing genomic equivalents as quantification standards, combined with the application of the live/dead discriminatory dye propidium monoazide (PMA), is here applied. This study focuses on recent experiences in our laboratory employing this PMA-qPCR approach, integrated with an internal sample process control for monitoring among others, possible DNA loss during sample processing. Data on validation of the method, coupled with its application on routine samples (both retail and slaughterhouse) will be presented. Additionally, preliminary studies on the feasibility and applicability of the assay, carried out in two laboratories (LGL and BfR) will be presented, as prelude to a proposed international ring trial according to ISO 16140.

Keywords: thermophilic Campylobacter, Quantification, PMA-qPCR, Validation

Impact of interventions during food production on microbial biodiversity

P4.55

Application of *Lactobacillus reuteri* and glycerol as a novel approach to control *Campylobacter* colonization in chicken gut

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Chicken meat is a good source of high-quality lean protein; however, consumption of chicken meat is the main source for *Campylobacter* food-infection. Current interventions in the poultry meat production chains have limited effect or are hampered by economic aspects or consumer acceptance. Novel approaches to naturally reduce *Campylobacter* contamination of chicken flocks and ultimately chicken meat are needed. *Lactobacillus reuteri* is a commensal in chicken gastrointestinal (GI) tract, and forms natural biofilms in the crop. *L. reuteri* of chicken have the ability to produce reuterin, a potent antibacterial system produced from glycerol. Reuterin is a dynamic multi-compound system consisting of hydrated and dimer forms of 3-hydroxypropionaldehyde (3-HPA). We hypothesize that administration of reuterin-producing *L. reuteri* isolated from chicken combined with glycerol feeding result in the *in situ* production of antibacterial reuterin which can reduce *Campylobacter* infection in chicken gastrointestinal tract.
To test our hypothesis, we isolated 51 reuterin-producing *L. reuteri* from crop and cecum of Swiss broiler chicken. Based on their ERIC-PCR profiles, 25 different strains were further characterized. The whole genome of those isolates was sequenced. *L. reuteri* isolates were screened for antibiotic resistance against erythromycin, vancomycin, ciprofloxacin, penicillin, tetracycline, colistin, sulfamethoxazole and cefotaxime using microbroth dilution test to determine the Minimal Inhibitory Concentrations (MICs); the occurrence of antibiotic resistance (AMR) genes was also verified by PCR. Among the isolates *L. reuteri* PTA6-C2 was selected for its high reuterin yield (400 mM) by biotransformation of 600 mM glycerol and absence of AMR genes. The efficacy of reuterin (3-HPA) was then tested against a broad panel of *C. jejuni* (n =14) and *C. coli* (n=3). The MICs and Minimal Bactericidal Concentrations (MBCs) of reuterin against *Campylobacter* spp. were low, 156 µM and 313 µM, respectively, indicating a high sensitivity of the species in particular compare to the sensitive indicator, *E. coli*.
Here we showed the frequent presence of reuterin producing *L. reuteri* in chicken gut and very high bacteriostatic and bactericidal effects of reuterin against *Campylobacter*. The effect of *L. reuteri* supplementation combined with glycerol will be tested on *Campylobacter* in a new fermentation model of chicken gut (PolyFermS) inoculated with immobilized cecum microbiota.

Keywords: Campylobacter, Biocontrol, Lactobacillus reuteri, reuterin

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P4.56

Application of nanotechnology to decontaminate food industry surfaces – Nanoclean

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Biofilms have been associated with several problems in the food industry as the conditions in food processing environment may support biofilm formation. Biofilms may posing a serious health hazard because upon subsequent de-attachment, that constitutes a significant source of food contamination and leading to foodborne disease outbreaks. To this respect, emphasis should be given on the removal of microorganisms from food industry surfaces by the use of disinfectants. In recent years, an effort has been made to find novel methods for controlling this phenomenon. Nanoparticles technology appears to be an attractive alternative one regarding surface disinfection in food processing industry, since nanoparticles possess potential effect as antimicrobial agents and inhibitors of biofilm formation. The project aims to create a new surface disinfectant consisted of TiO_2 nanoparticles and combined with UV irradiation. After the product development, the disinfecting and antibiofilm effectiveness against foodborne pathogens is being checked on surfaces and materials used in food industry. More specifically, in the current project, the potential antimicrobial effect of the developed product against growth and survival of *Salmonella enterica* ser. Enteritidis, *Listeria monocytogenes* and *Escherichia coli* is being monitored. In addition, pilot application is carried out in two food industries, at least. Through this plan, a more holistic approach is being implemented testing the effectiveness of nanoparticles for surface disinfection within the food industry, aiming thus in food safety enhancement.

Keywords: nanotechnology, decontamination, biofilm, food industry

Impact of interventions during food production on microbial biodiversity

P4.57

Modelling thermal inactivation of *Staphylococcus aureus*

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Slovak cheese Parenica, an artisanal pasta-filata-type of cheese, is made from full-fat raw milk curd. In the traditional way of production, the steaming and stretching of the curd at 60 to 65 °C is the only heat-treatment process used. In connection with consumption of raw milk cheeses the potential microbial threat can be also *Staphylococcus aureus* due to the production of heat-resistant enterotoxins. Therefore, the aim of our work was to study thermal inactivation of *S. aureus* isolates (14733, 2064 and 9V1). The data from experiments performed in glucose-tryptone-yeast extract broth (GTYE) and in UHT milk at 51 °C, 53 °C, 55 °C, 57 °C, 61 °C and 63 °C were modelled using Weibull's model and subsequently, *D*- and *z*- values were calculated.

A considerable heat-resistance of enterotoxigenic *S. aureus* 14733 isolate compared with other studied isolates especially in temperature range 51 – 57 °C was found. *4D*-values of 18.0 to 2.6 h in GTYE broth and 52.8 to 4.4 h in UHT milk were calculated at these temperatures, in order. On the other hand, the *4D*-values of 6.51 - 0.12 h and 8.60 - 0.51 h in GTYE broth and in UHT milk, respectively were found out for the *S. aureus* 9V1 isolate.

Lethality kinetic curves of non-toxigenic *S. aureus* 2064 were linear showing a significant higher heat-resistance at 61 and 63 °C in comparison with previous isolates. The *4D*-values of the isolate 2064 at 61 °C and 63 °C were 0.61 h and 0.51 h in GTYE broth and 2.37 h and 1.42 h in UHT milk, respectively. However, the enterotoxigenic isolate 14733 showed lower *4D*-value 0.28 h and 0.66 h at 63 °C in GTYE broth and UHT milk, respectively.

Our experiments confirmed the variability in survival of individual *S. aureus* isolates. Therefore, the obtained results could contribute to the evaluation of microbiological safety and quality of traditional Slovak cheeses manufacture.

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Keywords: heat-resistance; 4D-values; strain variability

Impact of interventions during food production on microbial biodiversity

P4.58

Innovative changes in cleaning to enhance production efficiency and reduce environmental impact

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Cleaning and disinfection is a time and money consuming process. A five-day working week normally requires 5 cleaning shifts per week. If the production time is increased from one to five shifts, traditional cleaning can be reduced to three shifts per week. This will lead to a 40% reduction in both time and water consumption used for traditional cleaning procedures.
The wording and the focus in national legislations and certification schemes differs, but the overall conclusion is, that it is possible to implement a risk-based cleaning regime for production facilities if acceptable levels of food safety and shelf life are maintained. DMRI has investigated if it is possible to extended production time by changing the traditional daily cleaning paradigm of abattoirs and meat processing companies. In the study the following topics were assessed to evaluate the potential of introducing a risk-based cleaning regime:
· Microbial baseline at the current ratio between production and cleaning time (microbiological data, temperatures and accumulation of product residues)
· Food safety (predictive microbiology and challenge test)
· Methods for ”in-process” cleaning (steam vacuum, alcohol wipes)
· Shelf life trials (microbiological number and diversity, sensory impact)
In the studied cases, the microbial baseline showed no increase in bacterial load from a few hours after production start-up to the end of production (up to 18 hours).
Predictive microbiology and challenge tests showed that *Yersinia enterocolitica, Salmonella* and *Listeria monocytogenes* are the major pathogens of concern. However, the growth of spoilage bacteria is faster and thus a critical parameter to maintain products with a good shelf life.
On smooth surfaces, steam vacuuming or cleaning with alcohol-based wipes may reduce the bacterial count between 1 - 3 log cfu/cm^2. This is a sufficient reduction to maintain a low microbial load in the production. A major challenge is to clean equipment that cannot be readily disassembled and to clean joints in modular conveyor belts and other non-smooth product contact surfaces.
Shelf life trials (sensory, microbial count and diversity) showed that production time could be extended without reducing the shelf life of the products when “in-process” cleaning was used at specified critical areas.
The project showed that it is possible to implement an extended production time, provided that the ”in-process” cleaning can be implemented in a cost-effective way.

Keywords: extended production time, risk-based cleaning, shelf life, microbial diversity, food safety

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P4.59

Prediction of *Listeria monocytogenes* growth in thermally processed, sliced at retail, deli meats during refrigeration

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Thermally processed meat products are classified as foods of a high risk for listeriosis. In particular, those cut at the retail outlet are related with five times more cases in comparison to pre-packaged ones. The objective of the study was to monitor and compare the chemical (nitrites), physicochemical (pH, water activity) and microbiological (Lactic Acid Bacteria, LAB) factors of thermally processed, retail sliced deli meat products and to evaluate their effect on *L. monocytogenes* growth during domestic storage of these products using predictive microbiology tools. Retail sliced deli meat products (turkey, ham etc.) of various brands were purchased from 3 super-market chains. Nitrite content was determined by ISO 2918-1975 reference method and LAB were enumerated using de Man, Rogosa & Sharpe Agar. The results showed that the parameters affecting *L. monocytogenes* growth ranged as following: pH 5.83-7.27 (mean 6.57), a_w 0.965-0.988 (mean 0.978), nitrites 0.47-207.55ppm (mean 35.6) and LAB 2.56-7 log (cfu/g) (mean 4.28). Additional experiments showed that the latter LAB levels were exclusively attributed to contamination during handling and slicing. The above data were further used to predict the growth of *L. monocytogenes* during a domestic storage scenario of 7 days at 7°C. The predicted growth of the pathogen ranged from 0.66 to 2.62 log (cfu/g) with a mean of 1.78 log (cfu/g). For nitrite concentrations above 50ppm, this factor was the most important for *L. monocytogenes* growth. For nitrite concentrations below 50ppm (89% of the samples), the most important factor for the pathogen's growth was the initial population of LAB. In conclusion, the present study provides quantitative data on the factors affecting *L. monocytogenes* growth in thermally processed, retail sliced meat products. In addition, the study provides predictions for the pathogen growth under domestic storage scenarios. These data can be the basis for the development of a risk assessment model which can be used to identify effective mitigation strategies for improving the safety of thermally processed meat products.

This research was conducted within the framework of a grant agreement "Evaluation of listeriosis risk related with the consumption of non-prepackaged RTE cooked meat products" (GA/EFSA/AFSCO/2016/04) commissioned by the European Food Safety Authority. The opinions expressed in this publication are those of the authors only and do not represent EFSA´s official position.

Keywords: Listeria monocytogenes, meat products, retail sliced, growth prediction

Impact of interventions during food production on microbial biodiversity

P4.60

Identification of tyramine producing microorganisms in fermented food

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Tyramine is produced by food-resident tyrosine decarboxylating microorganisms (MO) during food processing and storage. Fermented food such as cheese and salami-type sausages is a major source for poisonous tyramine, so far neglected, leading to health problems. It is still not clear which MO are the main responsible tyramine producer in cheese and salami and which production factors such as pH, salt content, temperature, affect the production of tyramine.
Objectives: To identify the major tyramine-producing MO in cheese and salami and to improve the knowledge about the tyramine producing mechanisms.

Methods: In a first step a variety of soft, semi-hard or hard cheeses from milk animals cow, goat or sheep and different treatments to get raw, thermized or pasteurized milk and fermented sausages were selected. These products were screened on selective media for lactic acid bacteria (LAB), including enterococci and also for staphylococci. All isolates were screened genotypically by PCR targeting the tyramine decarboxylase gene *tdc* and phenotypically by a pH induced differential medium. An Ion exchange chromatography (IC) procedure was developed to determine tyramine and other biogenic amines in food. In a second step, a mini cheese model was applied to examine the capacity of different isolates to produce tyramine in cheese like conditions.

Results/Conclusion: We screened 212 cheeses and 62 dry sausages by PCR and pH induced media for *tdc* and by IC on tyramine. A total of 144 cheeses and 29 sausages contained tyramine. The content in cheese was in a range between 5.7 mg/kg and 984.4 mg/kg and for sausages between 24.6 mg/kg and 785.3 mg/kg. In 87% of the cheeses containing tyramine a high content of enterococci between 3.7 x 10^3 and 3.5 x 10^7 cfu/g was found. Each *Enterococcus* isolate was positive for *tdc* gene PCR-amplicon and showed a color change in the differential medium. This suggests that enterococci play an important role in tyramine production in cheese. However, in sausages, it seems that coagulase-negative *Staphylococcus* spp. and LAB which served as added starter cultures or naturally occurred and act as spontaneous starter cultures have the main impact on tyramine formation. 66 out of 79 *Staphylococcus* spp. isolates from sausages generated *tdc* PCR-amplicons. Nevertheless, there is also a correlation between *Enterococcus* spp. and tyramine. In sausages mostly the higher the cell counts of *Enterococcus* spp., the higher the amount of tyramine.

Keywords: Biogenic Amine, Tyramine, Fermented Food, Cheese, Sausages, IC

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P4.61

Influence of stress response on the growth and survival of *Lactobacillus acidophilus* in fermented milk

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Lactobacillus acidophilus is a probiotic bacterium generally known and employed for the production of many fermented milk products. Some studies have reported that their exposure to stress prior fermentation could change their metabolism, leading to production of new compounds. Despite this, no studies have investigated the impact of these stresses on the kinetic growth parameters of probiotic bacteria. Therefore, the aim was to evaluate the influence of previous exposure of *L. acidophilus* (LA-5®, DSM 13241 strain) to three different stress conditions (acid, osmotic and oxidative) on its growth kinetic parameters during production of fermented milk. In addition, the acidification profile and probiotic survival during the 21-day storage period of fermented milk at 4°C were also evaluated. LA-5® was cultured in MRS broth at 37°C/10h, centrifuged and added to the respective broths with the stressing agent (HCl, NaCl and H_2O_2) at concentrations around 10^7 CFU/mL. The stress conditions were as follow: pH 4.5 for 3h; 3.5% NaCl for 6h; and 10.54 mM H_2O_2 for 30 min. (conditions previously optimized to result in 1 log cycle reduction in LA-5® population). Then, cells were centrifuged, suspended in whole UHT milk, following fermentation in a water bath at 42°C until pH reached 4.7-4.6. During fermentation, 10 mL aliquots of milk were collected for acidification and microbial analyses. Counts of LA-5® were carried out by pour plating in MRS agar, following incubation 37°C/48h. The acidification profile was evaluated by titrating the samples with 0.1 N NaOH solution and the pH was read using a digital pHmeter. These analyses were also performed throughout the storage period, whereas the enumeration of probiotic bacterium was done after 7, 14 and 21 days. Then, the counts of LA-5® were transformed into log and further plotted as a function of time along the fermentation. Baranyi model was fitted to the growth data using the add-in for Microsoft Excel DMFit. All experiments were repeated twice (independent duplicates). The stress conditions employed did not result in significant variations in the growth rate or acidification profile of LA-5® when compared to the control condition. In addition, all conditions tested led to stable viable probiotic counts during the storage period at 4°C. Ongoing analysis will reveal whether the stress conditions applied resulted in the modification of compounds' profile produced by LA-5® during milk fermentation.

Keywords: Predictive microbiology; lactic acid bacteria; milk product

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P4.62

New term for effect of temperature on pH_{min}-values in cardinal parameter growth models for *Listeria monocytogenes*

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Cardinal parameter models for growth and growth boundary of *L. monocytogenes* (CPM-*Lm*) are popular, extensively validated and widely used for various foods in the assessment and management of risk. Interestingly, available CPM-*Lm* includes very different pH_{min}-values from 4.3 to 5.0. This can be due to differences in the mathematical terns used to estimate pH_{min}-values and to strain variability as often suggested. However, the experimental conditions used to estimate pH_{min}-values remain little studied although the minimal pH-values supporting growth is known to depend on other environmental conditions including temperature. Therefore, the objective was to study the influence of temperature on pH_{min}-values of *L. monocytogenes* as used in CPM-Lm.
The combined effect of temperature and pH on maximum specific growth rate (μ_{max}) for eight different *L. monocytogenes* strains were determined experimentally by using Bioscreen C or collected from the literature (287 μ_{max}-values). 16 pH-values from 4.4 to 6.8 and eight temperatures from 5°C to 37°C were studied. At each temperature the pH_{min}-value was estimated by fitting a simple pH_{min}-model (AEM, 63, 2355-2360, 1997). pH_{min}-values decreased from 5.0 at 5°C to 4.3 at 20°C and then increased to 4.7 at 37°C. These changes in pH_{min}-values has major influence on predictions from CPM-*Lm*, particularly for products with low pH values of less than about 5 and a new pH_{min}-model to describe the influence of temperature on pH_{min}-values in CPM-*Lm* was developed as shown below.
$0°C \leq T < T_{ref}$; $pH_{minT}=pH_{min0}-T*((pH_{min0}-pH_{minR})/T_{ref})$
$T_{ref} < T < 37°C$; $pH_{minT}=pH_{minR}+(T-T_{ref})*((pH_{min37°C}-pH_{minR})/(37-T_{ref}))$
where T is the storage temperature (°C); pH_{minT} the fitted pH_{min}-value at T°C; T_{ref} the estimated reference temperature (°C); pH_{min0} and pH_{minR} the fitted pH_{min}-values at 0°C and T_{ref} (°C), respectively.
The fixed pH_{min}-value from an existing CPM-*Lm* including 12 environmental parameters (IJFM, 141, 137-150, 2010) was substituted by the new pH_{min}-model and the model performance has been evaluated for 33 growth/no growth responses of *L. monocytogenes* in a well characterized food with pH below 5.
Average bias and accuracy factor values were 1.16 and 1.27 for 30 growth curves at constant temperatures. The new pH_{min}-model can estimate the pH_{min}-value for *L. monocytogenes* based on temperature storage conditions and this markedly extend the limit of applicability of the existing CPM-*Lm* from pH 5.6 to pH 4.4.

Keywords: Mathematical modelling, validation, product development

Impact of interventions during food production on microbial biodiversity

P4.63

Effect of UV-C treatment on lethality of *Escherichia coli, Listeria monocytogenes, Bacillus subtilis* and *Aspergillus niger* in soymilk

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The main objective of this study was to evaluate the effect of flow rate (FR) on the effectivity of short-wave ultraviolet (UV-C) treatments on *Listeria monocytogenes*, *Escherichia coli*, spores of *Bacillus subtilis* and conidiospores of *Aspergillus niger* inoculated in soymilk (absorbance coefficient ($AC_{254\ nm}$) of 446.2 ± 42.3 cm^{-1}). Treatments consisting in different UV-C doses (20, 80, 120 and 160 J/mL) applied with different FR (10, 42 and 65 mL/s) were evaluated. Treatments were performed using an UV-C equipment consisting on a peristaltic pump that feeds a circuit composed by two serially connected UV-C reactors formed by an UV-C lamp (41 mW/cm^2 at 254 nm) each one, protected with a 765 cm long concentric quartz tube with 1 mm of path between tubes for samples. FR was adjusted increasing the speed of the peristaltic pump. The reduced thickness of the sample when it passes through the reactor (just 1 mm) does not allow the formation of a turbulent flow nor a high Re number even at the highest FR tested.
In general, conidiospores of *A. niger* showed to be most resistant ($p< 0.05$), followed by the spores of *B. subtilis,* that were at the same time more resistant than the vegetative *E. coli* and *L. monocytogenes*. The effectivity of the UV-C treatments increased when the FR and dosage were increased. Considering the same final dose (160 J/mL), the more times the matrix passed through the reactor the more effective resulted the treatment in all the tested microorganisms. After treatments at the highest FR (65 mL/s) and dosage (160 J/mL) lethality values exceeded 5 Log_{10} CFU/mL for all microorganisms tested. *L. monocytogenes* showed a higher sensibility when increasing the FR, achieving a 5 Log_{10} CFU/mL reductions when doses above 80 J/mL at a FR of 65 mL/s or 160 J/mL at a FR of 42 mL/s were used.
Generating turbulent flows has been suggested as a strategy to increase the germicidal efficacy of UV-C treatments for matrices with a high AC_{254nm}. In our work, the Re number generated was never high enough to consider the flow as turbulent ($Re < 2100$), but it was possible that increasing the number of passes caused a mixing effect that increased the exposure of the particles to UV-C, helping to overcome the lack of penetration of UV-C. From the results obtained, it can be inferred that in that way UV-C could guarantee not only the safety but also the stability of soy milk from the microbiological standpoint.

Keywords: UV-C, continuous reactor, soymilk, Aspergillus niger, Escherichia coli, Listeria monocytogenes

Impact of interventions during food production on microbial biodiversity

P4.64

Survey of blue pigment-producing *Pseudomonas* along a production chain of fresh mozzarella cheese

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The "blue" discoloration defect of mozzarella cheese is still a frequent and unsolved problem for the dairy industry. This defect was associated to the presence of strains of the *Pseudomonas fluorescens* group contaminating processing water. However, not all the strains/species of the *Ps. fluorescens* group are able to produce the blue pigment. This is due to both the wide phenotypic and physiological heterogeneity of the group and the influence of substrate and culture conditions on the production of the blue pigment. In this work, an ecological study to track the distribution in a dairy plant of blue pigment-producing *Pseudomonas* spp. strains was carried out. Samples of milk, cheese curd, processing waters, brine, preserving fluid, and finished products were taken and analyzed all over the production line. The causative agents of blue discoloration were preliminary identified by 16S rRNA gene sequencing as belonging to the *Ps. fluorescens*/*Pseudomonas gessardi* subgroups. The pigment-producing biotypes were found along the production chain over the stretching plant. The spoiling strains were isolated from the chilled water used for cooling and hardening of the mozzarella, which then contaminates the product and/or the preserving fluid during further cheese salting and conditioning. This conclusion was supported by the finding of genotypically identical, blue pigment-producing strains from samples of water along the cooling line, the preserving fluid, and mozzarella sampled at the dairy plant. Interestingly, other strains isolated from the cooling line in different sampling periods and not producing the pigment resulted genotypically similar to the *Ps. fluorescens*/*Ps. gessardi* subgroup strains giving the blue. These data showed a wide phenotypic heterogeneity between strains isolated from a mozzarella plant. A strict relationship between the dairy plant, the processing fluids and the environmental conditions, which may change according to seasonal variability, might explain this heterogeneity. Further in-depth studies to highlight the phenotypic and genetic differences between producers and non-producers of the blue pigment are underway.

Keywords: "blue" mozzarella, Pseudomonas spp., microbial spoilage, Mozzarella cheese.

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P4.65

Determination of the culture conditions favoring the production of bacteriocins by *Lactococcus lactis* QMF 11

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Lactic acid bacteria and their products (bacteriocins) could be eco-friendly antimicrobials for improving food safety. *Lactococcus lactis* QMF 11, isolated from Brazilian fresh Minas cheese, produces bacteriocin like substances capable of inhibiting the growth of foodborne pathogens, mainly *Listeria monocytogenes*. The present study aimed to identify, through the critical dilution essay, conditions that could favor the production of bacteriocins by *L. lactis* QMF11. The analyzed parameters were broth culture medium (APT, APT-EC, APT-YE, BHI, TSBYE, Nutrient, MRS), temperature and addition of surfactants (10 µL/mL of Tween® 80 or Tween® 20). *L. monocytogenes* ATCC 7644 was used as indicator microorganism. The results indicated that the production of bacteriocin like substances by *L. lactis* QMF 11 was higher in MRS broth than in the other evaluated culture media and that the production of the antimicrobial substances occurred between 20 and 30 °C, with the optimum incubation temperature between 25 and 30ºC. Adding Tween® 80 to the MRS broth, at an initial pH of 6.0 and incubation temperature of 25 °C, resulted to the highest bacteriocin yields.

Keywords: Lactic acid bacteria, bacteriocins, Listeria monocytogenes

Impact of interventions during food production on microbial biodiversity

P4.66

Characterization of a non-cytotoxic bacteriocin produced by the bacteriocinogenic *Enterococcus hirae* ST57ACC isolated from a Brazilian artisanal cheese

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Bacteriocins produced by *Enterococcus* spp. are being increasingly explored by the pharmaceutical industry and food industry due to their already known antimicrobial potential. *E. hirae* ST57ACC was isolated from a Brazilian artisanal cheese and it was previously characterized as a bacteriocinogenic strain, possessing some beneficial properties and free of virulent determinants and antibiotic resistance genes. Due to the beneficial potential of *E. hirae* ST57ACC, this study aimed at characterizing the expression, production and cytotoxicity of its bacteriocin. *E. hirae* ST57ACC was subjected to GeXP fragment and data analyses and presented expression of bacteriocin ABC-transporter and ATP-binding and permease protein, typical for two-component bacteriocins (class IIb). Cultures of *E. hirae* ST57ACC at 1, 2, 5 and 10% were inoculated in MRS broth, incubated at 37 °C, and the growth, pH and bacteriocin production were monitored in each 3 h; no relevant differences were observed for growth (expressed as biomass, mg/mL) and pH, but bacteriocin production was detected only after 9 h of incubation by cultures inoculated at 5 and 10%. In addition, cultures of *E. hirae* ST57ACC at 5% were inoculated in MRS and incubated at 37 °C in conventional flasks and in a bioreactor (constant stirring at 50 rpm and pH control to 5.5), and the growth (biomass, mg/mL), pH and bacteriocin production (AU/mL) were monitored in each 3 h; despite the difference in pH variation among fermentation systems, strain growth and bacteriocin production were similar. Bacteriocin produced by *E. hirae* ST57ACC was purified in a three-step protocol, involving cation exchange, gel filtration and RP-HPLC, with final yield of 3.05. The cell-free supernatant of *E. hirae* ST57ACC and the partially-purified fraction of its bacteriocin, obtained by elution with 60% acetonitrile, were tested for cytotoxicity in HT-29 cells, and presented 123.74 and 125.84%, respectively, of cell viability when compared to cells treated with Triton X100. Assuming that non-cytotoxic effects were observed on human cells, the bacteriocin produced by *E. hirae* ST57ACC can be considered safe in this aspect, highlighting therefore its potential as a biopreservative tool and control of pathogenic agents.

Acknowledgments: CAPES, CNPq, FAPEMIG, FUNARBE.

Keywords: Enterococcus, bacteriocin, cytotoxicity, production, ABC

Impact of interventions during food production on microbial biodiversity

P4.67

Effect of simulated cleaning in place on the germination of *Bacillus cereus* spores isolated from extended shelf life milk

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Bacillus cereus spores are resistant to heat and chemicals and can attach and form biofilms on stainless steel surfaces, indicating that the spores could potentially contribute to the contamination of Extended Shelf Life milk (ESL). *B. cereus* were found to be present in the aseptic ESL filler nozzles and ultimately they may be dispensed into final ESL milk and multiply at refrigeration temperatures as well as during the distribution chain. The objective of this study was to determine the effect of simulated cleaning in place (CIP) on the germination of *B. cereus* spores isolated from ESL milk processing with the aim of improving the shelf life and safety of ESL milk. *Bacillus cereus* strains were isolated from filler nozzles and raw milk. Spore isolation was done where after spores were subjected to simulated CIP processes. Flow cytometry, epifluorescence microscopy, scanning electron microscopy as well as transmission electron microscopy were used to analyse physical characteristics of spores following simulated CIP treatment. Biofilm formation and growth kinetics were also determined. CIP damaged at least 98% of all spores in all three *B. cereus* strains, however, 0,1% of spores remained intact. Spores were still capable of germination, regrowth and biofilm formation following CIP treatment and variation among strains in terms of response to treatment was evident. Due to some *B. cereus* spores still being capable of germinating and growing following CIP treatment it was concluded that CIP treatment was not entirely effective and that the final ESL milk product could potentially become contaminated which could lead to a reduced shelf life as well as food safety risks.

Keywords: Bacillus cereus, milk, ESL milk, CIP, spores, biofilms

Impact of interventions during food production on microbial biodiversity

P4.68

Impact of frozen storage on microbial quality of prepared cassava

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Cassava, *Manihot esculenta*, a shrubby tuberous plant of the Euphorbiaceae family is cultivated mainly for its starchy roots. It is an important raw material and is used to produce many staple products in Africa and Asia. The main challenge facing the usage of cassava roots by processors is that, it is highly perishable, deteriorating within three to four days of harvest making post-harvest losses high. Therefore further research is required to optimise its handling and processing to improve its quality and shelf life. In this study, assessment of freezing as a preservation method for cassava roots over a period of 3 months was carried out. Three cassava varieties: Bitter cassava (30572); Fortified cassava (1011371) and Sweet cassava (Isunikankiyan) were studied. The samples were prepared under good manufacturing practices and were held at -18°C for 90 days. Microbial enumeration was carried out monthly by checking total viable counts on Plate Count Agar (PCA) and Potato Dextrose Agar (PDA) for bacteria and fungi respectively, following decimal dilutions in Maximum Recovery Diluent (MRD). Colony counts of bacteria were checked after 48 hr and for fungi after 120 hr. Bacterial counts assessed monthly showed a decrease from 3.0 x 10^4 to 1.8 x 10^3 (cfu g^{-1}) in Bitter cassava, from 6.0 x 10^2 to 3.0 x 10^2 (cfu g^{-1}) in the Fortified cassava. Yeast counts declined from 6.9 x 10^3 to 1.0 x 10^3 (cfu g^{-1}) in Bitter cassava, from 5.0 x 10^2 cfu g^{-1} to levels below the detection limit in Fortified cassava and from 9.0 x 10^2 cfu g^{-1} to levels below the detection limit in the Isunikankiyan cassava. There was no mould found in all cases. The physical examination regarding colour, odour and texture was consistent throughout the period of study. All the above suggest that freezing at -18°C is a promising method of storing cassava roots intended for long-time use.

Keywords: Cassava, freezing, microbial enumeration

Impact of interventions during food production on microbial biodiversity

P4.69

Screening of Listeria *monocytogenes* strains for thermal inactivation and growth capacity under refrigeration evaluation in ice cream syrup

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Listeria monocytogenes is a psychrotrophic pathogen that can grow at low temperatures. The presence of this pathogen has been reported in ice cream and due to its ubiquitous nature, may be hard the elimination from process line. Even though some studies have reported on the D-value for *L. monocytogenes* in milk, there is no study aiming to assess the behavior of this bacterium during ice cream processing. Therefore, the purposes of this work were to characterize as (1) the ability of *L. monocytogenes* strains isolated from ice cream and popsicle samples to grow under refrigeration in ice cream syrup and (2) to select the most thermal resistant strains. For the tests, 14 strains of *L. monocytogenes* isolated from ice cream (n=4) and popsicles (n=10) were utilized. For the ability to grow under refrigeration, suspensions of vegetative cells of each isolate were individually inoculated in vanilla ice cream syrup at concentration of 2 log CFU/mL. The samples were incubated at 4°C and counts was done in TSA-YE after 24 and 355h of storage. For the selection of the most heat tolerant strain, aliquots of ice cream inoculated with 7 log CFU/g of each isolate were placed inside capillary tubes and submitted to thermal shock at 70°C/15s. The enumeration was done before and after thermal shock and the number of decimal reductions was calculated. All experiments were conducted in independent duplicates. The results indicated no significant difference regarding the ability to grow under refrigeration after 24 and 355h of storage among the strains. The average increase in the counts after 24 and 355h were 0.1 and 4.5 log, respectively. In the thermal resistance assessment, it has been found reductions in *L. monocytogenes* counts varying from 2.1 to 5.3 log, which enabled the discrimination between the most and least resistant strains. The growth variability in isolates studied was not significant during refrigeration, while these strains were found to behave differently when subjected to thermal resistance tests. These findings indicated that the selection of *L. monocytogenes* strains for further growth and kinetic studies during ice cream processing, should be based on their thermal resistance rather than on their growth ability characteristics. These strains are of interest for future studies of inactivation kinetics simulating real conditions of processing, such as pasteurization, maturation and freezing, generating important data for risk assessment studies.

Keywords: Predictive microbiology; foodborne pathogen; milk products

Impact of interventions during food production on microbial biodiversity

P4.70

Bioprotection in cooked ham

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Spoiled cooked ham sliced and packaged in protective atmosphere are usually colonized by lactic acid bacteria (LAB) such as *Leuconostoc*, *Lactobacillus*, and *Carnobacterium,* that show a preferential growth in psychotropic conditions. Depending on the strains, the colonization can result in a premature spoilage characterized by off-flavors formation, gas and slime production, discoloration, and pH decrease.
The purpose of this research was the isolation, identification and characterization of potential bioprotective starters able to prevent spoilages in cooked-ham. With the aim to provide an useful tool in food technologies and extend the shelf-life, physiological properties of the bacterial starters were studied in order to preserve the safety of the products.
The first step of the project aimed to identify the main microbial consortia both in the good-quality and in the spoiled lots by microbiological analysis. The interaction between consortia was performed in order to screen strains able to inhibit spoiled consortia. The isolated strains were at first selected for their antimicrobial activity *in vitro* against spoiled consortia (by the agar well diffusion assay method). Strains showing antimicrobial activity were identified and characterized by molecular methods and tested for their physiological features.
Among a total of about 150 strains isolated from good quality ham, six of them were selected together with four strains from the DISAFA's collection for their ability to inhibit a large number of spoiled consortia, to growth at 4°C and to produce bacteriocins.
The results provide information about the use of microbial starters for the extension of the shelf life's of meat products. The effectiveness of potentially protective starters will be evaluated by the use of challenge tests *in situ*, analyzing sensorial and microbial profiles of cooked-ham samples after bioprotective treatments.

Keywords: Bioprotection, Cooked Ham, Consortia Interaction

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P4.71

Shelf-life extension of frankfurters by the combined application of antimicrobials and high pressure processing by a product-specific approach

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Frankfurter sausages are perishable cooked meat products that can be post-processing contaminated with lactic acid bacteria (LAB), which can grow during shelf-life and spoil the product even if stored at chill temperatures.
The aim of the present study was to evaluate the inhibitory effect of different antimicrobials and high pressure processing (HPP) during refrigerated storage of smoked and non-smoked frankfurters. A challenge testing approach was used to quantify the shelf-life extension. Smoked and non-smoked frankfurters formulated without antimicrobials or with three different antimicrobials applied either in batter (lactate/diacetate batch and distilled vinegar batch) or on surface (spice extract batch) were inoculated with *Lactobacillus curvatus* CTC1755 (previously isolated from spoiled frankfurters) at 10^2 cfu/g (shelf-life study) and 10^6 cfu/g (HPP lethality study). Frankfurters were vacuum packaged, treated by HHP at 600MPa for 5 min or not treated, and stored at 5.5ºC. The levels of LAB were monitored in all the batches by plate count in MRS agar at selected sampling times. Growth rate at different temperatures was estimated by simulation using the Ratkowsky root-square equation secondary model. Commercial shelf life was assessed as time to increase 7 log the levels of LAB.
The growth rates of *L. curvatus* were up to 67% lower in smoked than non-smoked frankfurters not submitted to HPP. All the assayed antimicrobials had an inhibitory effect on the growth of *L. curvatus* being proportionally higher in non-smoked sausages and by the in-surface application of the spice extract, which kept the levels of the spoiler strain below the limit of detection for at least 10 days. The effect of the refrigeration temperature was remarkable and the shelf-life of the products stored at 4 or 8ºC was reduced 4 and 16-fold, respectively, when compared with storage at 2ºC. HPP produced a lethality of more than 5 logs in all the batches and an extension of the shelf-life. In contrast to non-HPP results, *L. curvatus* inhibition was higher in non-smoked than smoked batches, where detectable levels of *L. curvatus* were recorded earlier (i.e. day 15 in batch without antimicrobials).
Our findings highlight the importance of the application of a product-oriented approach when exploring different antimicrobial strategies to extend the shelf-life of a food product as interaction between intrinsic and extrinsic factors can lead to unexpected outcomes.

Keywords: High Hydrostatic Pressure, Cooked meat, Spoilage, Natural Antimicrobials, Shelf-life, Lactobacillus

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P4.72

Surface materials and environmental microbiota affect recovery of *Listeria monocytogenes* in multispecies biofilms after biocide treatment

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The viable but not culturable (VBNC) state is a survival strategy implemented by bacteria in response to adverse environmental conditions. VBNC bacteria remain metabolically active but can no longer be detected on conventional culture media. These characteristics of VBNC cells pose a risk for food safety as pathogens may not be detected in the processing environment or even in foodstuffs where they could end up resuscitating under appropriate conditions. This project aimed to identify the factors responsible for the entry into the VBNC state and that affect the ability of biofilms to recover after biocide treatment.
A sampling campaign performed in 6 shrimp-processing industries highlighted an increase in the occurrence of *Listeria* VBNC cells after cleaning and disinfection operations. The *in vitro* study of biofilms then demonstrated the role of quaternary ammonium compounds (QAC) and H_2O_2 as disinfectants on the entry of *L. monocytogenes* into the VBNC state, even though used according to the manufacturer's instructions.
Comparison of *L. monocytogenes* biofilms formed on stainless steel or PVC coupons showed that the material had an impact on the ability of the chlorinated alkaline detergent to eliminate attached cells but also affected the residual biofilm recovery after treatment with QAC or H_2O_2.
Analysis of environment samples highlighted a great qualitative and quantitative diversity in the bacterial contamination of surfaces. The environmental microbiota, identified by MALDI-TOF MS, was mostly composed of γ-Proteobacteria (mainly *Pseudomonas*, *Escherichia*, *Serratia* and *Acinetobacter*) and Bacilli (mainly *Staphylococcus* and *Lactococcus*). One to 3 strains of each of these 6 most represented genera were mixed with *L. monocytogenes* for the formation of multispecies biofilms. Susceptibility of microbiota to detergent or disinfectant treatments was shown to vary among selected species but none of the competing flora prevented *L. monocytogenes* from entering the VBNC state after exposure to biocides. However, presence of a competing flora strongly influenced the ability of *Listeria* to recover from treatments. For instance, *Staphyloccus pasteuri* and *warneri* as well as strains from the *Pseudomonas fluorescens* group promoted recovery of *L. monocytogenes* after exposure to QAC.
The innovative results obtained in this study thus provide new insights for a better understanding of mechanisms underlying persistence of foodborne pathogenic strains in food industries.

Keywords: Listeria monocytogenes, viable but not culturable, biocide, surface, microbiota

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P4.73

Listeria mitigation strategies on fresh and cold-smoked salmon

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Salmon contaminated with *Listeria monocytogenes* represents a potential health threat for consumers and is a serious microbial challenge for the salmon industry. Hygienic processing is essential for *Listeria* control, but cannot ensure absence of *L. monocytogenes* in salmon and salmon products. Therefore, the salmon industry has shown an increasing interest in methods with documented effects for elimination or reduction of *Listeria* when applied directly on salmon and salmon products. We have evaluated strategies for controlling *L. monocytogenes* on fresh and cold-smoked salmon under industry relevant conditions. Strategies leading to reduction of Listeria or growth inhibition and combinations of these were tested. The strategies included surface treatments with UV light (continuous UVC/pulsUV) and the use of organic acid salts/fermentates as an ingredient in cold-smoked salmon. Salmon was contaminated with a 10-strain mixture of *L. monocytogenes*. Listericidal and listeriostatic effects were determined by microbiological analyses during various relevant processing and storage conditions. Reductions of *L. monocytogenes* on salmon treated with UVC and pulsed UV light were similar (0.5-1.5 log), with higher effects on skin of raw salmon and on fillets of smoked salmon compared to fresh salmon fillets. Organic acid salts and fermentates inhibited *L. monocytogenes* growth on cold-smoked salmon during storage. Complete growth inhibition was obtained, and the study identified important parameters and strategies for effective *Listeria* control in fresh and cold-smoked salmon. No negative effects on the sensory quality of treated salmon were evident according to performed sensory analysis and consumer tests.

Keywords: Listeria monocytogenes; salmon; intervention; UV-light; organic acid salts

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P4.74

Influence of acid-lactic bacterias and yeasts on growth of spoilage fungi in bakery products

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Fungi like *Penicillium roqueforti* (PR) and *Aspergillus chevalieri* (AC) are the main microorganisms responsible for bakery products' deterioration. Biopreservation, which consists in the use of microorganisms to control the deterioration of foods, has been highlighted in recent years. Therefore, the aim of this work was to verify the influence of the formulation of bread and panettone with sourdough fermented by *Wickerhamomyces anomallus* or *Lactobacillus fermentum* on growth of PR and AC. Four different formulations of bread and panettone were produced: with commercial baker´s yeast (*Saccharomyces cerevisiae*) and preservative; with sourdough, being used *W. anomallus* (S1) or *L. fermentum* (S2) as starters; and with commercial baker´s yeast without preservative (control). After baking the products, a mix of strains of *PR* (PR06, PR11, PR67) and *AC* (AC32, AC33, AC35) at the concentrations of 10^5 spores/mL were separately inoculated on the product surfaces. The time required to appear the first visible colony (t_v) and colony size (mm) were measured through the storage time. Four replicates were performed per condition. Baranyi and Roberts model was fitted to the growth data using DMfit Excel add-in, finding growth rate (μ). ANOVA was performed, followed by mean test (Scott-Knott), with significance level $p< 0.05$. In all trials of both bakery products, S2 presented better or equal antifungal action to S1. The results in panettone indicated that S2 was able to reduce μ of PR from 0.25 to 0.13 mm.day^{-1} when compared to the control, this rate being lower than the conventional preservative. However, the t_v presented similar values for the 4 formulations. For AC, both sourdough (S1 and S2) had a reduction of μ in relation to the control (5.33 mm.day^{-1}), but these values were higher when conventional preservative was used. In this case, the t_v for S2 was 29 days, while the t_v in the other formulations was faster. In the bread formulations studied, there was no difference in μ of PR inoculated, being on average (20.5 mm.day^{-1}), though for AC, the S2 reduced μ value from 11.8 to 8.8 mm.day^{-1} in relation to the control formulation. For t_v in bread, both sourdough did not promote a significant increase over control. Although microorganisms tested here did not present antifungal action as effective as the conventional preservative, their use may comprise an extra barrier in reducing the use of chemical preservatives to avoid spoilage of bakery products.

Keywords: Bread, Panettone, Penicillium roqueforti, Aspergillus chevalieri, Biopreservation, Sourdough.

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P4.75

Serotype characterization of *Listeria monocytogenes* isolated from meat processing plants by multiplex real time PCR

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Listeria monocytogenes is the causative agent of listeriosis, a serious food-borne disease that shows a significantly increasing trend in the EU in the last 5 years (2012-2016). This ubiquitous bacterium can survive and persist in food processing plants for extended periods. In addition, slicing and packaging can be a source of cross-contamination on ready-to-eat (RTE) meat products, such as dry-cured ham which has been identified as a potential vehicle for this pathogen. *L. monocytogenes* strains differ in their epidemic potential and in their ability to cause disease, being the serotypes 1/2a, 1/2b 1/2c and 4b responsible for 95% of human listerioris cases. In the present work, the serotype diversity of *L. monocytogenes* in dry-cured ham processing plants using a multiplex qPCR method has been studied. In total, 7 plants located at different Spanish provinces where sampled. A total of 350 samples from RTE dry-cured ham and food contact surfaces was collected and processed according to the guidelines provided by USDA FSIS MLG 8.09. Genomic DNA of the presumptive *L. monocytogenes* isolates recovered from CHROMagar™ Listeria was extracted and the isolated were serotyped using a multiplex qPCR method which is able to differentiate the four major serovars. Results showed that 21 strains were presumptive *L. monocytogenes* from contact surfaces while none of the RTE food samples analysed was contaminated with *L. monocytogenes*. The serotypes 1/2a (n=8) and 1/2b (n=8) were the most frequently found (38.09% for each of the serotypes), whereas 5 out of the 21 presumptive *L. monocytogenes* belonged to the serotype 1/2c. Results showed a higher prevalence of the serotypes 1/2a or 1/2b depending on the processing plant sampled. This study reveals the existence of persistent strains of *L. monocytogenes* in environments related to processing of RTE dry-cured ham which could harbour, survive and contaminate this product.
This work was funded by the projects RTA2013-00070-C03-03 of the INIA, IB1649 and GR15108 of the Government of Extremadura and FEDER. A. Alía and I. Martín are supported by predoctoral fellowships from the Junta de Extremadura (PD16023) and the Spanish Ministry of Education, Culture and Sport (16-05303), respectively. L.R. Dos Santos is supported by a fellowship from CAPES/BRASIL (88881.121063/2016-01).

Keywords: Listeria monocytogenes, serotype, qPCR, RTE dry-cured ham, meat processing plants

Impact of interventions during food production on microbial biodiversity

P4.76

Characterization of lactic acid bacteria biodiversity during ripening period of traditional Tulum cheeses produced in goat skin in central Toroses region ripened

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Tulum cheese is the most consumed cheese after White pickled and Kashar cheese in Turkey. Although Tulum cheese is produced commercially, it is mostly produced at small scales by using traditional methods. Tulum cheeses produced in traditional ways are more preferred by the consumer due to superior taste and aroma. In this study, Tulum cheese samples produced in animal skin using raw milk by traditional methods (without starter culture) will be obtained from Konya (Ereğli)-Karaman-Mersin (Mut and Anamur) regions. Cheese samples will be ripened under the similar ripening conditions to that of traditional producers and cheese samples will be taken in 7th, 15th, 30th, 60th, 90th and 180th days of ripening. The samples will be analyzed in terms of chemical properties (protein, fat, ash, dry matter content, pH, titratable acidity and water activity). Furthermore, the isolation of bacteria from the cheese samples will be carried out using various media and incubation conditions. Isolated bacteria from cheese samples were identified by biochemical methods and 16S rRNA gene sequencing. Moreover, DNA and RNA isolation directly still performing from samples (uncultural methods) and V3 variable region of the 16S rRNA gene was analysed by Denaturing Gradient Gel Electrophoresis (DGGE). Microflora change depending on ripening period examined. The data of DGGE and chemical analysis of cheese samples were compared during ripening. Surprisingly, microbial dynamic with dependent methods showed that *E.faecalis* strains especially strain 2014 VREF-41 the most dominant bacteria in tulum cheese throughout the ripening. Following them, *E.hirae* strains (SNNU0261, SU354 etc.), *L. plantarum* strains (AAHED-10, D27, L21 etc.), *L. lactis* strains (strain CAU1157, SNNU0274 etc.), *E.durans* (strain CAU1724, CAU:4815) and Streptococcus gallolyticus (strain G8 etc.). And also culture independent methods indicated that Enteroccus faecium strains the most detected strains in traditional skin tulum cheeses samples during ripening.

Keywords: Microbial dynamic, pcr, dgge, lactic acid bacteria

Impact of interventions during food production on microbial biodiversity

P4.77

The effect of *L. casei* and *L. paracasei* on antioxidant activities and total phenolic contents of various vegetable juices throughout cold storage

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In this study, the juices of broccoli, cauliflower, red and white cabbage were obtained and fermented by probiotic *Lactobacillus casei and Lactobacillus paracasei*. The antioxidant activity analysis of the extracted vegetable juices were performed by two different radicals binding methods. Throughout the storage period, the results of the antioxidant activity determined by $ABTS^+$ scavenging activity method were increased, but the statistical results of antioxidant activity were showed no significantly differences between the vegetable groups ($p>0.05$). The higher $ABTS^+$ antioxidant activity results were found in red cabbage which fermentation by *L. casei* and *L. paracasei.* The antioxidant activity in all vegetable juices which determined with DPPH radical binding method showed not significant differences between the juices which fermentation with *L. casei* and *L. paracasei* ($p>0.05$). The highest level of antioxidant activity which examined with DPPH were found in white cabbage juice. Also white cabbage juice showed highest content of phenolic compound between the all fermented vegetable juices. During the storage period, some chemical and physical characteristics of the juices were analyzed. All the test (pH, titration acidity and water activity) were showed increase during the storage period ($p< 0.05$). The color parameters of all vegetable juices were determined and the values of L*, a* and b* were showed decrease throughout the storage period. According to the results, the vegetable juices which fermented with *L. casei* and *L. paracasei* can be stored up to 42 days at 4 ° C.

Keywords: antioxidant, broccoli, cauliflower, cabbage, probiotic

Impact of interventions during food production on microbial biodiversity

P4.78

Combined effect of high hydrostatic pressures and grape seasonings against *Listeria monocytogenes* strains of cheese industry

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In the last years, high pressure processing (HPP) has been proved as a valid method for food preservation against microbiological growth among others. Recently new natural compounds are under developing, not only due to their flavor properties but also as natural additives because of the antimicrobial and antioxidant properties that they have. In relation to this a newly developed grape seasoning has shown some kind of antimicrobial effect. *Listeria monocytogenes* is one of the most important bacterium in food industry, as it is responsible of many food outbreaks, an important number of these finishes in a deadly manner. So, the main objective of this research was to assess the combined effect of two high hydrostatic pressure treatments and two wine origin seasonings in vitro against 8 strains of *Listeria monocytogenes*, which were isolated from cheese industry in floors, surfaces, gloves and product. Two HPP treatments were selected, (400 MPa for 5 minutes and 600 MPa for 5 minutes) and combined with two types of grape seasoning (S5 and S6). A control with lowered pH was also carried out in order to establish the relation between the pH of the seasoning and the antimicrobial effect. The combination of the HPP and the seasonings achieved a high inhibition of *L. monocytogenes*. The seasoning S6 combined with 600 MPa for 5 minutes was the best combination to eliminate the pathogen. There was no significant inhibitory effect with the seasoning alone, neither on the pH samples control. Therefore, the combination of HPP and the seasoning could be a good alternative to increase the food safety of different products.
This study was financially supported by the research project BU056U16 from Junta Castilla y León (Regional government), Spain.

Keywords: Grape seasonings, Listeria monocytogenes, antimicrobial effect, cheese industry, HPP

Impact of interventions during food production on microbial biodiversity

P4.79

Impact of biocides on *Listeria monocytogenes* viability in biofilm and in seafood industrial environment

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Listeria monocytogenes (*L. monocytogenes*) is a food pathogen frequently isolated in the seafood industry. This bacterium adheres to surfaces and forms biofilms composed of an extracellular matrix. This extracellular matrix protects bacterial cells from environmental stress factors such as cleaning and disinfection operations (NαD). It is therefore essential for professionals to have effective biocides to eliminate these biofilms and limit the transfer of *L. monocytogenes* cells from surfaces to food. The objective of present study was to evaluate the impact of two biocides on the cell viability of *L. monocytogenes* in biofilm using two recovery methods recommended in ISO 18593: 2004.

Methods: Stainless steel or PVC coupons were incubated for 1 h at 8 ° C in the presence of filtered smoked salmon juice and then 48 h at 8 ° C in the presence of a bacterial suspension of *L. monocytogenes* associated with *Carnobacterium maltaromaticum* and *Carnobacterium divergens* to allow the formation of a mixed biofilm. Treatments with biocides (two) or water (control) were applied to the biofilms followed by application of recovery methods (contact plate or sponge stick). Bacteria were enumerated on agar medium for the cultivable population and by qPCR and PMA-qPCR, respectively for the total and viable populations of *L. monocytogenes*.

Results: In all conditions, the treatment with the two biocides tested did not allow removing *L. monocytogenes* cells on the surface but led to a change in the viability of the population with mostly viable but non-culturable cells. Quantification data and epifluorescence microscopy observations showed that the efficiency of recovery methods varied according to the biocide treatment used and the surface on which these treatments were applied.

Significance: These results have allowed us to better understand and manage the health risk associated with this bacterium in industries.

Keywords: food hygiene, biocide, biofim

Impact of interventions during food production on microbial biodiversity

P4.80

Technologically hazardous *Gluconobacter* spp. and *Kozakia* sp. strains as frequent microbial contaminants of industrial non-alcoholic beverages produced on aqueous and whey bases

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Bacteria from the *Gluconobacter* and *Kozakia* genera are taxonomically ranked in the Acetobacteraceae family, in the group called acetic acid bacteria. These bacteria represent technologically hazardous microorganisms causing decrease in quality of variety non-alcoholic drinks in a form of serious textural and sensory defects. Some *Gluconobacter* spp. are opportunistic pathogens for humans, for example *Gluconobacter frauterii* can cause endocarditis, whereas *Kozakia baliensis* is non-pathogenic strain.
The objective of this work is realization of basic characterization of technologically hazardous *Gluconobacter* spp. a *Kozakia* sp. strains, followed by detection of their sensitivity to various food industrial sanitation agents. Experiments were carried out in different model cultivation systems by use of modern instrumental as well as classical microbiological methods.
The research was mostly based on the optical microscopy method, the agar plate method, the optical spectrophotometric method in real time at A_{850} using the BioSan personal bioreactor and also the BioTek microplate system. The mentioned methods were used particularly for estimation of *Gluconobacter* spp. and *Kozakia* sp. characteristics and their minimal inhibition concentrations to sanitation agents.
During the experiments with *Gluconobacter* spp. and *Kozakia* sp. strains, the following characteristics were estimated: macroscopic features of colonies, microscopic features of cells, growth activity in Sabouraud broth with 4 % (w/w) glucose and also in sweet whey without/with 4 % (w/w) glucose, minimal inhibition concentrations to five industrial sanitizing agents; all during aerobic dynamic cultivations at 30°C. The obtained results of this work could be useful for effective assurance of health safety and quality of non-alcoholic beverages produced on aqueous or whey bases, for elimination of technologically hazardous bacteria from the *Gluconobacter* a *Kozakia* genera.
This work was supported by the Ministry of Agriculture of the Czech Republic/the National Agency for Agriculture Research, by the project QK1710156 (02/2017–12/2021, MZE/OK), in the programme ZEME (2017–2025).

Keywords: Gluconobacter spp., Kozakia sp., microbial contamination, defects, non-alcoholic drinks, whey drinks

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P4.81

Risk assessment of *Listeria monocytogenes* in ready-to-eat foods for better diet advice to vulnerable consumer groups in Norway

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The Norwegian Food Safety Authority (NFSA) provides diet advice to different consumer groups including "pregnant and vulnerable groups". The last revision was in 2009 and an update was needed. The NFSA asked the Norwegian Scientific Committee for Food and Environment (VKM) to perform a risk assessment for RTE products of fish and seafood, meat, cheese / dairy products and vegetables. In addition, the NFSA wanted to assess the effect of various measures that the consumer can take: storage at refrigerator temperature < 4 °C; eating the food early in the shelf life period; avoiding food leftovers kept in the fridge for several days; only eating small amounts of the food; choosing products with additives/modified atmosphere that reduce growth of Listeria; heating the food before consumption; reducing the storage time for leftovers in the refrigerator.The four food groups were divided into subgroups and products falling into the RTE group were assessed for growth of Listeria. The flow chart for each product, from farm to fork, was set up and the most likely steps for contamination and growth of L. monocytogenes were assessed based on literature and experience data. In addition, the Growth rate in each step was estimated using the FSSP tool with food characteristics like pH, water activity, smoke compound, packing atmosphere, storage temperature and content of organic acids as input parameters. As these parameters vary even for similar products, the estimations were carried out for the least, the most, and the middle favourable conditions for growth. This approach gave an overview of the reasonable variation of the Listeria growth in the foods and was less time and resource consuming than probabilistic modelling. Recent dose-response models indicate that the threshold for increased likelihood of listeriosis cases among vulnerable consumers was at L. monocytogenes concentrations above 1000 cfu/g. In line with this, RTE foods that, according to our estimations, could contain L. monocytogenes above this concentration were short listed as risk products. Further, an easy-to-use excel sheet for estimation of the effects of shorter storage time, growth after opening of a modified atmosphere packed food, etc was developed in order to visualise for the NFSA and consumers how the risk of listeriosis by consume of RTE products would change. The outcome of the assessment and a demonstration of the excel sheet will be given in the presentation.

Keywords: Listeria monocytogenes, risk assessment, ready-to-eat foods, diet advice, vulnerable consumers

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P4.82

Evaluation of growth of spoilage microorganisms in vacuum-packed beef subjected at different temperature and pH conditions

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The study was performed to provide scientific data to explain the factors that could influence the deterioration of Brazilian vacuum-packed beef and to develop predictive tools. For this purpose, the growth of Lactic acid bacteria (LAB) and *Enterobacteriaceae* was evaluated in vacuum-packed beef, coming from the meat cuts Longissimus dorsi, Psoas major and Trapezius thoracis, with initial pH range of 5.4-5.8, 5.8-6.1 and ≥6.1 that were stored at 0°C, 4°C, 7°C and 10°C. The primary model of Baranyi and Roberts (1994) was fitted to the microbial growth data using TableCurve 2Dv5.1. Subsequently, the maximum specific growth rate (μ_{max}, h^{-1}) was obtained for each experimental condition. Multiple linear regression analyses were done to characterize the influence of all factors on the square root transformed μ_{max}. The secondary square root model of Ratkowsky was applied to evaluate the effect of the temperature on $\mu_{max.}$ The results indicated that LAB was the dominant group of spoilage microorganisms at all conditions. LAB reached growth levels between 7.0 and 8.0 log CFU/g at the end of the storage in most samples. Of all factors tested, the storage temperature was the most influencing factor on the behavior of LAB and *Enterobacteriaceae* ($P < 0.05$). In the case of LAB, the μ_{max} values varied from 0.017±0.007-0.074±0.064 h^{-1} to 0.060±0.023-0.169±0.040 h^{-1} when the storage temperature increased from 0°C to 10°C. In this study was also noted a slower bacterial growth, at all temperatures conditions, when the initial pH range of meat was 5.4-5.8 ($P< 0.05$). The findings obtained will serve to the meat industry and the quality managers to predict the shelf life of vacuum-packed beef.

Keywords: vacuum-packed beef, spoilage microorganisms, temperature, initial pH, predictive modeling

Impact of interventions during food production on microbial biodiversity

P4.83

Carbon source utilization, niche overlap and interactions between ochratoxigenic *P. nordicum* and *P. chrysogenum* isolated from dry-cured ham

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A non-negligible colonisation of moulds on dry-cured meat products occurs during ripening. Among these moulds, *Penicillium nordicum* may produce ochratoxin A (OTA) that is rated as a Group 2B carcinogen. A strategy to prevent its presences is the use of non-toxigenic moulds isolated from these products as biocontrol agents. The competition by nutrients and space is a key mechanism responsible for the antagonistic activity of protective microorganisms. The nutritional partitioning of carbon sources (CS) may influence the ability of biocontrol agents to either co-exist or dominate against toxigenic moulds. In addition, abiotic factors may affect the interaction between microorganisms. The CS utilisation patterns in different environmental conditions could be used to determine niche overlap indices (NOI) and to explain the co-existence or niche exclusion among different moulds.

The objective was to study the use of CS, NOI, and the interaction between the biocontrol agent *Penicillium chrysogenum* and an ochratoxigenic *P. nordicum* at conditions of temperature and a_w usually reached in the processing of dry-cured meat products. Moreover, the influence of *P. chrysogenum* on OTA production by *P. nordicum* was investigated. The use of 20 CS was tested under different combinations of three temperatures (25, 20 and 15 ºC) and two water activities (a_w) (0.97 and 0.94 a_w) in microtiter plates using resazurin as a metabolic indicator. NOI was calculated according to the CS utilisation profile.

The utilisation of CS was similar for both moulds in the conditions evaluated. However, the number of CS used by *P. chrysogenum* was higher than by *P. nordicum* at 15 ºC and 0.97 a_w. Although according to NOIs, the co-existence or niche exclusion were influenced by environmental factors, *P. chrysogenum* coexists or is nutritionally dominant against *P. nordicum* at most of tested conditions. On the other hand, the interaction between both moulds showed mutual inhibition in a space of 2-3 mm depending on the temperature and a_w. Furthermore, the growth rate and OTA production of *P. nordicum* decreased when co-cultured with *P. chrysogenum*.

In conclusion, *P. chrysogenum* could be considered as a good candidate to control ochratoxigenic *P. nordicum* in dry-cured meat products due to their ability to compete against it and reduce OTA production.

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Keywords: ochratoxin A, biocontrol, competition by nutrients, NOI

Impact of interventions during food production on microbial biodiversity

P4.84

Salmonella cross-contamination as affected by temperature during the grinding of beef: Transfer modeling and bacterial diversity

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Although the reliability and economic strength of livestock market depends greatly on the hygienic sanitary control, beef is often associated with foodborne outbreaks in which *Salmonella* stands out as implicated microorganism. Several of these outbreaks may be related to occurrence of cross-contamination occurred during food processing/preparation. In industry, grinding may be conducted at different temperatures and whether this may affect the transfer phenomena, safety and shelf-life of the product is not known yet. The aim of this study was to determine the influence of meat temperature prior to grinding (4 and 20 ºC) on the transfer parameters of *Salmonella*, bacterial counts and bacterial diversity during the grinding process of meat (*Vastus lateralis*). First, a cocktail containing equal concentrations (10^9 CFU.mL^{-1}) of *Salmonella* Enteritidis 54, 96 and 101 were inoculated into five slices of meat in order to build up the contamination within the grinder. Subsequently, 58 *Salmonella*-free slices were ground and samples were collected to determine *Salmonella* (n=20) and the counts of specific microbial groups (*Pseudomonas* spp., *Enterobacteriaceae* and *Lactobacillus*) (n=10). The behavior of beef microbiota was also evaluated through sequencing of rRNA 16S V3/V4 region. A 5-parameter transfer model was adopted considering two environmental matrices inside the grinder. The fit was done using the Solver function in Microsoft® Office Excel® 2013, by minimizing SSE. In all the evaluated conditions, adjustments of the model with $R^2 > 0.95$ and RMSE < 0.25 were obtained. The probability values of pathogen transfer from contaminated meat to grinder internal environments (parameters a_1 and a_2) were, on average, 0.01 and 0.46, respectively. The transfer from grinder to meat during grinding (parameters b_1 and b_2) were on average 0.03 and 0.27, respectively. Even after grinding of 58 non-inoculated meat slices, *Salmonella* was found in the ground beef, and the temperature did not show a significant influence on the transfer probabilities. The results of *Salmonella* transfer will be correlated with data on bacterial counts and bacterial diversity aiming to gain insights on the transfer phenomena evaluated. The findings of this study may be useful to better understand cross-contamination and to control its occurrence aiming to protect public health.

Keywords: Salmonella, food safety, cross-contamination, recontamination, quality control

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P4.85

Supercritical CO_2: A tool combining drying and decontamination of herbs

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Context: Dried foods are often considered microbiologically stable. However, they may contain viable pathogens, which may proliferate upon rehydration. If not processed carefully, highly contaminated commodities, such as herbs, can pose a threat to consumers. Therefore, a need arose to develop drying techniques which can decrease the contamination to acceptable levels. Above 74 bar and 31°C, CO_2 becomes supercritical ($scCO_2$) and can be used as an extraction solvent for water, hence providing drying. Moreover, $scCO_2$ is already known for its microbicidal properties. In this study, $scCO_2$ was used to dry coriander and inactivation of 5 foodborne pathogens by the process was assessed.

Methods:
Coriander was inoculated with *Salmonella enterica* subsp. *enterica*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Bacillus cereus*, and *Staphylococcus aureus*. For each pathogen, 25 g of coriander was spiked with a cocktail of 3 strains to reach 10^6 CFU/g. After inoculation, coriander was treated in a $scCO_2$ dryer at 1) 80 bar and 35°C for 1 min, 2) 100 bar and 40°C for 1 min, and 3) 80 bar and 35°C for 150 min. The depressurization rate was kept at 5 bar/min. Significant drying was only observed for treatment 3. Enumeration was performed by the plate count method and unselective enrichment was realized in buffered peptone water. Experiments were independently repeated 3 times. Difference in treatments was assessed by 1-way ANOVA at = 0.05.

Results: All strains were significantly reduced by $scCO_2$. The highest reductions were observed after treatment at 100 bar and 40°C. *E. coli* O157:H7 was completely absent in treated samples with >7.7 log reduction. *Salmonella*, *L. monocytogenes*, and *S. aureus* were reduced by 4.7 ± 0.1, 5.0 ± 0.4, and 5.9 ± 0.1 log CFU/g, respectively. After 2.5 h at 80 bar and 35°C, the water activity of coriander was 0.21 ± 0.02 and reductions for *Salmonella, E. coli* O157:H7, *L. monocytogenes*, and *S. aureus* were 5.5 ± 0.3, 5.3 ± 0.2, 5.6 ± 0.4, and 4.2 ± 0.6 log CFU/g, respectively. The spore forming *B. cereus* was more resistant. Only vegetative cells could be reduced by 1.4 ± 0.2 log CFU/g, but spore counts were identical before and after treatment.

Conclusion: This study showed that herbs similar to coriander can be dried to low water activity with $scCO_2$, while also offering a powerful tool for the inactivation of vegetative cells. However, innovative improvements are required to inactivate spores, such as a combination of $scCO_2$ with ultrasound.

Keywords: Supercritical CO2, food drying, foodborne pathogens, food preservation

Impact of interventions during food production on microbial biodiversity

P4.86

Application of predictive food microbiology along the food supply chain, what SMEs need

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The lack of technical expertise by the informal food economy in Africa warrants the need for support mechanisms as SMEs identify the need for a system of food safety and quality control. Predictive Microbiology, a recognised scientific-based reliable tool for estimating course of bacterial growth in RTE foods could be the solution the SMEs require.
Challenge test study was conducted on RTE foods (beef lasagne and egg noodles) with *L. monocytogenes* during storage for 12 days at ± 5°C. Performance of 4 different types of software (ComBase, PMP, MicroHibro & FSSP) selected based on accessibility and availability, user-friendly and pathogens for its application was evaluated for use in shelf life estimation of these selected RTE foods. The predicted growths from the software were compared to observed growth of *L. monocytogenes* in beef lasagne and egg noodles during the challenge test study. Indices of performance such as Coefficient of Determination (R^2), Root Mean Square Error (RMSE), Bias (Bf) and Accuracy factor (Af) were used to evaluate the performance of these software.
Growth of *L. monocytogenes* predicted by ComBase, PMP, MicroHibro & FSSP in beef lasagne and egg noodles was in agreement with the observed growth from the challenge test study, with a fail-safe prediction.
Predictive Microbiology is a field of Food Microbiology that can be explored by the food industry in Africa most especially the SMEs. This will assist in decision making with regards to food quality and safety, thereby reducing the problem of food waste as result of product shelf life and at the same time protect public health.

Keywords: SMEs, Shelf life, Predictive Microbiology, Ready to Eat Foods

Impact of interventions during food production on microbial biodiversity

P4.87

SafeConsumE: Safer food through changed consumer behavior: Effective tools and products, communication strategies, education and a food safety policy reducing health burden from foodborne illnesses

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Food safety violations at the consumer stage are common and nearly 40% of food-borne outbreaks are occurring in the domestic setting. The overall goal of SafeConsumE (http://safeconsume.eu/) is to provide effective, science-based and sustainable strategies for food authorities, market actors and the research community to help consumers mitigate risk, thus reducing the health burden from food-borne illness in Europe. SafeconsumE will suggest, develop and evaluate:
1) Tools, technologies and products (e.g. sensors, apps, hygiene concepts, kitchen utensils) that stimulate safe practices;
2) Communication strategies that effectively stimulate adoption and market uptake of safer practices and tools/technologies;
3) Education programs increasing skills and knowledge aiding teenagers to handle food safely;
4) Dynamic, sustainable and inclusive policy models that stimulates and support national and EU level initiatives.
To achieve high implementation and innovation power, scientists will work together with consumers, authorities and different market actors under a new trans-disciplinary and multi-actor approach based on Theories of Practices combined with Design-driven innovation. Covering the five most important hazards causing food borne disease, consumer behavior across Europe will be described using a risk-based methodology and utilizing the strengths of high-throughput surveys together with in-depth qualitative methodology. New strategies will be developed taking into account their impact on risk reduction, documented consumer barriers for change and sustainability. SafeConsumE will support transformation towards a more healthy population and cost-efficacy by reduced foodborne illness, and a more sustainable community by less food-waste and environmentally friendly solutions.
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 727580.

Keywords: Food safety; risk mitigation; consumer, trans-disciplinary/multi-actor; Theories of Practices

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P4.88

The effect of polyethylene and polypropylene nanocoamposite packaging containing calcium silicate on shelf life of Iranian traditional white cheese

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Traditional cheese is the most popular type of cheese in Iran. The use of raw and unpasteurized milk to produce traditional cheese can be caused infections in human. On the other hand improper packaging of this type of cheese it would exacerbate microbial problems. The aim of this research was to comparing calcium silicate Nano composite packaging with conventional packaging to increase shelf life and reducing the microbial load in the maintenance period of traditional cheese that has been accomplished over a period of 20 days at refrigerator temperature. The study were included 4 treatment groups (Nano polyethylene, polyethylene, Nano polypropylene and propylene packages) . The initial chemical analyses of sample were included protein, fat, salt and humidity content. Chemical periodically assessment of treatments were include acidity and pH value and microbiological analyses were mold and yeast count, coliform count and total viable count. The treatments also evaluated as color, texture, smell and taste. According to the results Nano packages showed the least chemical and biological changes during storage time compared with other treatments (P<0/05). As well as the results proved that Nanocomposite packaging are better than common packages in sensory evaluation (P<0/05). So that common packaging had lowest points on the 20 day. Generally it can be concluded that the use of Nano composite packaging enhance the quality and acceptability of the product was compared to conventional packaging.

Keywords: Iranian traditional cheese, Nanocomposites, Calcium silicate, Nano polyethylene, Nano propylene ,She

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P4.89

Fate of *Bacillus cereus* spores after spray drying at different temperatures and carrier agents

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Milk powder is a shelf-stable product that can be used as an ingredient several food formulations. Spray drying is a widely used method for producing milk powder, which has been found to be contaminated with sporeforming bacteria. Even though spray drying is not aimed to cause microbial inactivation, it can be observed. This study determined the inactivation kinetics parameters of three *Bacillus cereus* strains (436, B63, 540) in three menstrua (whole milk, phosphate buffer and talc suspension) in capillary tubes at 90 ºC, 100 ºC and 110ºC. D-values for *B. cereus* in the three menstrua were not significantly different at the highest tested temperature ($p>0.05$). Thus, talc was chosen as a carrier agent to allow the recovery of *B. cereus* from spray dried materials given its low interference on inactivation kinetics. After that, this study characterized *B. cereus* inactivation during spray drying. Spores of *B. cereus* were inoculated in whole milk and skim milk following spray drying at 95°C, 105°C and 110°C (outlet temperature). After the spray drying processes, *B. cereus* spores were counted and the number of decimal reductions (γ) calculated. A correlation between the small diameter of the particles with the survival of spores of three *B. cereus* strains was found, and *B. cereus* 436 showed consistently the lowest γ no matter temperature and carrier agent. The highest γ was found when talc powder was used, which suggested that this carrier agent does not protect *B. cereus* spores during spray drying. Spray drying of milk can lead to up to 4 γ (strain 540) of *B. cereus* spores but depending on the strain less than one γ (strain 436) could be observed. This study contributed to the knowledge on the microbiology of low water activity foods by providing novel findings regarding the fate of three *B. cereus* strains to different spray drying conditions. The results reinforced the necessity of dairy industry to employ preventive measures to avoid raw milk contamination by *B. cereus* as well as the establishment of this bacterium in the dairy environment. These data can serve food industries to design thermal processes of formulated foods aiming to ensure their microbiological quality and safety.

Keywords: Thermal resistance, Dairy products, capillary tubes, Predictive Modelling

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P4.91

Impact of D-tryptophan on growth inhibition of *Vibrio* spp. in shucked and live oysters

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Vibrio vulnificus and *Vibrio parahaemolyticus* are important human pathogens that are frequently transmitted via consumption of contaminated raw oysters. We recently showed that a small amount of d-tryptophan (d-Trp) inhibits some foodborne pathogenic bacteria under high-salt environments. Therefore, in this study, we aimed to evaluate the antibacterial effect of d-Trp on *V. vulnificus* and *V. parahaemolyticus* inoculated in culture media, artificial seawater, shucked oysters, and live oysters. The effectiveness of d-Trp in growth inhibition of *Vibrio* spp. was highly dependent on environmental NaCl concentrations. Higher levels of NaCl (>4.0%) with d-Trp (>20 mM) resulted in higher and consistent growth inhibition of both the *Vibrio* spp. Treatment with 40 mM d-Trp significantly ($P < 0.05$) reduced viable *V. parahaemolyticus* cell counts in tryptic soy broth (TSB) with >4.0% NaCl at 25°C. In contrast, compared to *V. parahaemolyticus*, *V. vulnificus* was more sensitive to d-Trp (20 mM). d-Trp (40 mM) treatment with NaCl (>4.5%) significantly ($P < 0.05$) inhibited the growth of *V. parahaemolyticus* and *V. vulnificus* in shucked oysters immersed in peptone water at 25°C throughout the 48 h incubation period. Moreover, 40 mM d-Trp inhibited the growth of both *V. parahaemolyticus* and *V. vulnificus* in live oysters with shell immersed in artificial seawater at 25°C for 48 h. The results revealed that d-Trp may serve as a novel and an alternative food preservative to control *Vibrio* spp. in live oysters.

Keywords: D-tryptophan, oyster, Vibrio, growth inhibition

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P4.92

Antimicrobial activities of quince *(Cydonia onlonga)* and persimmon *(Diospyros kaki)* leaf extracts

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Antimicrobial properties of the leaves have been used extensively food, cosmetic and pharmaceutical industries. Many studies have focused on antimicrobial effects exerted by plant extracts on major food spoilage and pathogen microorganisms to reduce the use of synthetic chemical preservatives due to the pressure by consumers and legal authorities. The aim of this study was to determine the effect of aqueous and methanol extracts of quince *(Cydonia onlonga)* and persimmon (*Diospyros kaki)* leaves on *Staphylococcus aureus* ATCC 6538P*, Escherichia coli* ATCC 25922*, Salmonella* Typhimurium NRRL B4420, *Bacillus cereus* ATCC 10876*, Saccharomyces cerevisiae* NRRL Y-12632 and *Lactobacillus acidophilus* La-5. Leaves were dried subsequently ground powder of leaves were extracted with water and methanol for 15 h at 25°C. Then samples were sonicated using an ultrasonic bath (35 kHz, 15 min). Antimicrobial activities of leaf extracts were investigated for *in vitro* by using disc diffusion and microdilution techniques. MICs were found in methanol and aqueous extracts of quince leaf a range of 1.69 to 51.50 mg/ml against the tested microorganisms. The minimum inhibitory concentrations were a range of 1.29-6.5 mg/ml in methanol and aqueous extracts of persimmon leaf, however aqueous extracts of persimmon leaf showed weak activity against all the tested microorganisms. Our results demonstrated that methanol extract of persimmon leaves showed highest inhibitory activity against *B.cereus* with minimum inhibitory concentration (MIC) value of 1.29 mg/ml. Inhibition zone diameters of persimmon leaf extracts against all the tested microorganisms were in the range of 7.5-10.5 mm while quince leaf extracts showed varied in the zone inhibition from 7.0-11.5 mm. Minimum bactericidal concentration (MBC) of methanol extracts of persimmon leaf were 10.25 and 20.65 mg/ml against *L. acidophilus* La-5 and *S.* Typhimurium*,* respectively. By contrast, MBC of methanol extracts of quince leaf was 25.75 mg/ml only detected in against *L. acidophilus* La-5 among the tested microorganisms. The antimicrobial activity of methanol extract of persimmon leaf was higher than the antimicrobial activity of the other extracts. The result obtained in this study suggests a potential application of quince and persimmon leaf extracts to be used as a natural antimicrobial agent for food industry.

Keywords: Quince Leaf Extract, Persimmon Leaf Extracts, natural antimicrobials

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P4.93

Performance assessment of the 3M™ Petrifi lm™ lactic acid bacteria count plate according to ISO 16140-2:2016 standard in food products and environmental samples

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Introduction: Lactic acid bacteria (LAB) are non-spore forming Gram positive cocci or rods which produce lactic acid during carbohydrate fermentation. The 3M™ Petrifilm™ Lactic Acid Bacteria Count Plate is a self-contained, sample-ready-culture-medium system designed to enumerate LAB: nutrients, selective agents and oxygen scavenger compounds create an ideal environment for LAB recovery from food and beverage.

Purpose: An independent study was conducted to compare this new alternative method to the ISO 15214 (1998) according to the ISO 16140-2 (2016) standard for NF Validation approval.

Methods: Different matrices were tested: meat, dairy and seafood products, composite foods, meal components and environmental samples. The alternative plates were inoculated with 1 ml of successive dilutions in peptone salt and incubated 45 h at 30 ± 1 °C. Red colonies with gas (heterofermentative) or without gas (homofermentative) were enumerated. The possibility to store the plates for 1 week at -18 °C after incubation was evaluated. The study investigated the relative trueness, accuracy profile, inclusivity and exclusivity.

Results: Overall, 48 naturally contaminated samples and 49 artificially contaminated samples were analyzed by both methods. Depending on the tested food categories, the mean difference between alternative method and reference method counts ranged between -0.05 and 0.18 log CFU/g. After the storage at -18 °C, these values ranged between -0.05 and 0.16 log CFU/g. For accuracy profile study, the lower and upper β-ETI were comprised within the acceptability limits. Among the 57 tested target-strains, 52 gave similar results with both methods, 2 were enumerated only with the alternative method and 3 did not grow. No cross reaction was observed with the 34 tested non-target strains.

Significance: The alternative method is reliable for the enumeration of LAB and offers more practicability to the user than the reference method.

Keywords: Food spoilage, Lactic acid bacteria, Method validation

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P4.94

Antimicrobial efficacy of horseradish *(Armoracia rusticana)* extracts

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Horseradish (*Armoracia rusticana*) roots are traditionally used as a condiment for the different types of foods such as meat, pickled vegetables and some bakery products. There is an increasing demand on inhibition of microorganisms with natural antimicrobial materials. In this study, antimicrobial activity of horseradish aqueous and methanol extracts were investigated against various microorganisms: *Bacillus cereus* ATCC 10876, *Escherichia coli* ATCC 25922, *Salmonella* Typhimurium NRRL B4420, *Stapylococcus aureus* ATCC 6538P, *Lactobacillus acidophilus* La-5 and *Saccharomyces cerevisiae* NRRL Y-12632. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of extracts using microdilution method as well as disc diffusion methods were used for the determination of antimicrobial activity. MIC values were found in the range of 4.16-16.65 mg/ml and MBC values were found in the range of 8.32-33.3 mg/ml for horseradish methanol extracts. Even though MIC values of water extracts were found between 10.9-21.8 mg/ml, there was no inhibition in MBC assay for tested microorganisms. The maximum inhibitory activity was obtained for *B. cereus* in methanol extracts as MIC and MBC values of 4.16 and 8.32, respectively. Inhibition zone diameters were between 8 - 14 mm in horseradish methanol extracts and the strongest inhibition was seen in *S. cerevisiae* with a zone diameter of 14 mm. In horseradish aqueous extracts, there was an inhibition only for three species, 10 mm for *S. aureus* and *S. cerevisiae* and 7 mm for *L. acidophilus*. According to the results, horseradish methanol extracts are more efficient than the horseradish aqueous extracts and horseradish methanol extract has an inhibition effect on both pathogenic and beneficial microorganisms, inhibition zones also confirmed this conclusion. This study demonstrated that horseradish extract seems to be a promising natural antimicrobial that could be used in food industry for different processes such as integration in packaging materials and edible coatings and encapsulation to produce natural food preservatives.

Keywords: horseradish root extracts, minimum inhibitory concentration, natural antimicrobials

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P4.95

Stochastic application of double Weibull model to describe individual cell time to death heterogeneity as variability source in microbial heat inactivation

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A statistical modelling approach was applied for describing and evaluating the individual cell heterogeneity as variability source in microbial heat inactivation. Heat inactivation experimentations of *Salmonella enterica* ser. Agona with initial concentration of 10^9cfu/ml were conducted in tryptone soy broth without dextrose at various temperatures (55, 57, 61, 63 and 65°C). Four to six independent trials were performed for each temperature tested. A global regression model based on double Weibull distribution was fitted to the inactivation data. Double Weibull model enabled the fitting of most of the shapes of inactivation curves and described the inactivation of mixed bacterial populations consisting of subpopulations of different cell resistance. In contrast to traditional modeling approaches, the model was used and applied in a probabilistic manner which enables the quantification of the variability. More specifically, by estimating the parameters of the model, the fraction of the cells belonging two each subpopulation was determined and the distributions of single cell inactivation time at each temperature were defined. Using the above, the prediction of the inactivation of various initial populations of the pathogen at various temperatures was enabled using simulation techniques. The Monte Carlo simulation results for a population with N_0=10,000cfu/ml showed that the variability in the predicted behavior is negligible for concentration down to 100 cells due to the law of large numbers. As the concentration decreases below 100 cells however, the variability increases significantly. The results also indicated that the D-value used in deterministic first order kinetic models is valid only for large populations while for small populations D-value can be better characterized by a probability distribution rather than a single value. Apart from the variability stemming from single cell inactivation times, Poisson distribution was used to describe the variability due to experimental bacterial cell counts. For validation purposes, the predicted behavior and variability of the number of survivors at 60°C was compared to independent experimental data at the same temperature and it was found that the model described satisfactorily the variability in the inactivation behavior. It is expected that the present study will be useful in the development of a new generation of more realistic risk-based heat inactivation models leading to effective food safety management.

Keywords: double Weibull, Salmonella, variability, heat, single cell time to death

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P4.97

Detection of *Enterococcus* spp. in the probiotic *Lactobacillus* productions

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The quality of the fermentation process for the production of probiotics is commonly monitored by checking for microbial contaminations that may occur. Selective media are usually adopted to detect possible contaminants including *Enterococcus* spp. that may occur during fermentation causing the loss of the production.
As part of the Food Digital Monitoring (FDM) project, funded by the Piedmont Region, a rapid method for quantifying possible contaminants has been developed.
The objective of this study was the selection of sensitive methods to monitor the presence of contaminants during probiotic productions. Culture dependent and independent methods were adopted and ten probiotic samples (belonging to *Lactobacillus* spp.), artificially contaminated with *Enterococcus* spp., coming from the research laboratory of a pharmaceutical factory, were analyzed. Through viable count on selective media, *Enterococcus spp*. was detected in four samples among the ten analyzed. Isolates were identified by 16S rRNA sequencing and characterized by REP-PCR. The cluster analysis showed different profiles, confirming different contamination sources occurring during the productions.
Regarding culture independent methods, DGGE and Real-time PCR were tested for the detection of the contaminants. In both cases the enterococci were not found.
In order to simulate the conditions in the fermenter and to understand the detection limit of the contaminants, mixtures including a constant concentration of the probiotic *Lactobacillus spp.* and a decreasing concentration of *Enterococcus spp.* (10^9-10^2 cfu/ml) were analyzed. The results of the DGGE showed the presence of the contaminant only in the concentrations of 10^8-10^7 cfu/ml. The same matrices were analyzed using Real-time PCR. Different housekeeping genes with specific primers designed for *Enterococcus spp*. (16S, 23S) reported in literature were tested and then abandoned because of their poor selectivity against *Lactobacillus spp*. The primers designed on *recA* and *tuf* genes showed the best performance.
Analyzing the matrices, using the regression lines constructed, it was possible to detect the presence of *Enterococcus spp*. up to 10^4 cfu/g. The limits of the culture independent methods in these matrices are related to the high counts of the probiotic microorganism but in this study through Real-time PCR, it was possible to find the contaminant in concentration of about 10^4 cfu/g thus reducing the time needed in the culture methods.

Keywords: Enterococcus, contamination, probiotic, culture indipendent methods

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P4.98

Characterization of bacterial diversity in smoked salmon-processing environments

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Introduction: Bacterial biofilms are structured clusters of bacterial cells coated with an extracellular matrix and attached to a surface. This extracellular matrix confers tolerance to bacterial cells against cleaning and disinfection treatments and thus contributes to the persistence of these bacterial cells in food-processing environments.

Material and methods: Four smoked salmon-processing industries were requested to perform environmental samplings. Two 100cm² surfaces were swabbed before and after cleaning and disinfection steps. Sampling campaigns were carried out once a month for 8 months (from June to March) in each plant. Mesophilic, psychrophilic and lactic microorganisms were enumerated from each sample and the dominant flora was identified by MALDI-TOF mass spectrometry. *Listeria monocytogenes* was also enumerated and detected in each sample. Pulsotypes and serotypes of isolated strains were subsequently determined.

Results: Analysis of smoked salmon-processing environment samples highlighted a great qualitative and quantitative diversity in the bacterial contamination of surfaces. The main result of the study is the heterogeneity in the efficacy of cleaning and disinfection procedures revealed through this sampling campaign.

Significance: The presence of persisting bacteria in food plants questions the actual efficacy of applied cleaning and disinfection procedures

Keywords: smoked salmon-processing environments, microbial biodiversity, cleaning and disinfection procedures

Impact of interventions during food production on microbial biodiversity

P4.99

Mineral water: Concentration of heterotrophic microorganisms and their phenotypic characterization

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Water is a fundamental component to maintain biological functions and life on earth. Due to its microbial diversity, water can be considered as one of the critical disease vehicles, associated principally with antibiotic resistant bacteria. The nonpathogenic heterotrophic microorganisms are frequently found in mineral water. This study was undertaken to evaluate heterotrophic microorganism's concentration and their phenotypic properties in bottled water commercialized in the state of São Paulo, Brazil. A total of 1,010 samples of mineral water were analyzed. A total of five samples of 10 distinct brands was collected ffrom markets in different seasons (spring, summer, autumn and winter). Samples with different packaging sizes were collected (0.2 -0.310, 0.5 L, 1.5 L and 20L) and the pour-plate technique was used to determine the HPC as described by ISO 6222: 1999. HPC colonies with different morphological properties were selected, and the hemolytic activity was determined. Each different strain was inoculated onto sheep blood agar (Laborclin, Brazil), following incubation at 37°C/24 hours. Then, the type of hemolysis was recorded as alpha, beta or negative. Most of the samples had counts equal to or less than 500 CFU/mL (55.4% spring, 55.6% summer, 51.2% autumn and 61.6% winter) or between 1000 to 100000 CFU/mL (40.77% spring, 37.2% summer, 42.8% autumn and 37.2% winter). Among the HPC 972 isolates examined for hemolytic activity, 161 (16%) demonstrated beta hemolytic activity and 129 (13%) presented alpha hemolytic activity, showing virulence potential. High HPC in mineral water may cause odor and taste modification, and some HPC may turn to opportunistic pathogens with capability of threatening public health, especially immunosuppressed individuals.

Keywords: bacteria, bottled water, hemolysis

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P4.100

Prevalence and counts of *Pseudomonas aeruginosa* in mineral water samples marketed in the state of Sao Paulo, Brazil

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The contamination of mineral water with *Pseudomonas aeruginosa* can be influenced by water source conditions, microbial ecology, process conditions and storage temperature. Moreover, *Pseudomonas aeruginosa* can grow and survive in environments with few nutrients. Also, *Pseudomonas aeruginosa* can adhere to industrial pipes and filtration systems, and consequently biofilms can be formed. This study was undertaken to evaluate the prevalence and contamination level of *Pseudomonas aeruginosa* in bottled water available for purchase in state of São Paulo, Brazil. Furthermore, the isolated *Pseudomonas aeruginosa* strains were genotypically characterized. A total of 1,010 mineral water samples were analyzed, being five samples of each of 10 distinct brands. The samples that comprised different sizes of packaging (0.2 -0.310, 0.5 L, 1.5 L and 20L) were purchased from retail markets from different seasons (spring, summer, autumn and winter). The membrane filtration technique was used to detect and determine the counts of *P. aeruginosa* as described by the International Organization for Standardization (ISO) (ISO 16266:2006). *P. aeruginosa* typical colonies were identified by PCR based on the *oprL* gene. Out of 1,010, a total of 123 samples were contaminated by presumptive *P. aeruginosa* (0.2 -0.31 L (n=49), 0.5 L (n=14), 1.5 L (n=2) and 20L (n=58)). Among 282 isolates 85 (30.14%) ((0.2 -0.310 L (n=48), 0.5 L (n=8), 1.5 L (n=1) and 20L (n=28)) isolates were confirmed as *P. aeruginosa*. Further genotypic analysis will provide data regarding typing, virulence genes and biofilm formation properties of *P. aeruginosa* isolates from mineral water samples analyzed contributing to a better understanding of their potential health impact on consumers.

Keywords: bottled water, opportunistic pathoge,microbial ecology

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P4.101

Lis-RA: A software tool to predict listeriosis risk in different ready-to-eat food categories

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Listeriosis is a food-borne disease caused by *Listeria monocytogenes* of primary concern for Governments and Industry. The development of tools enabling food operators and risk managers to assess interventions and control measures is crucial to efficiently to reduce listeriosis incidence. Recently, a quantitative risk assessment model has been published aimed at estimating the listeriosis cases in Europe for different RTE food categories, in addition to providing wealthy source of models and scientific data (https://doi.org/10.2903/sp.efsa.2017.EN-1252). Based on this study, an user-friendly tool, called *Lis-RA*, was created for probabilistic risk assessment of food-borne pathogens, focused on *L. monocytogenes* in ready-to-eat foods, using Visual Basic for Applications (VBA) and including a customized Ribbon interface. The simulation capabilities were built upon functions from @Risk software.
The software can be freely accessed at the EFSA community for food safety tools, Knowledge Junction, in the Zenodo research-sharing platform (https://doi.org/10.5281/zenodo.822350). The software tool allows users to load risk model spreadsheets, select scenarios, define model inputs and simulation settings in an easy and intuitive way. The model notation is based on a simplified standard, making it possible to define models and customize software interface, showing only those variables and parameters of paramount interest for risk estimate, thereby creating a more understandable and easy-to-use environment for users who are not familiar with complex probabilistic models.
The annual number of listeriosis cases estimated with the application corresponded to 2,318 (95 confidence interval (CI): 1,450-3,612). Cooked meat and sausage presented most cases (median of 863 and 541, respectively). Importantly, the model evidenced that elderly population was the group most contributing to listeriosis cases in EU. Results confirmed the importance of controlling temperature and time in reducing listeriosis cases, but at the same time, showed that their effect varied depending on the type of food. This fact supports the hypothesis that more food specific approaches should be considered to mitigate risk by *L. monocytogenes.*
Food stakeholders can benefit from the use of *Lis-RA* to assess the risk by *L. monocytogenes* in different food products, providing a suitable scientific basis to better support risk management decisions and derive suitable control measures.

Keywords: Probabilistic risk assessment, Predictive software, Listeria, predictive microbiology, Excel Add-in

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P4.102

Antimicrobial effect against Listeria *monocytogenes* and *Bacillus cereus* of hydroethanolic extracts from fruit processing by-products

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Phenolic compounds are secondary plant metabolites that possess antioxidant, antimicrobial, antiviral and anti-inflammatory properties. Special attention is now focused on the extraction of these compounds from food processing by-products industry, such as the peels of several fruits and vegetables, which contain higher amounts of phenolics than the edible fleshy parts.
The aim of the present study was to evaluate the antimicrobial effect of hydroethanolic extracts of apple, peach, red pepper and cucumber, extracted with 70/30 (w/w) ethanol/water by ultrasound technology. Treatments were performed at 24 kHz and different amplitudes from 12 µm to 125 µm and times from 20 s to 120 s to find the optimum extraction conditions for each residue. The content of total phenolics in the extracts was determined spectrometrically according to Folin-Ciocalteu procedure and calculated as mg of gallic acid equivalents (GAE) per 100 g of extract. The antimicrobial effects of these extracts on *Listeria monocytogenes* ATCC 13932 and *Bacillus cereus* ACTC 11778 strains were evaluated using the agar disk diffusion and the "spot on a lawn" methods. Extraction of red pepper (1022 ± 42 mg GAE/100 g) yielded the highest amount of total phenolic compounds, followed by apple (472 ± 19 mg GAE/100 g), peach (322 ± 7 mg GAE/100 g) and cucumber (162 ± 5 mg GAE/100 g). However, only red pepper and cucumber showed halos of antimicrobial effect against both strains being cucumber extract the strongest one. The inhibition was more noticeable with the "spot on a lawn" method. These results reveal that hydroethanolic extract of cucumber, although its reduced phenolic content, is very promising for being used as a natural antimicrobial agent in foods.
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Keywords: food processing by-products; phenolic extracts; food pathogens; antimicrobial effects

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P4.103

LISTWARE project for developing a new *Listeria* assessment tool

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Introduction: The risk management of Listeria monocytogenes in ready-to-eat foods (RTE) involve large expenses and time spent for the food business operators (FBO). The FBOs should either perform expensive laboratory challenge tests or apply predictive modelling. In practice, the interpretation and compliance of the regulations by the European food industry and food authorities varies significantly. There are several software tools on the market for predicting Listeria growth. However, the software are of many regarded as difficult to use, and the application of them in FBOs are limited, at least in Norwegian food industry. The objective of the ListWare project is to develop a user-friendly risk management software for reliable assessments of Listeria monocytogenes safety and shelf life in RTE meat products and mixed products.

Methods: The project is working with establishing a database with quality assured data with product characteristics from the industry and the literature. Development of new predictive models of growth and interactions in product categories is performed by new challenge tests based on experimental design. Different categories of RTE foods will be tested. Also, durability studies of naturally contaminated products will be performed, A web based user-friendly application will be developed.

Results: The data collected from Norwegian studies show that there are several gaps in information of criteria for products and processes in earlier studies. Apparently similar products produce results with large variations. It is important to find the criteria that vary and influence the shelf lives in each study. The plan is to engage also other companies with similar software and databases, to discuss cooperation. There is also an idea to establish a European “library”. The ListWare team is interested in cooperation with others in this matter.

Conclusion: The ListWare software will mean saving of time and costs for the food industry. To secure the best use of the tool, ListWare will also include support and consultancy service for the users. When the database and interface is ready, licenses for access will be sold in both Norway and international. A business model will be made, which also include consultancy from ListWare team for the users.

Keywords: Listeria predictive modelling software

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P4.105

Assessment of selected bacteria species and risk factors associated with contamination of soured milk (Bongo) sold in retail diary shops in Makindye division, Kampala

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Aim: assess for occurrence of selected common bacteria species in soured milk sold out in retail dairy shops within Makindye division, Kampala district-Uganda.

Study Design:

A laboratory based cross sectional study.

Place and Duration of the study:

The study was done in Makindye division May to July 2017, 2017.

Method: A total of 174 soured milk samples were collected from retail dairy shops in Makindye division Kampala. These were cultured for isolation of bacteria species using standard bacteriological methods. The assessment of risk factors associated with contamination of soured milk was done using Chi-square tests. Odds ratios and their 95% confidence intervals were calculated. All statistical tests were two-tailed and *P values* of less than 0.05 were considered significant.

Results: In this study, 89.1% of all the soured milk samples showed significant bacterial contamination. Organisms isolated included E coli (47.1%); Klebsiella spp (28.4%), Shigella Spp (11.6%), Salmonella Spp (7.1%) and Enterobacter faecalis (5.8%). All the bacteria isolated were Enterobacteriaceae indicating probable faecal contamination of the soured milk as a result of poor hygiene. Risk factors that were associated with contamination of soured milk were age, level of education, working experience, source of information on milk handling, longevity of soured milk, presence of pests at facility, availability of hand washing equipment and general cleanliness.

Conclusion: The study has shown that soured milk sold by small retail diary shops in Makindye division Kampala is highly contaminated with pathogenic bacteria. There is a need for improvement of hygienic handling of soured milk during its processing and storage so as to ensure that the quality of soured milk sold out to the public is acceptable and safe for human consumption.

Keywords: bacteria species soured milk (bongo)

Impact of interventions during food production on microbial biodiversity

P4.106

Combining mathematics and physiology to predict the growth and sporulation of *Bacillus subtilis* BSB1 in dynamic conditions of temperature and pH

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Introduction and objective: Spore-forming bacteria can be involved in food poisoning and spoilage of processed foods. The physico-chemical conditions encountered during the spore formation define the spores yield, the resistance and germination abilities of the produced spores. Thus, it is interesting to identify where and when the spores can be formed during a food process. To do so, it is first necessary to predict the sporulation behaviour in dynamic conditions of temperature and pH.

Materials and methods: *Bacillus subtilis* BSB1 was used as a bacterial model. Growth and sporulation kinetics were performed in batch, in brain heart infusion with an initial inoculum of 10^3 CFU/mL, under 250 rpm agitation. The growth was monitored by enumerating total cells after plating on agar medium and sporulation was monitored by enumerating spores after a 10 minutes treatment of the total culture at 80°C. During incubation, two temperature profiles (a constant temperature profile at 37°C and a variable profile, alternating 37°C, 10°C and 37°C) and two pH profiles (at a constant pH profile pH 7.0 and a variable profile by applying pH, 7.0, then pH 5.0 and then pH 4.5) were applied.
The impacts of the environment temperature and pH on the growth and sporulation curves were previously assessed and modelled in static conditions. The predictions of growth and sporulation kinetics were computed and compared to the observations in dynamic conditions.

Results and discussion: We hypothesized that an environmental shift triggers a time necessary for bacterial adaptation which delays the sporulation. Then, the sporulation would continue according to the effect of the new environmental conditions. The growth and sporulation curves were efficiently predicted in dynamic conditions of temperature and of pH with RMSE values associated to the spores ($\log_{10}$ CFU/mL) below 0.45 and the maximal concentration of spores obtained at the end of the cultures were also correctly predicted with relative errors below 6.2%. These first results validate our hypothesis because the sporulation is indeed delayed when a bacterial culture is transferred to a new environmental condition. Then, the sporulation resumes following the sporulation curve which would be obtained in static conditions of the new environment applied.

Significance: This innovative work provides good tools to predict the formation of spores in the food industry where pH and temperature evolves through food processes.

Keywords: sporulation, dynamic conditions, models, Bacillus subtilis

Impact of interventions during food production on microbial biodiversity

P4.107

Potential of *Lactococcus lactis* strains to produce nisin in milk for a potential use as biocontrol agents

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The term “bio-preservation” refers to the use of microorganisms or their antimicrobial products in order to increase food safety and shelf life. The concept of “bio-preservation” offers a promising alternative to chemical preservation in food sector. Lactic acid bacteria (LAB) are certainly the most important bio-protective cultures for fermented (dairy products) and non-fermented foods (minimally processed fruit and vegetables) due to their ability to produce several metabolites, such as organic acids, fatty acids, hydrogen peroxide and diacetyl, as well as bacteriocins, which inhibit the growth of pathogens. In particular, *Lactococcus lactis* strains are recognized for their ability to produce nisin, particularly active against *L. monocytogenes*. In this view, principal aim of this research was to test the aptitude of some *L. lactis* strains, belonging to the Bologna (Italy) and LAVAL (Canada) Universities, to produce nisin in milk in order to select the best candidates to be used as adjuncts in cheesemaking. Moreover, since EFSA has established that the antibiogram is considered a prerequisite in protocols for the selection of starter, co-starter or functional microorganisms, the strains were investigated using a wide gamma of antibiotics. The results obtained showed that all the tested strains were susceptible (on the basis of CLSI guideline) to Ampicillin, Penicillin G and Chloramphenicol. Only the strain LSGA1B resulted intermediate to Tetracycline For what concerns Erythromycin the tested strains resulted susceptible with the exception of the strains LSGA1B, 9FS16 and 209 that resulted borderline. The tested strains resulted borderline with regard to Ciprofloxacin with the exception of the strain 3LC39 that resulted susceptible. The strains LSGA1B and 9FS16 were resistant to Gentamicin while all the other strains were susceptible. The main antibiotic resistance phenomena occurred with and Trimethoprim + Sulfamethoxazole. In fact, around 60% of the tested strains resulted resistant to Streptomycin and Trimethoprim + Sulfamethoxazole. The L. lactis strains were able to produce nisin in milk at different concentrations.
Although the interactions with the traditional starter used in cheesemaking such us S. thermophilus need to be verified, the present research permitted to select some strains as potential biocontrol agents.
This research was supported by the exchange project QU17MO02 Italy- Canada/Quebec.

Keywords: Nisin, antibiotic resistance, milk, L.lactis, biocontrol agents

Impact of interventions during food production on microbial biodiversity

P4.108

Determination of best process conditions for maximum flavour yield and safety in lafun produced with selected lactic acid bacteria (LAB)

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Lafun is one of a number of fermented cassava-based products. There is need to standardise the method of lafun production to a level where it can be adopted by industry, which is the focus of this study. The experimental design was broken down into three studies: Study 1: assessment of the pressing effect on drying time of fermented cassava and flavour quality of lafun, where cassava roots were fermented spontaneously and pressed before drying, while controls were processed without prior pressing; Study 2: Impact of freeze drying on the concentrations of flavour compounds in cassava fermentation and Study 3: determination of rate of acidification during cassava fermentation by LAB strains. Thirty-seven LAB strains were isolated from lafun produced through spontaneous fermentation. They were identified using API Fermentation Galleries 50 CHL as *Lactococcus lactis* (54.1%), *Lactobacillus collinoides* (21.6%), *Lactobacillus plantarum* (10.8%), *Leuconostoc mesenteroides* (10.8%), and *Lactobacillus brevis* (2.7%). Each isolate was assessed for acid and flavour production in lafun. Strains were selected based on lactic acid and succinic acid production for enhanced safety and flavour. Four strains were selected namely: *L. collinoides 36*; *L. lactis 43*; *L. lactis 56* and *L. brevis 58* based on these criteria and used in control fermentation of cassava in Studies 2 and 3. The results of study 1 suggest that pressing affects drying time as it was shortened from 96 to 24h. The concentrations of compounds such as lactate, citrate, malate and succinate were, however, reduced substantially by 1.57, 7.33, 3.30 and 2.62-fold respectively, when pressing was applied. In study 2 it was found that freeze-drying did not negatively impact the concentration of non-volatile flavour compounds in the fermented cassava. Finally, *L. collinoides 36* had the highest rate of acidification (reached pH 5.1 within 12h) followed by *L. lactis 43* (pH 5.2 within 12 h). Thus, lafun quality requires a steady fermentation for 48h without pressing prior to drying. Strains with the fastest acidification rate and highest flavour yield are important to achieve high quality lafun. Full genomic sequencing of the selected LAB strains used in this study is also underway to establish consumers' safety by ensuring the strains are not carrying antibiotic resistance genes.

Keywords: Cassava, lafun, Lactic Acid Bacteria, flavour compound, acidification, standardisation

Impact of interventions during food production on microbial biodiversity

P4.109

Risk assessment for *Salmonella* Senftenberg in sous vide cooked products at mild temperatures

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Sous-vide has evolved in recent years to an accurately controlled cooking technique at low temperatures in order to enhance even more sensory and nutritional quality of food. In view of this fact, food safety authorities and scientists have expressed interest in accurately define the microbiological safety of such delicate products. Salmon and pork loin portions were in-depth inoculated with *Salmonella enterica* serovar Senftenberg to a level of 5 - 6 log CFU/g. Then, they were portioned to 30 g pieces, vacuum packed and cooked at 55ºC and 60ºC in a convection-steam oven for 30, 60 and 90 minutes. *S.* Senftenberg counts were determined immediately after cooking and during storage at optimal (4ºC) and abuse temperature (8ºC) conditions to monitor lethality and recovery kinetics. pH and a_w values were also determined in control samples, ranging in salmon from 6.03 to 6.40 and from 0.998 to 0.992, respectively. Pork samples presented pH values between 5.60 and 5.82 and a_w values between 0.990 and 0.992. Microbial population densities along with time were modelled with the online application DMFIT (https://www.combase.cc/index.php/en/). Thermal inactivation at 55ºC presented moderate downward curves during the whole heat treatment. On the contrary, at 60ºC the initial cell population decreased more than four orders of magnitude in the first 30 minutes. S. Senftenberg cells could only recover and growth at 8ºC in salmon and pork loin samples after being cooked at 55ºC and in salmon samples after 60ºC cooking. However, counts exhibited high variability between replicates, with mean differences up to 2 log CFU/g. Experimental data were adjusted to the growth model of Baranyi and Roberts (1994). Probability density functions were generated for (i) the estimated maximum growth rate (Gr_{max}) and (ii) the predictions as a function of time, by using Monte Carlo analysis. Treatments of 30 minutes at both cooking temperatures showed smaller 95% CI for Gr_{max} and enclosed narrower zones of prediction than longer treatments. The heterogeneity observed in 60 and 90 minutes treatments can be attributed to a major presence of sub-lethal injured cells. Data obtained in this study are intended to improve quantitative risk assessment studies for mild temperature sous vide cooked products.

Keywords: Sous-vide cooking, predictive microbiology, Monte-Carlo analysis, risk assesment.

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P4.110

Microbiological quality assessment of minorly processed foods from supermarkets of the west zone of Rio de Janeiro, Brazil

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The market for minimally processed foods is growing rapidly and is increasingly gaining approval from consumers who opt for healthy foods that are quick and easy to prepare. Minimal processing comprises the handling, preparation, packaging of agricultural products, through selection, classification, pre-washing, slicing, sanitizing, rinsing, centrifugation and refrigeration, aiming at maintaining the product fresh, healthy and most often, ready for consumption.. However, non-monitored processing can become a public health problem In the last decades, processed food consumption has increased and consequently the problems of Foodborne Diseases (FBDs). Foods can be contaminated at various times and from a variety of sources, including inadequate handling, packaging, humidity and temperature. FBDs represent a significant economic loss and an important public health problem. The objective of this study was to determine the microbiological and parasitological quality of minimally processed foods sold in supermarkets of the municipality of Rio de Janeiro, Brazil. Food quality was determined according to norms established in RDC n° 12 (Brazil, 2001). Samples were acquired and sent under refrigeration to the Laboratory of Microbiology and Parasitology of Castelo Branco University, Rio de Janeiro. The quantification of total and thermotolerant coliforms and the detection of potentially pathogenic bacteria were conducted according to methodology described by the American Public Health Association (APHA, 1984). The detection of helminth eggs and protozoan cysts followed the methodologies of Lutz (1991) and Faust (1938), respectively. No helminth eggs or protozoan cysts were found in the samples. However all samples had fecal coliform count above the allowed standard (10^2 MPN/g), with *Escherichia coli* being identified and isolated, and most foods were positive for *Salmonella* sp. The presence of *Salmonella* sp and fecal coliform count above the limit accepted by the legislation indicates that the foods studied were not adequate for consumption. The results evidenced the low hygienic-sanitary quality of these foods and that training on good manufacturing practices is needed.

Keywords: Minimally Processed Foods, Foodborne Diseases. Food Quality

Impact of interventions during food production on microbial biodiversity

P4.111

Application of PMA-qPCR to evaluate the efficacy of novel non-thermal techniques on microbial inactivation of pelagic

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Novel non-thermal technologies such as cold plasma, ultrasound, high pressure and light based technologies, and their application in the improvement of the food safety profile of pelagic fish, present many challenges. In applying such technologies for enhancing the shelf life of pelagic fish, methods employed for testing the efficacy of new technologies are equally important. This application also requires a thorough understanding of the mechanisms that underpin cell injury and/or death in relation to the specific technology employed. To address these challenges, a molecular toolbox that addresses the ability of a cell to undergo sub lethal injury, as well as the induction of the Viable But Non Culturable (VBNC) state induced by sub lethal stress, is required. As well as confirming true inactivation, current monitoring tools must also improve upon specificity, sensitivity and rapidity to overcome the limitations inherent in relying upon culture methods alone. By employing a DNA binding viability dye (propidium monoazide) prior to quantitative PCR, the discrepancy between truly inactivated cells and VBNC cells can be addressed in the context of these non-thermal technologies. PMA-qPCR alongside imaging techniques such as SEM and Flow Cytometry allow us to elucidate the mechanism of inactivation for a number of novel non-thermal technologies. The degree of inactivation for each method could be quantified, and the relative efficacy of PMA-qPCR on various inactivation techniques could be established. Furthermore, cell reductions ascertained from the conventional plate count method allow for a direct comparison with PMA-qPCR results for both pathogenic and spoilage bacteria associated with pelagic fish.

Keywords: microbial inactivation, non-thermal technologies, PMA-qPCR, VBNC, pelagic fish

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P4.112

Antagonistic activity of *Lactobacillus:* Growth dynamics of antifungal strains and application of antibacterial strains in cheese and meat fermentations

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Biopreservation by food-grade lactic acid bacteria (LAB) is still a limited technology in fermented food since the living conditions for individual microorganisms vary greatly in different food matrices. Therefore, we aimed to develop an approach to screen large numbers of LAB strains for potential use as protective cultures in individual food categories. A phenotypic screening of more than 500 *Lactobacillus* strains for 26 food relevant growth conditions revealed variations and physiological limits for the *Lactobacillus* genus. We assessed the activity of antibacterial or antifungal strains from that screening in more detail, mainly against *Listeria, Enterococcus, Rhodotorula* and *Candida* species. 22 antibacterial and 42 antifungal strains were detected. In a co-culture competition-assay a minimal number of 1-2 cfu/ml of our isolate *Lactobacillus plantarum* RI-162 was able to inhibit the outgrowth of our *Rhodotorula mucilaginosa* test strain and reduce the cell number below the detection limit of 50 CFU/ml within 48 hours. This underlines the power of LAB and the requirement of proliferating conditions of this antifungal strain i*n vitro*.
In vivo, we applied findings from antibacterial *Lactobacillus* strains to industrial (1) meat and (2) cheese fermentations:
(1) the potential protective culture *L. sakei* RI-409 reduced the initial *Listeria ivanovii* DSM 12491^T concentration in an industrial-scale salami fermentation by 1.4 log within 5 days. In a further small-scale salami fermentation one *Lactobacillus sakei* and 5 *Lactobacillus plantarum* strains were tested as protective cultures. Four of them reduced the spiked counts of *L. ivanovii* DSM 12491^T from 10^5 cfu/g at the start of fermentation to below the detection limit of 100 cfu/g within 2 days.
(2) in a 1000-L small-scale raw milk soft cheese fermentation, the potential protective culture *L. plantarum* RI-271 reduced the endogenous enterococci concentration with 1.5 log compared to untreated raw milk.
In conclusion, we have developed an approach to select tailor-made antimicrobial protective cultures for biopreservation in fermented food products.

Keywords: Lactobacillus, protective culture, systematic approach, food fermentations, biopreservation

Impact of interventions during food production on microbial biodiversity

P4.113

When the hurdle theory works unexpectedly: The case of high pressure processing and biopreservation in deli cooked meat products

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Listeria monocytogenes is the most relevant hazard associated with ready-to-eat cooked meat products considered. Food industry needs to control this pathogen with strategies aiming to reduce its loads with post-lethality treatments, such as high pressure processing (HPP). In order to reduce or supress the growth of the pathogen during shelf-life, the use of antimicrobial agents such as organic acid (OA) salts are often used. Based on the hurdle concept, an additive or a synergistic effect may be expected by the combination of these strategies. In this work we present surprising results of the studies performed aiming to understand the behaviour of *L. monocytogenes* in cooked meat products with OA salts and HPP, in terms of HP-inactivation and the subsequent growth during storage.

A series of experiments were performed with cooked ham manufactured without OA (control), with lactate and with lactate plus diacetate at different concentrations. Products were sliced aseptically and inoculated with *L. monocytogenes* (strains CTC1034, CTC1011 or ScottA or a cocktail) and vacuum-packaged. To monitor inactivation kinetics, products were pressurized at 400 MPa at different holding times (0-10 min). To study the growth behaviour after HPP, products were treated at 600 MPa for 3 min and stored at 8, 12 and 20 ºC. *L. monocytogenes* was enumerated on chromogenic medium.

The shape of HP-inactivation curve was dependent on the strain and its magnitude depended on the product formulation and the strain. Overall, the presence of lactate caused a dose-dependent piezo-protection, as the inactivation rate was lower in cooked ham formulated with lactate than in the control product. During the storage of cooked ham, a higher number of samples showing growth of pathogen were recorded in HPP products formulated with lactate in comparison with control HPP products (without OA). At 20ºC, the growth rate of *L. monocytogenes* in HPP cooked ham was up to 4 fold-higher in the presence of lactate than in control products (without OA).

The piezoprotective effect of lactate in the immediate HP-inactivation of *L. monocytogenes* together with the piezo-estimulation of its growth during shelf-life in lactate-containing HPP cooked ham, makes the formulation with lactate not recommended for cooked meat products intended to be HPP. Additionally, the design, validation and implementation of HPP require a tailor-made approach, taking into account the specific product formulation.

Keywords: Listeria monocytogenes, safe-shelf life, RTE cooked meat products, high pressure, antimicrobials

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P4.114

Prevalence of pathogenic in camel, cows and goats fermented milk consumed in Burkina Faso

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The aim of this work was to evaluate the prevalence of *Staphylococcus aureus*, total coliforms, thermotolerant and *Salmonella* or *Shighella* in fermented milk consumed in Burkina Faso. A total of 114 fermented milk samples produced by traditional method, were collected from five localities in Burkina Faso; *Bobo Dioulasso, Djibo, Dori, Gorom-Gorom*, and *Sebba*. The research this pathogenic was monitored using standards methods. Microbiological analysis of fermented milks showed high average values of 1.97 ± 0.18 x 10^3 CFU/ml; 1.98 ± 0.25 x 10^3 CFU/ml, and 0.10 ± 0.09 x 10^3 CFU/ml for *Staphylococcus aureus*, total coliforms, and thermotolerant coliforms respectively. None of the samples were contaminated by *Salmonella* or *Shighella*. *Staphylococcus aureus*, and thermotolerant were not significantly different, but total coliforms was significantly different. Camel milk was contend high rate of *Staphylococcus aureus,* while that to thermotolerant coliforms was high in goat milk. This research contributed to evaluation the hygienic quality of local fermented milk. Results obtain this study confirms the need to set up the training program on the sanitary condition to traditional maker´s to ensure the good fermented milk with high organoleptic and nutritional qualities.

Keywords: Camel, Cow, Goat, Fermented milk, Sanitary quality, Burkina Faso

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P4.115

Effect of high hydrostatic pressure and grape seasonings on *Listeria monocytogenes* inoculated in soft cheese

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Listeria monocytogenes is a foodborne pathogen and its characteristics of ubiquity and resistance involve a challenge in the cheese industry. The combination of the addition of natural additives, such as wine industry by-products, with other preservation technologies, such as high pressure processing (HPP), could be effective to control its presence in some types of cheese.
The aim of this work was the evaluation of the effectiveness of grape seasonings and/or HPP on *L. monocytogenes* inoculated in soft cheese. Two types of grape seasonings, added as a coating to cheese, and a high pressure treatment of 600 MPa for 5 minutes were evaluated in soft cheese inoculated with 5 strains of *L. monocytogenes*. Along 28 days of chilling storage, microbiological and physic-chemical parameters were studied. Besides of *Listeria*, aerobic mesophilic microorganisms, lactic acid bacteria (LAB) and enterobacteria were analysed. Moreover, pH and a_w were determined.
The addition of seasonings did not have anti-microbial effect, being the aerobic mesophilic and LAB counts in these cheeses similar to the control cheese counts. In any of the samples there was presence of *Enterobacteriaceae*. Neither the 5 strains of *L. monocytogenes* inoculated were affected by the seasonings. The HPP treatment limited the development of aerobic mesophilic and LAB and determined an inactivation of *L. monocytogenes*, whose counts were reduced by three units logarithmic without increasing throughout the storage.
The combined treatment of high pressure and coating of cheese with the seasonings had a clear synergistic effect on aerobic mesophilic and LAB. These cheeses presented lower counts, especially in the early days of chilling storage. The synergistic effect was especially remarkable on the *L. monocytogenes*, since not only a greater initial inactivation occurred but there was an additional inactivation during storage. In the cheeses with combined processing (HPP plus seasoning), counts at the end of the storage were 2 log units less than in the cheese treated only with high pressures. There was not much difference between the two seasonings. The addition of coatings or treatment with high pressures largely did not change pH values. On the other hand, the a_w was not changed during the time of storage and not affected by the presence of seasonings or high pressure treatment.
This study was financially supported by the research project BU056U16 from Junta Castilla y León (Regional government), Spain.

Keywords: Grape seasonings, Listeria monocytogenes, antimicrobial effect, cheese industry, HPP

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P4.116

In vitro study of *Listeria* spp. inactivation with a combination of grape seasonings and high hydrostatic pressure

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Listeria monocytogenes is recognised as a foodborne pathogen worldwide and the causative agent for many food outbreaks and ready to eat products (RTE) as the main products involve in the latest. High hydrostatic pressure technology (HPP) is described as an effective alternative in the inactivation of *L. monocytogenes* along with the minimum deterioration of the sensory and nutritional characteristics of the food product. Moreover, natural additives, such as grape seasonings, are of interest in the food industry to control this pathogen in combination with other preservation technologies besides its health impact on the consumer.

The aim of this work was to study the *in vitro* effect of the combination of two HPP treatments (400 MPa-5 minutes; 600 MPa-5 minutes), and two natural grape seasonings (S5 and S6) against 8 *Listeria* spp. strains. Three *L. monocytogenes* strains isolated from RTE salmon, 4 from the ILSI collection and 1 *L. innocua* strain from meat origin were inoculated at a level of 8 log cfu/mL. A control with low pH was also included to a better attribution of the seasoning antimicrobial effect other than its pH reduction.

After the treatment, *L. monocytogenes* strains and *L. innocua* were completely inactivated at the highest pressure tested (600 MPa). Treated at 400 MPa, *L. innocua*, *L. monocytogenes* ILSI 17 (serogroup 1/2c, 3c) and the serogroup 1/2 a, 3a reached significantly lowest inactivation except the salmon strain S1 10 within serogroup 1/2 a, 3a that was the more sensitive to 400 MPa. The pH contributed to the complete inactivation of the 4 ILSI serogroups; however, salmon strains (serogroup 1/2 a, 3a) and *L. innocua* were not inactivated. In combination with S6 almost all the strains were completely inactivated with the exception of 3 strains within serogroup 1/2 a, 3a (S1 1, S1 6 and ILSI 18). The inactivation reached in combination with S5 was significantly higher than the controls, especially for S1 6 and *L. innocua*. Furthermore, S1 10 (serogroup 1/2 a, 3a) and ILSI 17 (serogroup 1/2c, 3c) were completely inactivated.

Therefore, the combined application of grape seasoning and the non-thermal technology of HPP could be a promising tool for the food industry with a view of replacing the artificial additives to improve the food safety of RTE products and the positive effect on the consumer's health.

This study was financially supported by the research project BU056U16 from Junta Castilla y León (Regional government), Spain.

Keywords: natural additives, food safety, hurdle technology

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P4.117

Effect of red grape seasonings and high hydrostatic pressures in the levels of *Listeria monocytogenes* inoculated on marinated salmon

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Listeriosis is a foodborne disease caused by *Listeria monocytogenes* in different ready-to-eat foodstuffs such as smoked fish and cheeses. Listeriosis is the major cause of death by foodborne pathogens. For this reason, food industry is interested in searching for new strategies to avoid the growth of *L. monocytogenes.* Some strategies are the use of high pressures, the use of natural antimicrobial compounds mainly from plant origin, and the application of hurdle technologies, such as the combination of the two strategies mentioned before.

Therefore, the main aim of this work was to study the antimicrobial effect of two different red grape seasonings and high hydrostatic pressures, on *L. monocytogenes* inoculated on marinated salmon, which is one of the foodstuffs with more prevalence of this pathogen.

To achieve this goal a challenge test was carried out. A pool of 4 different strains of *Listeria monocytogenes* and one of *Listeria innocua* were inoculated on marinated salmon which was made with and without grape seasoning (4%). In addition, salmon was treated or not with high pressure processing (HPP, 300 MPa, 5 minutes). The growth of *Listeria* was evaluated during 27 days. In order to evaluate global quality of salmon, Total aerobic mesophilic bacteria, Lactic acid bacteria, *Enterobacteriaceae* and TBARS levels were also measured.

The results obtained indicated that applied HPP treatment so as the concentration of seasoning used to marinate the salmon were not enough to significantly reduce the levels of *Listeria* in the marinated salmon. However, the combination of the high pressures with the seasoning favored the bacteriostatic effect on *Enterobacteriaceae* and *L. monocytogene*s. It was also observed that HPP brought on lipid oxidation, effect that was not observed on salmon marinated with the seasonings.

This study was financially supported by the research project BU056U16 from Junta Castilla y León (Regional government), Spain.

Keywords: Grape seasonings, Listeria monocytogenes, antimicrobial effect, salmon, HPP

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P4.118

Study of sublethal damage in *L. monocytogenes* strains treated with the combination of HPP and grape seasoning

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Minimally processed food products are in increasing demand due to convenience and fresh-like organoleptic characteristics. On the other hand, *Listeria monocytogenes* is recognized as a foodborne pathogen worldwide. Its importance in the food industry is due to the ubiquity and resistance to adverse conditions. Nowadays, the use of multiple barriers against microbial growth (hurdle technology) is the best strategy for maintaining the balance between safety and quality. High pressure processing technology (HPP) is described as a very effective alternative in the inactivation of *L. monocytogenes* along with the minimum deterioration of quality characteristics of the food product. However, many HPP potential applications would require long treatment time and high pressure intensity to ensure an adequate inactivation level of pathogens and spoilage microorganisms. For that, the combination of new grape seasonings and the non-thermal technology HPP, could be very useful for the food industry with a view of replacing the artificial additives. For this purpose, the behavior of 8 strains of *L. monocytogenes* isolated from a cheese company in in floors, surfaces, gloves and product treated with two HPP, (400 MPa for 5 minutes and 600 MPa for 5 minutes), two wine seasonings (S5 and S6) and the combination of both, were tested in an in vitro experiment after some time storage in refrigeration (19-26 days). The results of cell damage were different depending on the strain and the treatment applied. *L. monocytogenes* grew in most samples kept at 4 ºC after the storing time, except in HPP samples which did not show a significant proliferation. No damage was detected at 600 MPa, no significant growth was observed in the samples treated by high pressures at 600 MPa, *Listeria* was totally inhibited. In the samples treated at 400 MPa there was a significant subletal damage but combined with grape seasoning was lower. A possible relationship between the damaged cells and a later reduction in the final time counts was established.
This study was financially supported by the research project BU056U16 from Junta Castilla y León (Regional government), Spain.

Keywords: Grape seasonings, Listeria monocytogenes, antimicrobial effect, cheese industry, HPP

Impact of interventions during food production on microbial biodiversity

P4.119

Salmon gravlax biopreservation: Impact on organoleptic properties, microbial ecosystem and volatilome

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Seafood and fishery products are very fragile commodities with a short shelf-life as the consequence of organoleptic and microbiological qualities quick deterioration. Spoilage, resulting from microbial growth and activity, is responsible for up to 25% of food losses in the fishery industry. In this context and to meet the consumer's demand for minimally processed food, developing mild preserving technologies such as biopreservation, chitosan coating and superchilling, represents a crucial challenge. In this work, we studied the use of 6 lactic acid bacteria (LAB), selected for their properties as bioprotective agents, for salmon dill gravlax biopreservation. Naturally contaminated dill gravlax with a commercial shelf-life of 21 days, sliced and vacuum packaged, was purchased from a French retailer. Unpacked gravlax slices were inoculated aseptically by spraying (airbrush) 2% of LAB suspension in saline buffer to reach a final concentration of 10^6 log UFC/g in the product. Inoculated sliced were stored under vacuum packaging at 8 °C until the end of the experiment. A control was also realized in the same conditions without bacteria inoculation nor saline buffer addition. At each sampling date:

1) microbiological analyses were performed: lactic flora (NAP), total flora (LH), *B. thermosphacta* (STAA), Enterobacteria (VRBG), *P. phosphoreum* (q-PCR). Bacterial ecosystem evolution was determined using Illumina Technologie Miseq (V4 region of 16S rRNA gene);

2) A complete sensory analysis was conducted with a conventional profiling test on appearance, and odor;

3) Volatile organic compounds (by HS-SPME/GC-MS), Total Volatil Basic Nitrogen (TVBN), trimethylamine (TMA), biogenic amines (by HPLC) and pH were also measured;

In parallel, challenge tests were performed for the 6 LAB against a cocktail of 5 strains of *L. monocytogenes*.

Some of these LAB strains showed interesting abilities to improve the quality or safety of this product. In order to study the effect of these LAB strains on organoleptic properties as well as on biochemical and microbiological aspects, statistical tools for multidimensional analysis were used on the whole data set.

Keywords: Fish, food safety, spoilage, inhibition, protective bacteria, microbial communities

Impact of interventions during food production on microbial biodiversity

P4.120

In vitro study of *Listeria* spp. sublethal damage and its recovery after high hydrostatic pressure treatment combined with grape seasonings

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Clean labels, currently demanded by consumers, present a great scientific challenge to the food industry that has also to deal with the production of safety products. Natural additives, such as grape seasoning, and novel technologies, like high hydrostatic pressure technology (HPP), have been developed in the last years to control the presence of *Listeria monocytogenes* in food products, especially in those ready to eat (RTE).

The aim of this work was to study the sublethal damage on 8 *Listeria* spp. strains after two HPP treatments (400 MPa-5 minutes; 600 MPa-5 minutes) combined with two natural grape seasonings (S5 and S6). Its recovery after a period of 19-26 days at 4 °C was also studied. Three *L. monocytogenes* strains isolated from RTE salmon, 4 from the ILSI collection and 1 *L. innocua* strain from meat origin were inoculated at a level of 8 log cfu/mL. A control with low pH was also included to a better attribution of the seasoning antimicrobial effect other than its pH reduction.

After the treatment, *L. monocytogenes* strains and *L. innocua* were completely inactivated at the highest pressure tested (600 MPa) thus, no sublethal damage was observed. 400 MPa induced sublethal damage statistically higher in ILSI 29 (serogroup 4b, 4d, 4e), ILSI 9 (serogroup 1/2b, 3b,7), ILSI 17 (serogroup 1/2c, 3c) and S1 10 (serogroup 1/2 a, 3a) than in the other 3 strains from serogroup 1/2 a, 3a and *L. innocua*. A reduced pH in combination with 400 MPa produced 100 % sublethal damage in two salmon strains, however a 68.28 % and 6.26 % was observed in the other salmon strain and *L. innocua*, respectively. S6 in combination with 400 MPa resulted in 100 % sublethal damage as well as in the case of S5 except for S1 6 and ILSI 18 (serogroup 1/2 a, 3a) with 55.61 % and 7.34 % damage respectively. On the other hand, after storage at 4 °C the S6 controlled the growth of *Listeria* except for S1 1. No growth was observed when treated at 600 MPa combined with S6 expect for ILSI 18 and *L. innocua*. S1 1 and ILSI 17 were the more sensitive strains and were not recovered after the storage. S5 was effective during the storage against 3 strains in combination with 600 MPa.

Therefore, food safety could be improved during the shelf life of the products by the combination of a natural grape seasoning and HPP providing consumers a clean label product minimally processed.

This study was financially supported by the research project BU056U16 from Junta Castilla y León (Regional government), Spain

Keywords: natural additives, food safety, hurdle technology, shelf life

Impact of interventions during food production on microbial biodiversity

P4.121

Occurrence of anti-*Listeria* bacteriocins in LAB: Screening of genetic determinants and preliminary protein characterization

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Bacteriocins are bacterially produced antimicrobial peptides expressing narrow or broad host ranges. Lactic acid bacteria (LAB) are well known for the production of bacteriocins with two main classes. Class IIa comprises pediocin-like bacteriocins that are small peptides with a narrow spectrum of activity but a high specific activity against the food pathogen *Listeria monocytogenes*. Bacteriocin producing LAB are thus highly interesting for application as beneficial microorganisms in biocontrol concepts aiming at increased food safety concomitant with a reduced use of artificial additives. In this study, the presence of genes encoding class IIa bacteriocins was determined in 52 LAB strains belonging to the species *Lactobacillus sakei*, *Lb. plantarum*, and *Lb. curvatus.* LAB strains were previously isolated from a broad variety of fermented meat and fish products and sowed high activity against strains of *L. monocytogenes* serovars 1/2a (ATCC15313), 1/2b (SLCC2755), and 4b (ATCC19115) in an agar spot assay in meat simulation medium (MSM) and in a well diffusion assay. In an overview, the presence of the structural genes of pediocin AcH/PA-1 (5 strains), plantaricin A (7 strains), plantaricin E/F (8 strains), plantaricin JK (2 strains), plantaricin S (6 strains), plantaricin W (10 strains), curvacin A (4 strains), sakacin G (16 strains), sakacin Q (13 strains), and sakacin P (10 strains) was observed. Furthermore, 12 strains belonging to the species *Lb. sakei* and *Lb. curvatus* revealed no amplicons under the conditions tested supposing novel bacteriocins. A preliminary characterization of a putative unknown bacteriocin of *Lb. plantarum* strain 25 based on ammonium sulfate precipitation (80%) followed by dialysis (MWCO 500Da) and ultrafiltration
(MWCO 3 and 30kDa) revealed an anti-*Listeria* active band in a renaturated SDS PAGE at »16 kDa. This study showed a broad variety of class IIa bacteriocins within LAB naturally present in fermented meat and fish products including unknown bacteriocins. The potential of selected strains in food model systems is currently under investigation.

Keywords: anti-Listeria bacteriocins

Microbiological spotlights

P5.1

Virulence potential and antibiotic resistance of Shiga toxigenic Escherichia *coli* (STEC) isolates from raw cow milk in Ghana

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Shiga toxigenic *Escherichia coli* (STEC) is an important food-borne pathogen of public health concern in both developed and developing countries. STEC can cause haemorrhagic colitis and haemolytic-uremic syndrome, which can lead to kidney failure and death, particularly in young children. Currently, there is little or no information on the virulence potential and antibiotic resistance profile of STEC isolates in raw milk collected from agro-pastoral farms in Ghana. This study therefore sought to investigate the prevalence, virulence potential and phenotypic antibiotic resistance of STEC isolates in raw cow milk collected from agro-pastoral farms in Ghana. A total of 210 raw cow milk samples were collected from 42 agro-pastoral units made up of 15 small scale (1-20 cattle), 23 medium scale (21-100 cattle) and 4 large scale (> 100 cattle) farms in Ghana. *Escherichia coli* was isolated from raw milk and identified using the Vitek II Compact system. PCR analyses were performed using specific primers targeting the major STEC virulence genes that encode Shiga toxin (*stx*1 and 2), intimin (*eae*A), and STEC autoagglutination adhesin (*saa*). The O-serotypes of STEC isolates were determined by using *E. coli* antisera. Antibiotic susceptibility profiles of STEC isolates was examined by the determination of the minimal inhibitory concentrations (MIC). In total 58.1% (122/210) of raw milk samples collected from agro-pastoral farms were positive for *E. coli*. Escherichia coli was frequently detected in milk from large-scale farms (42/60; 70%), medium-scale farms (47/90; 52.2%) and small-scale farms (33/70; 47.1%). Ten of 423 (2.4%) *E. coli* isolates harbored the *stx* genes. The *stx* genes were detected in *E. coli* isolates originating from large-scale (6/130 isolates; 4.6%) and medium-scale (4/185 isolates; 2.2%) farms, while no *stx* gene was detected in *E. coli* isolates originating from small-scale farms. All 10 STEC isolates belonged to different serogroups, with no O157 serotype detected. Again, all STEC isolates harbored *eae* gene but not *saa* gene. In addition, 40% (4/10) harbored only *stx*1 gene, 10% (1/10) harbored only *stx*2 gene and 50% (5/10) possessed both *stx*1 and *stx*2 genes. STEC isolates showed phenotypic antibiotic resistance to ampicillin (10/10; 100%), streptomycin (10/10; 100%) and tetracycline (8/10; 80%). All STEC isolates showed resistance to at least two different antibiotics.

Keywords: STEC, virulence potential, raw milk, antibiotic resistance, serogroups

Microbiological spotlights

P5.2

Incidence and antibiotic susceptibility pattern of medically important bacteria in meat pie: Implications for public health

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Meat pie is a widely consumed ready-to-eat food in Nigeria. Its popularity cuts across all strata of the society with a wide acceptance in Makurdi. However, the manner in which it is produced suggests the possibility of risks to the health of consumers. Ninety samples of retailed meat pie were examined to investigate its bacteriological quality, determine the antibiotic susceptibility pattern of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* species in the samples and evaluate observance of point of sales best practices by retailers. Samples were collected from eateries, supermarkets and street hawkers, and isolates were identified by cultural and biochemical characteristics. *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus* spp, *Enterobacter* spp, *Proteus* spp, *Pseudomonas* spp, *Citrobacter* spp, *Edwardsiella* spp, *Bacillus* spp, *Klebsiella* spp and *Shigella* spp were identified. *Bacillus* spp (85.0%) occurred most. Mean viable counts of fresh meat pie samples ranged from 6.63×10^7 to 1.01×10^9 cfu/g for both fillings and crusts. Although Analysis of variance (ANOVA) and Chi-square tests revealed no statistically significant differences in contamination rates for samples from different sources ($p > 0.05$), samples sold by hawkers had the highest mean viable counts. Contamination with isolates in the samples differed statistically. Antibiotic susceptibility tests revealed multiple drug resistance and showed that 87.1% of *Staphylococcus aureus* isolates were resistant to Cloxacillin while 88.6% were susceptible to Ofloxacin. *Escherichia coli* was resistant to Amoxycillin (100%), Tetracycline (100%), Cloxacillin (100%) and Augmentin (100%) but susceptible to Gentamicin (80%) and Ofloxacin (80%). None of the vendors (90:100%) used hand gloves, none (90:100%) used an apron, 89 (98.9%) used no cutlery and 89 (98%) had uncovered hair while serving the product. All samples were found contaminated beyond acceptable limit (10^3 to $< 10^4$) and some vital point of sale best practices were negleted by vendors. Meat pie could serve as a vehicle for antibiotic resistant bacteria. Production and sales of meat pie should be appropriately regulated and observance of established point of sales practices enforced for the sake of the public health.

Keywords: Resistance, Meat, pie, hygiene, antibiotic, contamination,

Microbiological spotlights

P5.3

Certification of rapid methods for pathogens in foods

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There are many rapid/alternative methods for detection of pathogens and indictor bacteria in food and environmental samples. In order to use these methods in the context of official controls and for business operators, the methods need to be validated against reference methods according to internationally accepted protocols and assessed/certified by independent parties or authorized by the competent authority ((EC) 2073/2005).
In a third-party certification, the alternative method is compared to a reference method through extensive studies, reviewed by technical experts and finally certified by an organization like NordVal International. Hereby, it will be documented how well the method performs; such as the detection level and how suitable the method is in detecting the bacteria strains of interest and no other interfering strains. The validation is carried out according to ISO 16140-2:2016 Microbiology of the food chain - Method validation - Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method. Independent reviews also assess that the performances comply with the claims of the manufacturers.
Some methods are not granted a certificate, however, can still be on the market. Use of certified methods are beneficial for all laboratories and their business associates; due to both the quality aspect and hence possible less costs related to false or inaccurate results, as well as immense resource saving for the individual laboratories in not performing extensive validations. The presentation will be about the validation and certification process, as well as interpretation and use of the results given in the certificates.

Keywords: Certification, rapid methods, validation

Microbiological spotlights

P5.4

The role of host matrix metalloprotease-9 and Zpx in *Cronobacter* spp. mediated sepsis in a morphant zebrafish model

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Bacteria belonging to the genus Cronobacter (C.) have been recognized as causative agents of life-threatening systemic infections primarily in premature, low birth weight and immune-compromised neonates. Elucidation and validation of putative bacterial virulence components as well as host factors possibly involved in the response to infection has been hampered in the past by the availability of suitable neonatal animal models. In the current study the recently developed zebrafish embryo model was employed to study the interaction of the zinc metalloprotease Zpx present in Cronobacter spp. with MMP-9, a protease cleaving extracellular matrix gelatin and collagen. RT-PCR revealed differential expression of the zebrafish mmp-9 upon infection with Cronobacter, as well as an increase of the processed, active form of the MMP-9 over the course of infection. Employing a C. turicensis zpx knock out mutant revealed that the presence of this bacterial protease induces the expression of the mmp-9 but also increases the levels of processed MMP-9 during infection. Most striking, experiments using a morpholino knock down of zebrafish mmp-9 suggest involvement of the MMP-9 in induction of the expression of the bacterial protease. This study identified host MMP-9 as a substrate of Zpx and demonstrated yet undescribed mutual cross-talk between these two proteases in Cronobacter mediated infections.

Keywords: Cronobacter mediated sepsis, matrix metalloprotease (MMP) 9, zinc metalloprotease Zpx, zebrafish

Microbiological spotlights

P5.6

A preventative approach to promote food safety bacterial contamination of domestic refrigerators

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Domestic refrigerators could be considered as one of the major potential sources of food-borne diseases, in addition limited data are available regarding the level of contamination of domestic refrigerators in Iran. The purpose of this paper is to detect some of bacterial contamination in domestic refrigerators. In total, 104 households were randomly selected from ten health centers in five areas of Tehran, Iran. Visual inspection and temperature evaluation of the households' refrigerators were done. In addition, the refrigerators were swabbed and analyzed for contaminants using polymerase chain reaction (PCR) method. Screening of the domestic refrigerators by PCR method showed that 51.7 percent of the samples were positive for pathogens as follows: *L. monocytogenes* 41.6 percent, *S. aureus* 5.5 percent, *Salmonella spp* 4.6 percent, and *E. coli* O157:H7 0 percent; consequently, none of mentioned pathogens were detected in 48.3 percent of the refrigerators. Results of the visual inspection indicated that 57 percent of the refrigerators were on desirable, 36.5 percent were acceptable, and 7 percent were weak conditions. Most of the refrigerators about 44 percent had desirable temperatures. There were no significant correlations between the visual inspection scores, temperature and frequency of isolation of specific pathogens in the domestic refrigerators. A significant correlation was observed between contamination and education of parents ($p \leq 0.05$). Determination of the bacterial contamination and evaluating the temperature of domestic refrigerators in Iran can be considered as a novel approach of current study. These findings could be employed in designing and implementing appropriate educational interventions to promote food safety and diminish the risk of food-borne illnesses. Also, obtained results might be applied as introduction for further investigations.

Keywords: Temperature, Domestic refrigerators, Food-borne pathogens, PCR analysis

Microbiological spotlights

P5.7

Detection of *Cryptosporidium* oocysts by acid-fast staining method and PCR in surface water from Tehran, Iran

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Statement of the Problem: Cryptosporidium is a coccidian protozoan parasite; its oocysts in surface water are a global health problem. Due to the low number of parasites in the water resources and the lack of laboratory culture, rapid and sensitive method for detection of the organism in the water resources is necessarily required. We applied modified acid-fast staining and PCR for the detection of the Cryptosporidium spp. and analysed the genotypes in 55 samples collected from surface water.

Methodology & Theoretical Orientation: Over a period of nine months, 55 surface water samples were collected from the five rivers in Tehran, Iran. The samples were filtered by using cellulose acetate membrane filters. By acid fast method, initial identification of Cryptosporidium oocyst were carried out on surface water samples. Then nested PCR assay was designed for the specific amplification and analysed the genotypes.

Findings: Modified Ziehl-Neelsen method revealed 5-20 Cryptosporidium oocysts detected per 10 Liter. Five out of the 55 (9.09%) surface water samples were found positive for Cryptosporidium spp. by Ziehl-Neelsen test and seven (12.7%) were found positive by nested PCR. The staining results were consistent with PCR. Seven Cryptosporidium PCR products were successfully sequenced and five gp60 subtypes were detected. Our finding of gp60 gene revealed that all of the positive isolates were Cryptosporidium parvum and belonged to subtype families IIa and IId.

Conclusion: Our investigations were showed that collection of water samples were contaminated by Cryptosporidium, with potential hazards for the significant health problem. This study provides the first report on detection and genotyping of Cryptosporidium species from surface water samples in Iran and its result confirmed the low clinical incidence of this parasite on the community.

Keywords: Cryptosporidium spp., membrane filtration, subtype, surface Water, Iran

Microbiological spotlights

P5.8

Porcine gastric mucin stimulates toxin production of enteropathogenic *Bacillus cereus*

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Bacillus cereus is a ubiquitous, spore-forming, Gram positive bacterium, which can be found in a variety of different foods. It causes two types of gastrointestinal diseases, an emetic and a diarrheal form. The latter is caused by enteropathogenic strains. For this, spores are ingested with the contaminated food. These survive the stomach passage, germinate in the intestine and subsequently produce locally high concentrations of enterotoxins, which attack the epithelial cells by forming pores. So far it is unclear which signals trigger the bacterial toxin production in the intestine. It is believed that specific host factors play an important role, such as temperature and the intestinal cells. This work focused on the impact of the mucus layer surrounding the epithelial cells towards toxin production of *B. cereus*.

0.5 % PGM (porcine gastric mucin) in minimal medium had only little influence on the growth of a *B. cereus* reference strain, while the amount of secreted enterotoxins was significantly increased, as determined in enzyme immuno assays. Mucin degradation assays showed that *B. cereus* was able to degrade PGM via secreted metalloproteases. Furthermore, PGM "protected" the enterotoxins from enzymatic digestion by trypsin and pancreatin, as well as CaCo-2 cells from the cytotoxic action of *B. cereus* culture supernatants. Differential gene regulation upon contact with PGM was determined via RNA sequencing. After 3 h, 805 genes were down-regulated in the presence of PGM, while 787 genes were up-regulated. Of the latter, the most represented categories were carbohydrate transport and metabolism, amino acid transport and metabolism, and energy production and conversion. Also genes involved in chemotaxis and motility, as well as resistance and defense were found. Furthermore, putative virulence factors such as proteases, collagenases, haemolysins and enterotoxins were identified.

These results suggest complex interaction of *B. cereus* and the mucus layer in the intestine. Mucin seems to be one important factor by which *B. cereus* senses the host environment and starts producing enterotoxins.

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Keywords: Bacillus cereus, food poisoning, porcine gastric mucin, mucus layer, cytotoxicity, enterotoxins

Microbiological spotlights

P5.9

Antibiotics susceptibility study of bacteria isolated from packaged milk products sold in Sokoto metropolis, Nigeria

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Packaged milk products commonly referred to as yoghurts are fermented milk products produced by bacteria fermentation of milk which is consumed throughout the world. Fermented milk, like the fresh milk from which they are produced, is liable to contamination. Evaluation of the bacterial quality of yogurts become apparent due to the high risk associated with consuming substandard or unhygienic yogurts containing pathogenic organisms and health complications associated with this include serious infections that are difficult to treat with antibiotics. This study was conducted to determine the antibiotics susceptibility of bacteria isolated from packaged milk products sold in Sokoto metropolis. Twenty seven packaged milk product samples were bought from three locations (nine samples for each location) in Sokoto metropolis. Isolation and identification of the bacteria species were carried out using standard microbiological procedures. Antibiotics susceptibility of the isolates was determined using a single disc of 7 antibiotics by disc diffusion method following Clinical and Laboratory Standards Institute guideline. Forty six (46) bacterial isolates were identified from the yoghurt samples. The result obtained showed that the major contaminants of yoghurt samples analysed were mostly Gram-positive organisms comprising *Bacillus* spp (30.4%) and *S. aureus* (21.8%) where as among Gram-negative organisms, *E coli* had the highest percentage of 15.2%. Susceptibility result showed that high percentage of the isolated bacteria were resistant to erythromycin (73.9%) but were however susceptible to ciprofloxacin (91.3%) and tetracycline (89.1%). The finding of the study showed that yoghurts sold at Ungwar Rogo, Rungin Sambo and Sokoto Central Markets in Sokoto metropolis contained pathogenic bacteria, some of which were resistant to commonly used antibiotics in the study area, and therefore posing a serious health risk to the consumers. So to ensure the proper quality of yoghurt, there should be a complete check on the methods through which yogurts are being produced and sold in local markets and major streets.

Keywords: Antibiotic susceptibility, Antibiotics, Bacteria, Packaged milk products, Sokoto metropolis.

Microbiological spotlights

P5.10

Diagnostic techniques and antimicrobial susceptibility profile of *Helicobacter pylori* infection in Gashua town

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Diagnostic techniques of *Helicobacter pylori* and antimicrobial susceptibility profile test with direct the applicability of recommended treatment regimens in our setting. To date to our knowledge, there is no published data on the culture and local antimicrobial susceptibility pattern of *Helicobacter pylori* in the Gashua: One hundred and twenty (120), dyspeptic adult patients undergoing endoscopy from the Outpatient of the Gastroenterology clinic of General Hospital Gashua underwent multiple gastric biopsy and specimens were transported for gram stain, culture, antimicrobial sensitivity testing, rapid urease test and histology. Antimicrobial susceptibility testing carried out Epsilometer testing (E-test) method against metronidazole, omeprazole, Ranitidine, and tetracycline. About Sixty percent (55%) of the study population was positive for *H. pylori* infection (mean age of 44 years ± 13), 60% were males. *Helicobacter pylori* culture showed a sensitivity of 45% (95% CI [29.5- 62.1]), specificity of 98% (95%CI [81.5-100%]), positive likelihood ratio of 19.93 (95% CI (1.254- 317.04) and a negative likelihood ratio of 0.56 (95% CI (0.406-0.772). All *Helicobacter pylori* strains isolated were sensitive to metronidazole, Omeprazole, ranitidine and tetracycline. It is imperative to have indebt knowledge on diagnostic method and antibiotic susceptibility patterns in our setting, this will allows us to be more cautious in the choice of first-line agents. Information on antibiotic susceptibility profile plays an important role in empiric antibiotic treatment and management of *Helicobacter pylori* refractive cases.

Keywords: Helicobacter pylori, isolate, susceptibility, antibiotic.

Microbiological spotlights

P5.11

High prevalence of a gene cluster conferring resistance to streptomycin, sulfonamide, and tetracycline in *Escherichia coli* isolated from indigenous wild birds

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Wild birds have been considered as an important source of antimicrobial resistance, posing a significant risk to human and animal health. *Escherichia coli* is a normal intestinal inhabitant in most animals and humans and antimicrobial resistant *E. coli* has been increasingly common worldwide. In the present study, we analyzed the prevalence and mechanism of antimicrobial resistance in commensal *E. coli* from indigenous wild birds in Korea.

A total of 116 *E. coli* isolates from cecal contents of 81 indigenous wild birds were tested for antimicrobial susceptibility. The prevalent resistance phenotype of the isolates was genetically characterized by polymerase chain reaction (PCR) and sequence analysis.

Seventy-one isolates from sparrows (*Passer montanus*) and one isolate from doves (*Columba livia*) were resistant to three antimicrobials, including streptomycin, sulfonamide, and tetracycline (SSuT). PCR and subsequent sequence analysis revealed the SSuT gene cluster region (approximately 13 kb) harboring genes encoding resistance to streptomycin (*strA* and *strB*), sulfonamide (*sul2*), and tetracycline (*tetB*, *tetC*, *tetD*, and *tetR*). In particular, tetracycline resistance genes were located on the transposon Tn10-like element. All 72 SSuT-resistant isolates of *E. coli* belonged to usually non-virulent phylogenetic group A.

Although commensal *E. coli* prevalent in wild birds, in particular, sparrows may not be virulent, it can be an important source of the transmission of antimicrobial resistance to other pathogenic bacteria. Therefore, strict sanitary measures in human and animal environments are necessary to prevent the spread of the resistant bacteria through fecal residues of wild birds.

Keywords: Escherichia coli, streptomycin, sulfonamide, tetracycline, antimicrobial resistance, wild birds

Microbiological spotlights

P5.12

Sequential transmission of *Salmonella* into and through the chicken slaughter line

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Salmonella is one of the most common foodborne pathogens worldwide. *Salmonella* infections in humans are mainly associated with the consumption of poultry products contaminated with *Salmonella*. Therefore, strict sanitary measures are necessary to control *Salmonella* contamination during the poultry slaughter process. The present study was to determine the occurrence and transmission of *Salmonella* at a series of steps in the chicken slaughter process.

A total of 601 samples were collected from a series of slaughtering steps (10 sampling sites) of 26 chicken slaughterhouses throughout Korea. *Salmonella* was isolated from the samples and its distribution was analyzed along the slaughter process. The isolates from each sampling site were tested for susceptibility to 15 antimicrobials by the broth microdilution method. They were also genotypically characterized by pulsed-field gel electrophoresis (PFGE).

One hundred and nineteen isolates of *Salmonella* were obtained from the 601 samples. Sixteen serotypes were identified and six isolates were untypable. *Salmonella enterica* serovars Montevideo (n = 29) and Virchow (n = 27) were the most common serotypes. Relatively high rates of *Salmonella* contamination were found in shackles (75.0%), feathers near plucking machine (68.5%), and feces from crates (44.0%). Eighteen antimicrobial resistance patterns were recognized and 40 (33.6%) isolates were resistant to five or more antimicrobials. There were the same serotypes of *Salmonella* distributed along the slaughter process of each *Salmonella*-positive slaughterhouse. Most of those isolates belonging to the same serotype had identical or closely related PFGE profiles. They also shared common antimicrobial resistance patterns.

Overall findings indicate that *Salmonella* has been sequentially transmitted through the chicken slaughter line. Routine microbiological monitoring and sanitization should be applied to a series of the slaughter process to block the flow of *Salmonella* through the line. This study also demonstrates the necessity to control *Salmonella* in chicken production facilities.

Keywords: Salmonella, serotype, distribution, transmission, chicken slaughterhouse

Microbiological spotlights

P5.13

Listeria monocytogenes prevention by fish processing plant selfchecking system measures and offi cial food control – Identifying gaps and strengthening control measures

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Incidence of severe foodborne illness caused by *Listeria monocytogenes* has increased in Finland and elsewhere in Europe this decade. Vacuum-packaged cold-salted and cold-smoked fish products are typical vehicles, but implementation of listerial preventive measures in their production has not been comprehensively studied. How actions of food control authorities impact *L. monocytogenes* occurrence in fish processing, is also not known. We aimed to assess the prerequisites for *L. monocytogenes* prevention in the fish industry: two independent studies were carried out to identify methods to improve the efficacy of official food control (A) and control measures in fish processing plant self-checking systems (B).
(A) For the study of efficacy of official control in 21 Finnish fish processing plants, *L. monocytogenes* occurrence in 2011-2013 was determined retrospectively from official inspection records, which were also used for extracting the occurred food safety violations and measures conducted by food control authorities. (B) To investigate the association of fish processing plant self-checking system preventive measures with *L. monocytogenes* occurrence, we conducted a questionnaire in 2015 including an in-depth inspection protocol on operational procedures and processing parameters. During 14 months in 2014-2015, detection and enumeration of *L. monocytogenes* were performed from product samples (ISO/FDIS 11290-1 (1996, 2004a) and 11290-2 (1998, 2004b)). Information derived from the inspection reports (A) and the questionnaire (B) was analysed using generalized linear modelling with *L. monocytogenes* occurrence during each respective study time-frame as the dependent variable.
(A) *L. monocytogenes* had appeared 1 to 14 times in products or facilities of 9/21 fish processing plants in 2011-2013. Increase in *L. monocytogenes* occurrence was associated with deficiencies in processing machinery and correction of violations. (B) During the 14-month follow-up in 2014-2015, *L. monocytogenes* occurred in the products of 7/21 fish processing plants. Risk of having product contamination associated with frequency of processing environment sanitation, poor processing machine sanitation, and unhygienic passage of employees.
Preventive measures and official control were not satisfactory in fish processing plants where *L. monocytogenes* occurred. They can be strengthened by improving working hygiene, sanitation of processing machinery and environment, and the correction of violations.

Keywords: Listeria monocytogenes, fish production, food control

Microbiological spotlights

P5.14

Meta-analysis of occurrence of ochratoxin A, zearalenone, deoxynivalenol and total afl atoxin in cereal-based products

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This study aimed to estimate the prevalence of total aflatoxin (TAF) ochratoxin A (OTA), zearalenone (ZEN) and deoxynivalenol (DON) in bread, cornflakes, breakfast meal and pasta-based products through meta-analysis. The required database including (Google Scholar, Scopus, and PubMed databases) was used to collect data on the concentration or prevalence of mentioned mycotoxins in cereal-based products. Among 17,472 explored articles in identification step, 38 articles with 9,627 samples were included in the conducted meta-analysis. The incidence of studied mycotoxins varied with the cereal-based food studied. TAF in the pasta, cornflakes, bread and cereal breakfast was 28%, 62%, 26%, and 7%, respectively. The incidence of ZEN in the pasta, cornflakes, bread and cereal breakfast was 17%, 55%, 39%, and 33%, respectively. The incidence of OTA in the pasta, cornflakes, bread and cereal breakfast was 60%, 50%, 50%, and 40%, respectively. The incidence of DON in the pasta, cornflakes, bread and cereal breakfast was 62%, 72%, 50%, and 37%, respectively. The findings of this meta-analysis may be useful for the building of exposure assessment models aiming to derive data for the development of specific actions to reduce the exposure to OTA, ZEN, TAF, and DON through the consumption of the cereal-based products.

Keywords: systematic review; mycotoxin; bread; cornflakes; cereal breakfast; pasta

Microbiological spotlights

P5.15

Impact of unit operations during processing of cereal-based products on the levels of deoxynivalenol, total afl atoxin, ochratoxin A, and zearalenone: A systematic review and meta-analysis

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The study aimed to perform a meta-analysis on the fate of ochratoxin A (OTA), zearalenone (ZEN), deoxynivalenol (DON) and total aflatoxin (TAF) during steps of bread, cornflakes, breakfast meal and pasta-based products processing. A total of twenty and eight articles (549 data) collected from 1983 through June 2017 were included in the meta-analysis. The type of product (flour, dough, bread, pasta, and biscuit), process (milling, baking, cooking, fermentation), continent, limit of detection (µg/kg), limit of quantification (mg/kg), and method of measurement were reported. The outcomes of our systematic review and meta-analysis showed that the mycotoxins and their fate were influenced differently by the unit operations steps involved in the preparation of the different cereal-based products The outcomes of our systematic review and meta-analysis showed that the mycotoxins and their fate were influenced differently by the unit operations steps involved in the preparation of the different cereal-based products. This comprises an important source of variability that should be better described and accounted to improve exposure assessment studies. Some of the investigated processing such as milling and fermentation caused an increase in the concentration of DON and TAF; they reduce the concentration of ZEN and OTA. Although heat processing (cooking) cause decrease in DON, OTA and TFA in bread and increase in the concentration of ZEN, it reduces the concentration of DON and ZEN in biscuit. Cooking of pasta reduce the content of DON, but it increases the concentration of TFA. This study also indicates specific combinations of mycotoxins and cereal-based foods for which the generation of prevalence data are required. It has also been indicated the need for data on the effects of unit operations of cereal-based foods on the modification of mycotoxins. Due to observed conflicts in the reported results by the investigated studies, the estimated average effect of processing should be used with caution. The obtained results in the current study can be used in exposure/risk assessment mycotoxin studies aiming to generate scientific-based measures to be implemented in the production chain of cereal-based products to safeguard public health.

Keywords: Mycotoxin; bread; cornflakes; dough; pasta; biscuit; exposure assessment; food safety

Microbiological spotlights

P5.16

Occurrence, serotypes and characteristics of *Listeria monocytogenes* in meat and meat products in South Africa between 2014–2016

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Listeria monocytogenes is an intracellular pathogen that cause life-threatening disease called listeriosis. However, in South Africa, there is a dearth of information concerning the potential role of products as potential sources for human listeriosis. The aim of the study was to investigate the occurrence and level of *L. monocytogenes* found in meat and meat products in SA and to characterise *L. monocytogenes* strains according to serogroups, antimicrobial resistance profiles and virulence genes. A total of 2, 017 from imported and locally produced raw and ready to eat meat samples were collected from 2014 to 2016 across nine provinces of SA. The samples for *L. monocytogenes* were isolated using microbiological techniques and real-time PCR. Antimicrobial resistance profiles of the isolates were determined by testing 19 antimicrobial impregnated discs using Kirby Bauer disc diffusion method. Characterisation in terms of serogroup typing and virulence profiling was done using conventional PCR. The overall occurrence of *L. monocytogenes* was 14.7% (296/2017) which varied between meat collected on the domestic market (15.0%; 264/1758) and directly at the ports of entries (12.4%; 32/259). The contamination level of the positive samples ranged from 1 to 3.7 log CFU/g and 1 to 4.1 log CFU/g for samples collected from the domestic and imported meat respectively. All positive isolates were serotyped by multiplex PCR, of which majority of the isolates belonged to molecular serogroup ½a-3a (45.5 %), followed by 4b-4d-4e (24.2%), and ½c-3c (15.2%). Most of the isolates harboured the *inlJ* (98.7%) and *ipa* (95.6%) genes. However, at least one of the other internalin genes (*inlB*, *inlC* and *inlA*) were present in most of the isolates. All the tested isolates showed resistance to at least three of the 19 antibiotics, with five (1.7%) of the tested isolates displaying resistance to 13 of the 19 antibiotics. Resistance to streptomycin (99.0%), clindamycin 97.3%, fusidic acids (95.6%), nitrofurantoin (79.7 %) and gentamycin (74.4%) were commonly observed while high rates of sensitivity were observed for ampicillin (85.6%), kanamycin (84.6%), amikacin (77.6%), vancomycin (74.2%), and tetracycline (62.5%). The presence of *L. monocytogenes* in various meat products in SA pose a risk to human health. The present research provides useful baseline information that will help in the development of policies and regulations for monitoring of *L. monocytogenes* in meat and meat products in SA.

Keywords: Listeria monocytogenes; meat and meat products; resistance genes; antimicrobial resistance profiles

Microbiological spotlights

P5.17

Cold-shock domain family proteins (Csps) contribute to regulation of virulence, cellular aggregation, and flagella-based motility in *Listeria monocytogenes*

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Listeria monocytogenes is an important foodborne pathogen that causes listeriosis and high rates of mortality amongst those with weakened immunity. Proteins of the cold shock domain family (Csps) are global gene expression regulating proteins that implicated in various functions including stress protection and virulence responses in bacteria. The role of Csps in virulence, cellular aggregation and flagella- based motility of *L. monocytogenes* was investigated. A mutant deleted in all three *L. monocytogenes csp* genes (Δ*cspABD*) was completely attenuated in virulence as assessed in human macrophage and zebrafish based infection models. Without Csps *L. monocytogenes* was also incapable of cellular aggregation and did not exhibit swarming motility or express cell surface flagella. Further evaluation in mutants that produce single *csp* genes revealed both redundancy as well as differences in the function of the three *L. monocytogenes* Csps with respect to virulence, cellular aggregation, flagella production, and swarming motility. Expression analysis at protein and mRNA levels showed an impaired production of key virulence and motility genes in the different *csp* mutants that are indicative of Csp-dependent expression regulation of key motility and virulence genes at both transcriptional and post-transcriptional levels. In a mutant lacking all three (Δ*cspABD)* as well as those possessing single (Δ*cspBD*, Δ*cspAD*, and ΔcspAB) *csp* genes we detected reduced levels of proteins or activity as well as transcripts derived from the *prfA*, *hly, mpl*, and *plcA* genes suggesting a Csp-dependent transcriptional regulation of these genes. The *csp* mutants also had reduced or completely lacked ActA proteins and cell surface flagella but contained elevated *actA* and *flaA* mRNA levels compared to the parental wild type strain suggesting Csp involvement in post-transcriptional regulation of these genes. Overall, our results suggest that Csps contribute to the expression regulation of virulence and flagella-associated genes thereby promoting host pathogenicity, cell aggregation and flagella-based motility processes in *L. monocytogenes*.

Keywords: Listeria monocytogenes, Cold shock domain proteins, Virulence, Flagella

Microbiological spotlights

P5.18

Comparison of *Campylobacter jejuni* isolates from different sources in Poland using MLST, fl aA and porA SVR sequencing methods

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A number of genotyping systems are currently in use for epidemiological surveillance of *C. jejuni*, the most common foodborne bacterial pathogen worldwide. In recent years multilocus sequence typing (MLST) is one of the most extensively used molecular typing method. However, this technique has sometimes inadequately discriminatory power for local epidemiological studies. It can be increased by including sequence-based typing schemes with a more variable loci, e.g. *flaA*-SVR or *porA* sequencing.
In the present study, 572 *C. jejuni* isolates from broilers (n = 148), poultry carcasses (n = 139), chicken meat (n = 139), and humans (n = 146) were subtyped using MLST, *flaA*-SVR and *porA* sequencing to obtain a better insight into the *C. jejuni* population structure in Poland. MLST was carried out as described by Dingle et. al. (2001), whereas *flaA*-SVR typing was performed by PCR amplification and subsequent sequencing as described by Meinersmann et al. (1997) and *porA* sequencing was performed according to the protocol available at https://pubmlst.org/campylobacter/ info/porA_method.shtml. The discriminatory ability of MLST, *flaA* SVR and *porA* sequencing was calculated using Simpson's index of diversity (SID).
Overall, 105 different sequence types (STs) based on MLST scheme were found among 572 isolates tested, covering from one to 58 of *C. jejuni* isolates. The single locus sequence-based typing schemes for *porA* and *flaA* generated 109 and 67 different alleles, respectively. The most prevalent alleles consist of 37 (*porA*) and 54 (*flaA* SVR) strains. The SID with 95% of confidence interval (CI) were 0.964 (0.958-0.969) for MLST, 0.959 (0.954-0.964) for *flaA* SVR and 0.967 (0.962-0.972) for *porA* sequencing. Significant differences ($p < 0.05$) with discriminatory power between used typing methods was found between *flaA* SVR and *porA* sequencing as well as between *flaA* SVR and MLST.
This study combined three molecular typing methods based on *C. jejuni* DNA sequencing. It was shown that inclusion of a highly variable *porA* locus increased the discriminatory power of this scheme and is useful for differentiation of closely related isolates.
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Keywords: C. jejuni, molecular typing, disciminatory power

Microbiological spotlights

P5.20

Quick colorimetric-molecular detection kits: A versatile technique for detection of *Fusarium* sp. contaminattion in grains

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The genus *Fusarium* can infect different crops and is responsible for economic losses, as well as, for contamination of stored grain cereals, by toxins production, which can be harmful to human and animal health if they enter the food chain. Approximately 25-50% of the produced commodities, especially staple foods, are contaminated with mycotoxins. The synthesised mycotoxins of Fusarium in harvested goods are classified in three major groups, the trichothecenes, the fumonisines and zearalenone. Different isothermal amplification methods have been developed to quickly amplify DNA to detectable levels in a single temperature, such as: Loop-Mediated Amplification (LAMP) and Recombinase Polymerase Amplification (RPA). In contrast with other techniques, RPA offers several important advantages for point-of-care applications: it requires a lower amplification temperature between 25°C and 42°C, is tolerant to impure samples, amplifies targets to detectable levels more rapidly (15-30 min), and uses lyophilized enzymes without cold chain storage and transport. This work aimed the development of rapid detection kits based on LAMP and RPA to identify different species of Fusarium present in seeds and grains of wheat, beans and corn. Amplification of DNA during the reaction was indirectly detected in situ by using *Neutral Red* as a marker without the necessity of time-consuming electrophoretic analysis. The colorimetric detection of specific DNA sequences of mycotoxin-producing microorganisms allows epidemiological studies and the tracking of food quality. The results of the LAMP kits were compared to the other methods already established, showing greater commercial usefulness, speed and sensibility and was shown to detect the presence of less than 2pg of purified target DNA per reaction within 30 min. Within 5 fungal species tested, exclusively DNA isolated from cultures of F. graminearum resulted in a fluorescent signal after amplification with the LAMP assay.

Keywords: mycotoxins; detection kits; fusarium; corn; seeds;

Microbiological spotlights

P5.21

Detection of human enteric viruses in blue mussels sold on Polish market

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Shellfish become a popular food type even in countries such as Poland where there is no tradition in growing and consumption of seafood. The majority of edible shellfish sold in Poland originate from European Union. Among different shellfish species present on the market, blue mussels (*Mytilus edulis*) are mainly consumed.
The aim of the study was an assessment of the occurrence of human enteric viruses such as noroviruses (NoV), hepatitis A and E viruses (HAV, HEV) in shellfish sold on Polish market. Samples of blue mussels were collected from national wholesalers distributing shellfish which were harvested in the Netherlands, Spain, France and Denmark. Samples were collected twice per year during summer (from May to August) and winter (from September to February) seasons from 2013 to 2017. In total, 32 batches of shellfish were sampled. From each batch a set of six subsamples composed of pooled digestive glands was prepared. Samples were individually analysed. A detection of human enteric viruses was performed according to an ISO method (ISO/TS 15216-2:2013). For HEV a real-time RTPCR according to Jothikumar et al. (2006) was employed. The correct method performance was controlled by using an internal amplification control and feline calicivirus as a sample process control. The molecular typing of NoVs and HEV was carried out using virus specific RTPCR protocols followed by sequencing and sequence analysis of PCR products.
The presence of human enteric viruses was shown in 33 out of 192 samples. NoV GI was detected in 20 (10.42%), NoV GII in 12 (6.25%) and HEV in 1 (0.52%) of samples. HAV was found in none of the tested samples. A seasonal distribution of viruses in shellfish was observed. Regardless the shellfish origin, 97% of positive mussels were harvested during a winter season. The highest number of contaminated shellfish originated from Denmark (42.4%) and the Netherlands (30.3%). The viruses were also detected in samples derived from France (15.1%), Ireland (9.2%) and Spain (3.0%). A fluorescence threshold with Cq > 42 was obtained for 27% of positive samples. Sequencing of NoV and HEV PCR products was not successful.
Human enteric viruses were detected in blue mussels sold on Polish market. Shellfish were mostly contaminated by NoV GI. Although cases of human infection related to the shellfish consumption were not recorded in Poland so far, the detection of viruses in mussels may indicate a risk of food-borne infection.

Keywords: shellfish, detection, human enteric viruses, occurrence, retail

Microbiological spotlights

P5.22

Adaptation of enteric pathogenic bacteria on salad leaves and involvement of conjugative multiple-antibiotic resistance plasmids

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Food-borne infections caused by contamination of fresh produce like green salad by pathogenic enteric bacteria (*Salmonella*, STEC - Shiga toxin-producing *E.coli*) appeared since a few years (Van Overbeek *et al,* 2014). Presence of these bacteria in this unusual environment is an emerging major health risk, especially given that enteric bacteria, pathogenic or not, frequently present antibiotic resistances (Laroche Ajzenberg *et al*, 2014 ; Flores Ribeiro *et al*, 2011).
In our study, we were interested **i)** in evaluating the sanitary quality of salad primary production in 4 farms in Normandy (France) and **ii)** in characterising adhesion on salad leaves of *E. coli* strains bearing or not a conjugative multiple-antibiotic resistance plasmid and their survival capacity.
i) Sampling campaigns were carried out on salads from Normandy crops and in irrigation water for 6 months. *E. coli* (faecal contamination indicator) and the two pathogenic enteric bacteria *Salmonella* and STEC, were investigated. Overall, 60 salad samples were analysed by cultural and molecular methods (chromogenic media and real-time PCR). A very large majority of them present a satisfactory quality. Thus, no STEC were found. *Salmonella* was detected in only one sample and *E. coli* was enumerated up to 610 UFC/g in a few samples. Determination of their resistance profiles are in progress. In parallel, we were able to monitor irrigation water in one farm. *E.coli* were found at variable density (10^2 to 10^3 UFC/100mL). Depending of the campaign, some of these strains (from 3% to 18%) are antibiotic resistant and even multi-resistant (patterns harbouring 3 or more resistances), indicating the likely human origin of contamination (Flores Ribeiro *et al*, 2011).
ii) Voluntary contaminations of young seeding were performed in an experimental greenhouse with different *E. coli* strains obtained by transformation of the laboratory strain DH5αMCR with conjugative plasmids extracted from environmental antibio-resistant *E.coli* strains recovered during previous works (Laroche Ajzenberg *et al*, 2014).
Preliminary results show that:
- *E. coli* is able to survive on the leaf surface at least 1.5 month
- The presence of conjugative plasmid harbouring antibiotic resistances seems to promote adhesion of the host strain
To confirm these results, microscopy essays with fluorescent strains are in process as well as survival studies of *E.coli* in soil and water.

Keywords: Food-borne infections, antibiotic resistancce,pathogenic enteric bacteria, E.coli

Microbiological spotlights

P5.23

Development of a long amplicon quantitative PCR method with propidium monoazide for enumeration of viable *Listeria monocytogenes* in food systems

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Cross-contamination with *Listeria monocytogenes* continues to be a problem in the ready-to-eat food industry due to its persistence in food processing plants. Rapid molecular methods for detection and enumeration of viable *L. monocytogenes* in the production environment are needed. Use of longer amplicons in quantitative PCR (qPCR) protocols with a propidium monoazide (PMA) treatment step of samples prior to DNA extraction has recently been shown to be superior in discriminating between viable and dead cells of other foodborne pathogens.

The purpose of this study was to develop a long-amplicon PMA-qPCR method for specific enumeration of viable *L. monocytogenes* in food processing facility and product testing. For comparison, a short-amplicon PMA-qPCR method was also developed and tested.

Primers specific for *L. monocytogenes* targeting the listeriolysin (*hly*) gene were designed to amplify a short (199 bp) or long (1561 bp) fragment. The short- and long-amplicon PMA-qPCR methods were then tested for their ability to discriminate between viable (no heat) and heat-killed cells (90°C, 10 min). The PMA-qPCR methods were subsequently used to assess the survival of two outbreak strains of *L. monocytogenes* during desiccation (33% RH, 15°C) on stainless steel surfaces for ten days.

The long-amplicon (1561 bp) PMA-qPCR method had a limit of quantification (LOQ) of 1.32 log CFU/reaction (efficiency 91.6%, R^2 99.1%), while the LOQ for the short-amplicon PMA-qPCR method was 1.44 log CFU/reaction (efficiency 101.9%, R^2 99.8%). The long-amplicon PMA-qPCR suppressed the signal from heat-killed cells, while the ability of the short-amplicon PMA-qPCR to discriminate between viable and heat-killed cells was poor. Enumeration of viable cells during desiccation by use of the long-amplicon qPCR method was not significantly ($p > 0.05$) different from plate counts. However, the short-amplicon PMA-qPCR significantly ($p < 0.05$) overestimated the desiccation survivors. Omission of the PMA treatment prior to DNA extraction resulted in both the short and long amplicon qPCR methods significantly ($p < 0.05$) overestimating the number of viable cells.

This method can aid in the detection and enumeration of viable *L. monocytogenes* cells, which may be used to further understand the survival and persistence of *L. monocytogenes* in food processing facilities. The developed method can be used on both heat killed and desiccated cells and highlights the importance of amplicon size in PMA-qPCR.

Keywords: Listeria monocytogenes, quantitative PCR, propidium monoazide (PMA), amplicon length, viability.

Microbiological spotlights

P5.24

Analysis of rapid alert system for food and feed (RASFF) notifi cations on products contaminated with *Listeria monocytogenes* affecting Germany, 2001-2015

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The Rapid Alert System for Food and Feed (RASFF) is a tool for supranational communication of food safety risks within the European Union and allows for a coordinated response to emerging threats in the food chain. *Listeria monocytogenes* (*Lm*), a bacterium widely distributed in the environment, is the causative agent of the foodborne disease listeriosis. As a result of disease severity and the increasing number of human listeriosis cases in Germany since 2011, an effective reporting system on *Lm* contamination in food products has become more important than ever to counteract this trend. In our study, we analysed RASFF notifications on food products contaminated with *Lm*, assessed trends in the reported data and addressed options for improvements in the current notification system.

From 2001 to 2015, a total of 226 *Lm* notifications for products distributed in Germany were published in RASFF. The majority of *Lm* notifications concerned milk products (54%) followed by fish products (23%) and meat products other than poultry (12%). In almost 75 % of notifications, country of origin was France, Germany, Italy or Poland. While *Lm* contamination in products of French origin was mainly notified by France, *Lm*-contaminated German, Italian and Polish products were mainly notified by Germany. This divergence between country of origin and notifying country indicates that food safety risks are not always recognized at the earliest possible time point of the product's life span. For our dataset, most *Lm* notifications were driven by official controls when the respective product was already on the market but there was an increasing trend for company's own checks. Making food manufacturers accountable for the detection and notification of contaminated products in the production line might help to reduce the number of contaminated food products that enter the market. Information about packaging and food processing was only provided for a minority of *Lm* notifications. Hence, no reliable identification of risk factors was possible. In the future, it would be useful to establish a comprehensive database including additional metadata to be able to tackle the problem of *Lm* contamination at its root.

Although a solid basis for the surveillance of *Lm*, there is still room for improvement in RASFF to speed-up the flow of information. This would help to identify food safety risks that can be harmful to European consumers much faster and consequently reduce the burden of listeriosis.

Keywords: European food safety, reporting, notification, RASFF, Listeria monocytogenes, contamination

Microbiological spotlights

P5.25

Multiplex TaqMan qPCR assays for sensitive and rapid detection of pathogenic foodborne viruses associated with fish and hutting meat

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Food and food environments are a major source of viral transmission to humans [1-4], being norovirus (NoV) the most frequently reported agent in foodborne outbreaks worldwide [4], although foodborne outbreaks caused by hepatitis A (HAV) and E (HEV) are also significant, being these viruses associated with a more severe disease [3].

In Portugal virus control in food is still poorly made and with no legislation making that control compulsory, namely in the cases of fish and hutting meat, two important food matrices, since: Portugal leads fish consumption rate among the EU, with an annual average consumption of ≈60 kg/capita [5], being fishing a cultural and traditional habit; while hunting is a rich tradition spanning hundreds of years, being Monteria's the primary hunting form in the country. Both these activities have a high impact in the country's economy, being important to ensure their safety as food concerning virologic hazards.

Therefore the main goal of this study is to develop and optimized *Taqman* multiplex qPCR protocols for the identification/quantification of the referred viruses in different food matrices, namely: gilthead and sergeant fish, two of the most extensively farmed species of fish in the Portuguese aquacultures; the European pilchard, the main species used in the canning industry [6]; the horse mackerel, one of the most consumed fish species in the country; Hares, highly in danger species, considered almost extinct in Portugal due to virological diseases; and wild boars, an growing species, due to the abandonment of agriculture, and monocultures of eucalyptus and pine that do not feed this animals. Also, 4 more viruses responsible for major economic losses in fisheries/aquaculture due the high morbidity/mortality [7,8] will be detected, namely: Infectious Pancreatic Necrosis Virus, Viral Hemorrhagic Septicemia Virus, Infectious Hematopoietic Necrosis Virus, and Viral Nervous Necrosis Virus.

For this purpose, animal´s tissues samples were already dissected and RNA extraction was optimized using an in house protocol based in trizol extraction with alterations, for further application in qPCR protocols. These protocols are now optimized as singleplex, where in vitro transcribed RNA transcripts were used as template to generate standard curves to HAV, HEV, NoV GI and NoV GII, whose limit of detection (LoD) ranged from 10^4 to 10^2 genome copies/µl. Regarding the 4 fish viruses the qPCR protocols optimization are still ongoing.

Keywords: Foodborne viruses, Fish and hutting meat hazards, Multiplex TaqMan qPCR protocols

Microbiological spotlights

P5.26

The relationships between antibiotic resistance, genetic proximity and the origin of *Campylobacter jejuni* isolates

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Campylobacter jejuni is one of the most common causes of food-borne gastroenteritis in many countries of the world. A high incidence of campylobacteriosis and possible development of post-infectious complications are the reasons why the disease refers to the infections with a high social and economic impact. Most of the cases are self-limiting and do not require a medical intervention. However, hospitalization and intensive antibiotic treatment is often necessary when immunocompromised patients, newborns and elderly people are infected. Increasing antibiotic resistance of campylobacters leads to inefficient therapy resulting in further complications.

The aim of this study was to investigate possible spread of antimicrobial resistance among *C. jejuni* isolates regarding their origin and genetic proximity. The susceptibility profiles of the isolates were determined by disk diffusion method and evaluated according to EUCAST guidelines. The phylogenic relationship was ascertained by multiplex PCR binary typing (mP-BIT), based on the multiplex detection of 18 different genes, together with Pulsed field gel electrophoresis (PFGE), which is based on the restriction and migration profile of the whole genome.

In total, 64 isolates originating from clinical samples were examined. Of these, 22 isolates were resistant to ciprofloxacin, 1 to erythromycin and 12 to tetracycline. In total, 9 isolates were multiresistant including one isolate resistant to all the three tested antibiotics. In conclusion, we did not find possible relationship between genetic lineages and resistance to antibiotics.

In addition, the results of clinical strains were compared to the isolates originating from outlet of waste water treatment plants and recreational pond.

Keywords: Campylobacter jejuni, antibiotic resistance, PCR binary typing, Pulsed-field gel electrophoresis

Microbiological spotlights

P5.27

A quantitative analysis of cross-contamination, recontamination and consumer exposure of ESBL/AmpC E.coli during a house-hold barbecue in Germany

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The presence of multidrug resistant bacteria in retail meat is one of the current concerns of the public health authorities. Bacterial cross-contamination and recontamination during house-hold food preparation could play an important role within the dissemination of such bacteria, and therefore could contribute to a serious health problem, more specifically for immunocompromised people.

In order to evaluate the importance of such events, a quantitative analysis was carried out to estimate the likelihood and extent of cross-contamination and the burden of Extended-Spectrum-β-Lactamase (ESBL) and AmpC-β-Lactamase (AmpC) -producing *Escherichia coli* (*E.coli*) from contaminated raw chicken breasts via hands and kitchen utensils in a serving (consisting on a piece of bread and a piece of grilled chicken meat) during a house-hold barbecue. For this purpose we adapted the model for cross-contamination during food preparation developed for *Campylobacter* spp. by Mylius et al. (2007). A probabilistic approach was used and Monte Carlo simulations were applied. A modular design was used, taking into account the chronological order of the routines during the barbecue event. Available data on the prevalence and burden of ESBL/AmpC *E.coli* in chicken meat at retail in Germany were used as starting point. The probabilities and extent of bacterial transfer between food items and kitchen utensils (referred to as "Objects") and the routines performed during food preparation (referred to as "Actions") specified by their probabilities of occurrence, were incorporated as input parameter values and probability distributions. In order to provide transparency and consistency to the modelling process, and to facilitate the reusability of the model, it was set up in R 3.4.0 following the structure required by the information exchange format called Food Safety Knowledge Markup Language (FSK-ML), including all the model metadata in Excel format.

The model will allow for an estimation of the exposure of consumers to ESBL/AmpC forming *E. coli* through food once contaminated levels has entered the household kitchen. It will therefore also highlight the importance of good kitchen hygiene for consumer food safety. The incorporation of the developed model into a complete Quantitative Microbial Risk Assessment Model will greatly help to estimate the risk of consumer exposure to ESBL/AmpC *E.coli* through the consumption of contaminated food, allowing to develop strategies for reducing its spread.

Keywords: Cross-contamination; Kitchen hygiene; Consumer Exposure; Model; Resistant bacteria; ESBL/AmpC E.coli

Microbiological spotlights

P5.28

Inactivation of *Coxiella burnetii* during short-time heat-treatment of milk

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Coxiella burnetii is a gram-negative, intracellular bacterium and the causative agent of the zoonosis Q fever. The disease mainly affects ruminants and is known for its atypical aetiopathology. Infected animals can shed the organism amongst others via the milking process, therefore, it is necessary to pasteurize raw milk products before they enter the human food chain. Early experiments for establishing regulations for milk pasteurization were performed during the fifties and sixties of the last century and until now they are still the international standard.
The intention of this study is to analyse the short-time heat treatment conditions for *Coxiella burnetii*-contaminated milk in agreement with the specifications of the Codex Alimentarius. Furthermore, the heat resistance mechanisms of the organism shall be tested by using comparative studies on the spore-like (SCV) and the metabolic active (LCV) form, using transcriptional analyses.
First experiments involved the reactivation of six different *C. burnetii* isolates via cell culture following axenic propagation. The breakpoint of an avirulent *C. burnetii* test strain after pasteurization was determined by the use of viability PCR, embryonated hen´s eggs and Real-Time PCR in combination with growth in axenic medium. Additionally a LCV/SCV-mixed culture and a SCV-enriched culture were generated and evaluated by transmission electron microscopy (TEM).

Keywords: Coxiella burnetii, pasteurization, milk

Microbiological spotlights

P5.29

The activity of a commercial bacteriophage against strains of listeria monocytogenes found in the South African food environment

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Listeriosis is a serious infection in humans caused by the bacterium *Listeria monocytogenes.* In South Africa, a national *Listeria* outbreak has been declared in December 2017 and is believed to be the largest-ever global outbreak of listeriosis. To date, there has been 948 laboratory confirmed cases, with a mortality rate of 27%. Prior to December 2017, listeriosis was not a notifiable disease, and published data relating to food borne listeriosis is lacking in South Africa.

The main objectives of this study will be to determine if there is a genetic relationship between isolates screened from ready-to-eat (RTE) food, the food processing environment and isolates from human listeriosis cases (obtained from a state Hospital in the Western Cape region). Food and environmental samples testing positive for *L. monocytogenes* are being acquired from food industries in the Western Cape Province. By applying molecular techniques such as polymerase chain reaction (PCR), the DNA extracted will screened for the virulent *hly* gene. Isolates obtained will be categorized into differentiating lineage groups by restriction fragment length polymorphism (RFLP) application. Pulsed field gel electrophoresis (PFGE) and sequencing methods will also be applied for epidemiological investigation.

The antibiotic resistance or susceptibility patterns of *L. monocytogenes* from the samples tested will also be monitored against different antibiotics currently used to treat listeriosis. The findings would indicate whether certain strains contribute to the real burden of disease in the human population. To investigate, control and prevent outbreaks of listeriosis, it is vital to have information about the spread of the pathogen in the food setting and the sequence diversity among strains.

The result will be an increase in the knowledge of the distribution of *L. monocytogenes* strains in the food environment in the Western Cape and the associated public health risk. This is particularly important since there is a growing concern about virulent strains of *L. monocytogenes* linked to food-borne outbreaks. Additionally, the findings of this study will be published since it has been reported that published research on this organism in the South African food setting is lacking.

Keywords: Listeria monocytogenes, serotypes, PFGE, antiobiotic resistance

Microbiological spotlights

P5.30

Detection of *Salmonella* spp. in food by loop-mediated isothermal amplification (LAMP)

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Salmonella is one of the most common food-borne pathogens worldwide. They can be transmitted by food of animal origin, such as raw milk, raw or insufficiently heated meat or eggs, as well as by contaminated vegetable foods such as tea, sprouts or tomatoes as well as chocolate. On site detection methods that are not only specific and reliable but can also be carried out rapidly, can contribute to an increase in food safety. Compared to conventional methods, the application of the official qPCR-based method for the detection of Salmonella spp. in foodstuffs according to §64 LFGB 00.00-98 already results in significant time savings. However, the laboratory equipment required for this analytical method does not permit mobile use of the method.
The loop-mediated isothermal amplification (LAMP) process is a promising alternative as LAMP can be carried out without major technical aids. In addition to a high reaction speed and thus a very short analysis time, it is characterized by its high specificity and simple detection of reaction products. Because of the advantages mentioned above, there is currently an increasing interest in applying this method in the assessment of food safety.
First, several LAMP systems and mastermixes for the detection of salmonella were evaluated. The sensitivity and selectivity of the optimized LAMP was then determined. The LAMP assay was 100% specific based on 35 *Salmonella* strains including 27 different S. *enterica* spp. *enterica* serovars and 49 non-*Salmonella* bacteria. The detection limit of LAMP was 39 KBE/ reaction for *S.* Typhimurium and 39 KBE/ reaction for *Salmonella bongori*.
For the comparison with the official qPCR method according to §64-LFGB different matrices were spiked with defined CFU (chocolate, black tea, black pepper, minced meat). The inhibitory effect of black tea was reduced for the LAMP compared to the qPCR. Finally, 42 routine samples were examined in parallel with LAMP and the qPCR according to §64 LFGB 00.00-98.
The results revealed that the optimized LAMP assay is a rapid, specific, and sensitive method for the detection of Salmonella in foods.

Keywords: Salmonella spp., Loop-mediated isothermal amplificaion (LAMP)

Microbiological spotlights

P5.31

Nature versus nurture – The effect of pre-growth conditions on pathogen survival during challenge and validation studies

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Effective control of foodborne pathogens requires science-based validation of intervention and control strategies. It has previously been shown that strains or genetic lineages of a pathogen may differ in their ability to survive different stress conditions. We hypothesize, however, that pre-growth conditions have a significantly greater effect on subsequent stress survival than genetic diversity. The strain collection is comprised of *Salmonella enterica* (n=23)*, Listeria monocytogenes* (n=11)*, Escherichia coli* (n=13), and surrogate, indicator and index organisms (n=8). Strain diversity was assured by including 10 most common serotypes (*Salmonella*), representation of all lineages (*Listeria*) and inclusion of the "Big six" (*E. coli*). All isolates in the collection were characterized by whole genome sequencing. Based on core SNP, a subset of representative strains was selected for (i) intervention with sanitizer and (ii) assessment of growth and survival on produce.

Strains were grown under 7 different conditions (e.g., high salt, low pH) before exposure to peroxyacetic acid or inoculation on produce (i.e., tomatoes, lettuce and cantaloupe). A generalized linear model was fitted to the binomial proportion of surviving cells using a $\log_e$ link function; independent variables were crossed random effects of strain and condition. For all pathogen groups the condition variance component was larger than the strain variance component, indicating that condition contributes more to variability in log-reduction than strain: *Salmonella* (condition 10.0 and strain 3.0), *Listeria* (condition 12.1 and strain 1.4), *E. coli* (condition 23.9 and strain 0.0). Pre-growth conditions also showed a larger effect on the variation of growth and survival as compared to the variation attributed to different strains grown under a given condition. For example, *Salmonella* strains inoculated on tomatoes showed less strain variation within one condition (for pre-grown to stationary phase, Day7 recovery of strains ranged from 6.98 to 7.74 log). For a single *Salmonella* strain pre-grown under different conditions, recovery on Day 7 ranged from 5.1 to 8.15 log (for pre-growth at 21°C and NaCl, respectively).

In combination with previous data, our results indicate that pre-growth conditions affect both pathogen stress resistance as well as growth and survival in challenge studies. This knowledge now needs to be translated into guidance information that can be used in the design of validation studies.

Keywords: Intervention studies, E. coli, Salmonella, Listeria, sanitizer, produce

Microbiological spotlights

P5.32

Media for aerobic recovery of *Campylobacter* from mixed bacterial cultures

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A non-selective medium for culturing Campylobacter aerobically was recently described. The objective of the present study was to determine if a selective medium could be formulated by supplementing the new medium with the Bolton antibiotic mixture.
Basal medium containing beef extract, 50 g; tryptose, 10 g; soluble starch, 10 g; sodium lactate, 3.0 g; and agar, 0.5 g dissolved in 900 ml of distilled water was dispensed in 9 ml aliquots in screw capped test tubes and autoclaved. The medium was cooled, and 1 ml of 1.5% sterile sodium bicarbonate was added. The selective medium was prepared by adding the Bolton antibiotic mixture to the basal medium. Both media were transferred to 25 ml culture flasks before inoculation with bacteria.
Mixed bacterial cultures were prepared by adding either Campylobacter fetus, Campylobacter coli, Campylobacter jejuni, or Campylobacter lari to bacterial suspensions containing Enterococcus faecalis, Escherichia coli, Listeria monocytogenes, Pseudomonas aeruginosa Salmonella Kentucky, and Staphylococcus aureus. Separate culture flasks containing10 ml of the basal medium or the selective medium were inoculated with the mixed cultures, then incubated aerobically at 37C for 48 h. After incubation, Campylobacter were enumerated on blood agar with Blaser-Wang antibiotic mixture with microaerobic incubation. Aerobic incubation was used to enumerate E. faecalis on m-Enterococcus agar, E. coli on Levine EMB agar; L. monocytogenes on Listeria Selective Agar, P. aeruginosa on Pseudomonas agar base with Pseudomonas C-F-C supplement, Salmonella Kentucky on XLT4 agar, and S. aureus on Mannitol Salts agar.
Results indicated that all isolates were recovered from basal medium after aerobic incubation and that significantly ($p \leq 0.05$) fewer Campylobacter spp. were recovered than all other isolates, except S. aureus, in the mixed culture. However, after incubation in media supplemented with the Bolton antibiotic mixture, significantly more Campylobacter than the other bacterial isolates were recovered. Furthermore, there was no significant growth of the other bacteria in the mixed culture and no E. coli, Salmonella Kentucky, or S. aureus were recovered in most experiments.
Findings indicate that supplementing the basal medium with the Bolton mixture produces a selective medium that can be used to aerobically isolate Campylobacter from samples containing other bacteria.

Keywords: Campylobacter, aerobic incubation, selective medium

Microbiological spotlights

P5.33

Anti-staphylococcal activity of lactic acid bacteria isolated from Brazilian artisanal cheeses

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Artisanal cheeses (AC) have strong historical and social connections with the communities in which they are produced. AC are produced from raw milk and highly consumed in some regions of Brazil, but some of them have been regularly found to harbor foodborne pathogens, such as toxigenic *Staphylococcus*. On the other hand, AC can carry indigenous lactic acid bacteria (LAB) with antimicrobial features, which may enable their application in biopreservation strategies. The aim of this study was to evaluate the capacity of LAB strains isolated from Brazilian AC as anti-staphylococcal agents. A total of 1,639 LAB strains were isolated from 582 Brazilian AC samples: *coalho* and *manteiga*, from Northeast; *Marajo*, from North; *colonial* and *serrano*, from South; *Minas*, from Southeast and *caipira*, from Midwest. These LAB strains (810 isolated using MRS agar and 829 using M17 agar) were tested qualitatively for anti-staphylococcal activity by the deferred antagonism assay at 30 °C (24 h) against S*taphyloccous aureus* FRI 361 (enterotoxin C2 producer). In summary, 83.53% of the strains were able to inhibit the pathogen growth, being 86.85% isolated from M17 agar and 80.12% from MRS agar. Most of them were isolated from *Marajó* (97.14%), Manteiga (93.51%), Coalho (93.50%) and Minas artisanal cheeses from Cerrado (89.92%), Serro (87.26%), Canastra (81.40%) and Araxá (80.77%). These results suggest that LAB isolated from Brazilian AC have a great potential to inhibit the enterotoxigenic S*taphyloccocus aureus,* which may enable their use as biopreservative agents in cheese production.

Keywords: Brazilian artisanal cheeses, Food safety, Anti-staphylococcal activity, Endougenous microbiota

Microbiological spotlights

P5.34

MLST genotypes of *Campylobacter jejuni* isolated from broiler, carcasses, chicken meat and humans in Poland

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C. jejuni representing the most reported bacteria causing foodborne diseases in the European Union since 2005. Among genotyping methods, sequence-based typing methods are preferred for reliable data production and interlaboratory transferability. Multilocus sequence typing (MLST) utilizes nucleotide polymorphisms in relatively-conserved housekeeping genes, and is a powerful technique for bacterial population studies. The present investigation was aimed to investigate the relationship, based on the MLST analysis, among *C. jejuni* isolated from different sources in Poland. A total of 617 *C. jejuni* from humans (n = 157), broiler caeca (n = 150), broiler carcasses (n = 155), and chicken meat (n = 155) were genotyped using MLST as described by Dingle et al. (2001). The sequencing data were analyzed and assigned by MLST database using the BioNumerics software v.7.6.
Among 617 *C. jejuni* isolates 121 distinct sequence types (STs) were identified. The most common ST464 represented 9,6% of the strains. This ST was the most frequently found in *C. jejuni* from human cases and broiler caeca. Among isolates from carcasses and chicken meat ST50 and ST137 were predominant, covering 9.7% and 7.7% of strains, respectively. In addition, 33 *C. jejuni* representing 26 different STs were reported for the first time in the PubMLST database. No distinctive separation was observed between the sources and identified STs but in all origins the unique STs were found. STs only observed in separate origins were identified among meat (20 STs) and caecal (19 STs) samples where 30 and 20 of the isolates, respectively were found. Furthermore, 18 STs representing 386 (62.6%) *C. jejuni* were identified in all sources tested.
This study provided information on MLST type distribution and genetic relatedness of *C. jejuni* from broiler and human origins in Poland. The analysis revealed substantial genetic diversity of the examined bacterial population and showed that isolates from humans mostly represented the same STs which were also identified among *C. jejuni* from broiler food chain.

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Keywords: C. jejuni, MLST, food, humans

Microbiological spotlights

P5.35

A-type procyanidin analogues: Synthesis and antimicrobial properties against resistant bacteria from organic foods

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The growing number of foodborne-illness outbreaks caused by some pathogens, coupled with the high rate of antibiotic-resistance associated with foodborne infections, has increased the interest in developing new kinds of effective antimicrobial compounds derived from natural sources. Being aware of the current interest in proanthocyanidins, natural polyphenols structurally based on flavan-3-ols, and according with our previous results in the study of natural procyanidin B-2 and cinnamtannin B-1, we have designed a set of analogues to dimeric A-type procyanidins. We have obtained six analogues (**1–6**) and their antimicrobial and antibiofilm activities against a selection of resistant bacteria from organic foods have been analysed.These compounds have been synthesised by the nucleophilic attack of (+)-catechin or phloroglucinol on several flavylium salts, which were prepared by the acid-catalyzed condensation of *o*-hydroxybenzaldehyde and derivatives with 3',4'-dihydroxyacetophenone and a chloro derivative.The antimicrobial activities of the analogues were tested by the standard agar-diffusion method and Minimal-Inhibitory-Concentration (MIC) values were determined by the broth microdilution method. The inhibition of bacterial biofilm formation and the disruption of preformed biofilms by commercial procyanidin A-2 and analogues **1–6** was measured by the crystal-violet-stain method.Analogue **4**, which had a NO_2 group on ring A, proved to have the best antimicrobial activity against *B. cereus*, *Enterococcus* sp., *S. aureus*, *S. saprophyticus*, and *Enterobacter* sp. Analogues **1** and **5** also showed antimicrobial activities, although they were less effective and mainly against Gram-positive strains. Analogues **1**, **4**, and **5** were the most active compounds against biofilm formation and analogue **4** also showed high activity on the disruption of preformed biofilms, along with analogues **5** and **6**. These results suggest that some structure-activity relationships for the tested compounds may be deduced, so that the absence of electron-donating groups attached to ring A enhances the antimicrobial activities of the analogues.In all cases, synthetic compounds were found to show higher antimicrobial activities than procyanidin A-2, what may stimulate their use as food preservatives or sanitizers for processing equipment where foodborne pathogens reside.

Keywords: A-type procyanidins, antimicrobial activity, antibiofilm activity, resistant bacteria, organic foods

Microbiological spotlights

P5.36

Impact of nutrient availability on *Clostridium botulinum* neurotoxin production

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Botulinum neurotoxins (BoNTs), among the most powerful natural toxins known to mankind, block signal transmission from motor neuron to muscle cells and cause flaccid paralysis called botulism in humans and animals. Botulism may lead to death upon failure of the respiratory muscles.

BoNTs are produced by the anaerobic, spore-forming, gram-positive bacterium *Clostridium botulinum* during growth in anaerobic environments like food, the gastrointestinal tract or tissues of humans and animals, and decomposing materials. *C. botulinum* is ubiquitous in nature and its spores can be found in different environments all over the world. Favorable conditions allow outgrowth of spores into viable culture and BoNT production. The most well-known form of botulism is an intoxication caused by ingestion of pre-formed BoNT with food or drink.

It is known that the availability of nutrients in the bacterial environment can have an impact on the level of toxin production. Here we identified carbohydrates and amino acids as sources of carbon and nitrogen that have a concentration-dependent influence on BoNT production in *C. botulinum* cultures. Using a metabolomics approach, we aim to understand which intracellular metabolites and metabolic pathways can contribute to BoNT production and how these processes are regulated. The results will help to better understand the molecular network regulating *C. botulinum*'s pathogenicity and contribute to the development of countermeasures to prevent botulism.

Keywords: Clostridium botulinum, botulinum neurotoxin, botulism, nutrient, spores, metabolic pathway, pathogen

Microbiological spotlights

P5.37

Sporadic occurrence of Hepatitis E virus in pork meat supply chain in Poland

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Hepatitis E virus (HEV) is recognised as a zoonotic pathogen transmitted via food. Its presence in food mainly relates to food of animal origin, due to an animal reservoir of the virus. The aim of the study was a quantitative assessment of the virus prevalence in a raw pork meat and variety of processed meat products containing blood and / or pork liver. In addition, the possibility of HEV introduction to the pork meat supply chain was assessed based on the presence of porcine adenovirus (pAdV), which is an index virus of faecal contamination. Ready-to-eat (RTE) pork meat products totaling 157 samples represented two food categories such as raw meat products (60 samples of white sausage and onion spreadable sausage) and variety of processed meat products (97 samples of black pudding and sausage liver) were tested for the presence of HEV and pAdV. Pork liver and blood (50 samples) as an incoming raw material used for food production were also included in the analyses. Samples were collected from meat processing plants. Virus extraction and isolation of viral nucleic acid from food samples were performed using TRIzol (TRI Reagent®) and a NucliSens kit (BioMérieux). A detection of HEV and pAdV was conducted using the duplex real-time (RT) PCRs. The correctness of the methods performance was monitored using a sample process control virus.

HEV was detected in 1.4% of tested raw material and food samples. The virus was present in black pudding (1/50) and sausage liver (1/47) at the highest concentration of 6.6 x 10^2 viral GC/g. Higher virus load (1.4 x 10^4 GC / ml) was only found in one out of three tested samples of pork blood. HEV RNA was not detected in any sample of pork liver (0/47), white sausage (0/35) and onion spreadable sausage (0/35). PAdV was present in white sausage (4/35), black pudding (9/50) and sausage liver (4/47). Phylogenetic analyses of the nucleotide sequences of the ORF 1 and ORF 2 fragments of the HEV genome derived from pork blood indicated at HEV subgenotype 3e strain.

The presence of HEV and pAdV in RTE pork products indicates an insufficient thermal processing of food. Nevertheless, sporadic HEV detection and low virus concentration in tested food may indicate at negligible risk of infection associated with their consumption.

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Keywords: HEV, meat products, occurrence, detection

Microbiological spotlights

P5.38

Genetic profiles and virulence potential of *Salmonella* spp. tracked in a Brazilian pork production chain

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Salmonella spp. is a relevant pathogen in the pork production chain, demanding tracking of the potential contamination points to lead proper control. In such context, molecular tools are useful to characterize the genetic profiles and virulence traits of *Salmonella* spp., in order to predict the potential impacts of contaminated products on human health. The present study aimed to track the origins of *Salmonella* spp. contamination in a pork production chain in Brazil, and to characterize the virulence aspects of isolates. A total of 800 samples from pig farms (floor, feed and water from barns), slaughterhouse (lairage floor and different utensils), pig carcasses (after bleeding, buckling, evisceration and final washing, and palatine tonsils and mesenteric lymph nodes) and pork cuts was obtained and subjected to *Salmonella* spp. detection according to ISO 6579. *Salmonella* suspect isolates were subjected to PCR targeting *invA* and *ompC*. One confirmed *Salmonella* isolate per sample was selected and subjected to *XbaI* macrorestriction and PFGE, serotyping and PCR reactions to detect virulence related genes: *sitC, pagC, tolC*, *sifA, msgA, orgA, spiA, sipB, prgH, iroN*, *spaN, cdtB*, *spvB, spvC*. *Salmonella* spp. was detected in barns floor from 3 pig farms (03/10), lairages floor (07/10), mesenteric lymph nodes (43/100), palatine tonsils (45/100), carcasses after bleeding (2/100) and final washing (1/100), utensils (3/120) and cuts (4/40). PFGE of selected *Salmonella* isolates (n = 112) grouped them in 15 clusters (more than 90% of similarity) and allowed the identification of isolates with identical genetic profiles from different pig farms, as well as the persistence and contamination routes of these pulsetypes in the studied pork chain. The most prevalent identified serovars were Typhimurium (59%) and Anatum (14%); 27% of the isolates belonged to other serotypes. Virulence related genes were present in high levels among tested isolates, indicating their pathogenic potential: all tested isolates (n = 112) were positive for *sitC, pagC* and *tolC*; 111 for *sifA, spiA, msgA, orgA* and *sipB*; 108 for *prgH* and *iroN*; 107 for *sopB*; 104 for *spaN*; 26 for *cdtB*; and 4 for *spvB* and *spvC*. The obtained results allowed the identification of contamination routes and distribution of *Salmonella* spp. in the studied pork production chain, and highlighted the pathogenic potential of the obtained isolates.

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Keywords: pork; Salmonella; virulence; PFGE

Microbiological spotlights

P5.39

Study of viability and infectivity of *Toxoplasma gondii* oocysts in a complex food matrix: *Mytilus edulis*

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Toxoplasma gondii is a protozoan parasite that has been highlighted as emerging foodborne pathogen by the Food and Agriculture Organization of the United Nations and the World Health Organization. However, no standardized method is available to detect the oocysts in food, and hence no regulation exists. Nevertheless, considering their low infective dose, ingestion of foodstuffs contaminated by low quantities of these parasites can lead to human infection. To evaluate protozoan risk in food, efforts must be made towards exposure assessment to estimate the contamination with infective oocysts along the food chain, from raw products to the consumers.

In vivo inoculation is the method of choice to investigate infectivity with the ability to detect only one infectious oocyst, but it is time-consuming, expensive and raises many ethical problems. It is therefore essential to develop complementary method, easy to implement in the laboratory and accessible to food industries, to assess the risk associated with the presence of these parasites in food [1]. One alternative approach to animal bioassays is *in vitro* cell infection, which has been successfully used to quantify viable *Cryptosporidium* and *T. gondii* oocysts in water [2,3,4]. However, no such method has been proposed to detect viable and infective oocysts in food matrices [1].

The objective of this work was to develop a cell infection assay coupled to qPCR detection (CC-qPCR) as sensitive as bioassay, that can quickly evaluate the viability and infectivity of *T. gondii* oocysts in a complex food matrix such as the mussel *Mytilus edulis.* The developed method was then applied to assess the survival of *T. gondii* oocysts in experimentally exposed mussels and during depuration period.

The designed CC-qPCR assay was able to detect only live *T. gondii* oocysts at low dose. Infection of cells was observed within 72h compared to four weeks with *in vivo* inoculation. This method allowed to show that oocysts that are accumulated by mussels remain infective.

The developed CC-qPCR assay appears as a promising alternative to bioassays, to assess the risk linked to *T. gondii* oocysts in foods.

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Keywords: viability, infectiosity, Toxoplasma gondii, food matrix, shellfish, Mytilus edulis

Microbiological spotlights

P5.41

Whole genome MLST provides ample resolution for typing and outbreak detection of *Cronobacter* spp.

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Cronobacter spp. is linked with serious infections such as meningitis, septicaemia and necrotizing enterocolitis in neonates and immunocompromised adults. The organism has been isolated from powdered infant formula, and various environmental sources. Although infections are rare, they are often very serious for young infants, leading to death. Following the association of Cronobacter spp. to several fatal outbreaks in neonatal intensive care units (NICU), the World Health Organization in 2004 requested the establishment of a molecular typing method to enable international control of the organism. Nowadays, whole genome sequencing can be performed for the equivalent cost of classical 7-loci MLST typing.

We developed a whole genome MLST (wgMLST) scheme for the Cronobacter genus by expanding the cogMLST scheme developed by Forsythe et al. (2014) to the pan-genome. In total, we identified 13,862 accessory loci, complementing the 1,865 cogMLST loci. By also capturing the accessory loci, the discriminatory power increased. At the same time, the extended scheme also allows for the detection of subtype or outbreak-specific markers, enabling more powerful classification and detection of outbreaks.

The quality of the scheme was first assessed by comparing its performance to whole genome SNP (wgSNP) approaches. In each case it resulted in the same phylogenetic outcome as wgSNP. In fact, our wgMLST approach proved to be more easily scalable and computationally less intensive than the wgSNP approach. The performance was further assessed by applying the scheme to one of the largest described outbreaks in a NICU in France (1994) that lasted over 3 months and claimed the lives of three neonates. wgMLST analysis of the 26 outbreak isolates led to the same conclusions as the original study: phylogeny suggested 3 distant clusters of isolates associated with the outbreak and pointed towards extrinsic contamination of reconstituted infant formula from the NICU environment and personnel as a likely source of the outbreak.

In conclusion, the wgMLST scheme for Cronobacter spp. provides ample resolution for typing and detection of outbreaks. It provides better resolution compared to classical 7-loci MLST and cogMLST and is more scalable compared to wgSNP approaches. Moreover, the possibility to extract traditional typing data, resistance and virulence data from the wgMLST scheme reduces the total analysis time and cost, and may lead to more efficient outbreak detection.

Keywords: outbreak detection, typing, whole genome MLST

Microbiological spotlights

P5.42

Promoting effect of air-solid biofilm culture on persister cell formation in *Escherichia coli*

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Persister cells, or persisters, are a fraction of genetically homogeneous bacterial cells that are tolerant to lethal concentrations of antibiotics despite not expressing any typical antibiotic-resistant genes. This tolerance is rather a temporary phenotype; therefore, persisters revert to being antibiotic-sensitive cells when the drug is removed. Thus, persisters can be distinguished from permanent antibiotic-resistant cells, which are induced by either genetic mutations or by horizontal gene transfer.
A large amount of antibiotics are used in stockbreeding and fisheries, not only for curing or preventing bacterial infections but also for promoting the growth of animals and aquatic products. These practices increase the risk of development of antibiotic-tolerant cells, including persister cells, which has a negative influence on the human health and the food environment.
In this report, we have studied persister cell formation of *Escherichia coli* strains in an air-solid biofilm, wherein the bacterial cells often demonstrate unique heterogeneous phenotypes. The occurrence of persister cells in an air-solid biofilm or liquid culture was assessed by the survival assay in antibiotic-containing media. Using common laboratory *E. coli* strains, we found that the occurrence of persister cells was approximately 10^1-10^6 times greater in an air-solid biofilm than in a liquid culture, which suggests the promoting effect of an air-solid biofilm culture on persister cell formation in *E. coli.* To examine the generality of this effect in *E. coli* species, we used the *E. coli* collection of reference (ECOR), which is a standard collection of 72 natural strains of *E. coli*. We found similar promoting effects in majority of the ECOR strains, thereby demonstrating the generality of this effect.
Since air-solid biofilms are usually present in food environments, for example, as a growth on the surfaces of various foodstuffs or food-related implements, our results suggest the potential of bacterial persister cells entering the human environment or even in the human bodies through the food chain. (This work was supported by JSPS KAKENHI Grant Number 15K14694.)

Keywords: persister, Escherichia coli, air-solid biofilm

Microbiological spotlights

P5.43

Effect of environmental conditions on *E. coli* O157:H7 ATCC 43888 and *L. monocytogenes* ATCC 7644 cell surface hydrophobicity, motility and cell attachment on food-contact surfaces

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Biofilm formation is a major source of materials and foodstuffs contamination, contributing to occurrence of pathogenic and spoilage microbes in food processing resulting in food spoilage, transmission of diseases and significant food hygiene and safety issues. This study elucidates biofilm formation of *E. coli O157:H7* and *L. monocytogenes* ATCC 7644 grown under food related environmental stress conditions of varying pH (5.0;7.0; and 8.5) and temperature (15, 25 and 37 °C). Both strains showed confluent biofilm formation at 25 °C and 37 °C, at pH 8.5 after 5 days. *E. coli* showed curli fimbriae production at various temperatures, while *L. monocytogenes* did not show pronounced expression. Swarm, swimming and twitching plate assays were used to determine strain motilities. Characterization of cell hydrophobicity was done using the microbial adhesion to hydrocarbons (MATH) assay using *n*-hexadecane. Both strains showed hydrophilic characteristics as they fell within a < 20 % interval. FT-IR revealed COOH at 1622 cm^{-1}, and a strong absorption band at 3650 cm^{-1} - 3200 cm^{-1} indicating the presence of both -OH and -NH groups. Both strains were hydrophilic and could form biofilm at different combinations of temperature and pH. EPS produced in both species proved to be an acidic hetero-polysaccharide.

Keywords: Biofilm, E. coli, L. monocytogenes, Hydrophobicity, Motility

Microbiological spotlights

P5.44

Impact of environmental conditions on growth and toxin production of *Aspergillus carbonarius* and *A. flavus* isolates on wheat and rice grains

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The purpose of this study was to investigate the impact of environmental factors (temperature and a_w) on the germination, growth and toxin production (ochratoxin A & aflatoxins) of two common mycotoxigenic fungi on wheat and rice grains obtained from the Greek market. Fungal species, namely *Aspergillus carbonarius* and *A. flavus*, were selected with criteria, their occurrence in cereal grains and their toxin severity to health. Mycelium expansion recordings, growth rates and lag phases calculation and quadratic modelling were used to assess growth and germination of the fungi on grains, while HPLC analysis were used for toxin quantification.

The wheat and rice grains, were surface decontaminated, left to dry under sterile conditions, and then hydrated to the desired a_w levels (0.800, 0.850, 0.900 & 0.980). A 10 μl aliquot from a 10^5 spores/ml stock spores suspension was used to inoculate centrally the plates with the grains and incubation followed at 20, 25, 30 and 35°C. Fungal growth rates and lag phase duration were measured and analysis of variance was used to reveal any statistical significant differences between the factors studied and their interactions, while 3D contour plots after quadratic modelling of growth rates were built to present graphically growth ranges under the factors studied. Additionally, toxin production at different sampling times under the environmental conditions studied, determined with HPLC.

The low limit of a_w for *A. flavus* to grow was 0.800 regardless the temperature or the cereal tested while for *A. carbonarius* was 0.850 on wheat and 0.900 on rice in the whole temperature range studied. Although lag phases were similar for both fungi regardless the cereal used, their growth rates were significantly lower on rice. Additionally, *A. carbonarius* had slower growth rates compared to *A. flavus*, with optimum temperature for growth at 30 and 35°C, respectively. The maximum levels for both mycotoxins found at 0.980 a_w, and at 25°C for ochratoxin A and 30°C for aflatoxins, respectively.

As a conclusion, in order to have a safe, from fungal infections, storage of wheat and rice, grains should maintain a water activity lower than 0.850 for wheat and 0.900 for the rice. Above these values, fungal growth can take place within all the range of temperatures tested.

Keywords: Fungi, A. carbonarius, A. flavus, kinetics, ochratoxin A, aflatoxins, wheat, rice, food safety

Microbiological spotlights

P5.45

Characteristics of the microbial contamination on chinese cabbage in Korea

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This study was carried out to investigate microbial contamination on Chinese cabbage and changes of microbial density during the cultivation periods in Korea. Two hundreds six samples were collected from August 2016 to March 2017 at six different regions to investigate microbial contamination and total 55 samples were collected at three designated farms to determine changes of microbial density from August to October in 2017. The samples were examined the population of coliforms and *Bacillus cereus* and the presence of *Escherichia coli*, *E. coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes*. Out of 208 samples, coliforms were detected in 142 samples (68.9%) ranging from 1.0 to 5.9 Log CFU/g. *E. coli* was detected in 25 samples (12.1%). The density of coliforms and the detection rate of *E. coli* were relatively higher in August and October compared to those in November and March, respectively. The density of coliforms decreased while the density of *B. cereus* increased on Chinese cabbage during cultivation periods. *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* were not detected in any of the samples. These results provide the fundamental data for microbiological risk assessment (MRA).

Keywords: Chinese cabbage, microbial contamination, foodborne pathogen

Microbiological spotlights

P5.47

Sale of raw milk by vending machines in northern Germany: Food safety implications and other aspects

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The safety of raw milk sold in northern Germany was investigated in relation to hygiene quality parameters, the presence of foodborne pathogens (*Salmonella* spp., *Yersinia* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, thermotolerant *Campylobacter*, shigatoxin-producing *Escherichia coli* (STEC)) and by way of consumer interviews.

With regard to EU regulation 853/2004, 2 of 50 milk samples exceeded the limit of the somatic cell count, which is 400,000 cells/ml. The aerobic mesophilic count was higher than 100,000 CFU/ml in 17 (34%) of the milk samples with a mean value of 4.7 $\log_{10}$ CFU/ml. Counts of *Enterobacteriaceae*, pseudomonads and yeasts were detected at mean values of 2.5 $\log_{10}$ CFU/ml, 3.7 $\log_{10}$ CFU/ml and 3.1 $\log_{10}$ CFU/ml, respectively. However, 24% of the raw milk samples exceeded *Enterobacteriaceae* counts of 3 $\log_{10}$ CFU/ml, with up to 6.1 $\log_{10}$ CFU/ml in one sample. More than 100 CFU/ml of *E. coli* were observed in 16% of the samples, whilst levels of *Pseudomonas* spp. greater than 5 $\log_{10}$ CFU/ml were detected in 26% of raw milk samples. Overall, only every second raw milk sample tested met the previously mentioned hygiene criteria.

Staph. aureus occurred in 11% of raw milk samples, while foodborne pathogens such as *Salmonella* spp., *Listeria monocytogenes* and STEC could not be cultivated. *Campylobacter jejuni* was determined in an enrichment broth of one raw milk sample, while *Yersinia enterocolitica* was present in 66% of the raw milk samples, which was confirmed by 16S PCR analyses. However, for the *Y. enterocolitica* isolates, the virulence associated *ail* gene was not detected, which suggested that the isolates belonged to the apathogenic biovar 1A.

Evaluation of consumer habits, as determined in the interviews, revealed that their behavior may enhance the risk of infection linked to raw milk consumption, as about 75% of the interviewed consumers did not boil milk before consumption.

Although vending machines dispense raw milk, the consumers are generally instructed to boil the milk prior to consumption. If consumers follow these instructions, the microbiological risks associated with raw milk would be eliminated. Improved risk communication to consumers is therefore recommended.

Keywords: raw milk sale, food hygiene, food safety, consumer habits

Microbiological spotlights

P5.48

RAPID'B.cereus agar: N new sensitive chromogenic medium for the detection of *Bacillus cereus* group in food

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Introduction: The *Bacillus cereus* group includes organisms that are widely distributed in the environment and some species are well known as human and/or animal foodborne pathogens. The increasing demand for food with a fresh-like quality and the desire to produce low-heated food has increased interest for these bacteria. Thus, *B. cereus* is considered a "Quality Indicator" by different national or international regulations and should be quantified in different foodstuffs. The main challenge for detection is the heterogeneity of this group consisting of species that are very different morphologically and biologically: *B. anthracis, B. cereus sensu stricto, B. cytotoxicus, B. mycoïdes, B. pseudomycoïdes, B. thuringiensis* and *B. weihenstephanensis.*

Purpose: This study evaluated the performance of a new selective chromogenic agar - RAPID'*B. cereus* (RBC) - in comparison to the standard MYP Agar (ISO 7932). Data about the growth of the little-known *B. cytotoxicus* on RBC and other commercial products were also collected.

Methods: Inclusivity/exclusivity studies were performed with 50 *B. cereus* group strains and 50 non *B. cereus* group strains, respectively. The sensitivity and selectivity studies were carried out on a total of 50 food samples (artificially and naturally contaminated) representative of the matrices with a high risk of *B. cereus* contamination.

Significance: Exclusivity data demonstrated a much better selectivity on RBC than MYP. Inclusivity data also shows that Bio-Rad's RAPID'*B.cereus* agar allows the growth of most sensitive strains, bringing a new and effective tool for the detection of *B. cereus* group in 24h at 30°C. The current reference medium for *Bacillus cereus* group detection, MYP agar occasionally gave uninterpretable results depending on the food matrix due to its very limited selectivity. Nevertheless, even most sensitive *B. cereus* strains can grow on MYP. Conversely, most existing chromogenic media are strongly selective and do not allow the growth of every species in particular *B. cytotoxicus* or other challenging strains. On contaminated samples, RBC shows good enumeration of *B. cereus* group bacteria compared to the ISO7932 reference method.

Keywords: Bacillus cereus group, Bacillus detection, chromogenic media, Bacillus cytotoxicus

Microbiological spotlights

P5.49

Antimicrobial resistance of *Listeria monocytogenes* isolated from food in Poland

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Resistance of *L. monocytogenes* to antimicrobial agents is increasingly observed. Resistance genes can be transferred between *Listeria* and other Gram-positive and Gram-negative bacteria. Therefore, it is important to monitor the prevalence of resistant *L. monocytogenes* strains, especially isolated from food.

A total of 146 *L. monocytogenes* isolates from various types of food of animal origin were collected during 2013-2016. The isolates belonged to the serotypes most commonly caused food poisoning in humans: 1/2a, 1/2b, 1/2c and 4b. They were recovered from ready-to-eat food (n=105), raw meat (n=17), raw sausages (n=16) and seafood (n=8). Antimicrobial resistance was tested using the microbroth dilution metod with Sensititre GPN3F plates (Thermo Scientific, USA) containing 17 antimicrobials. The minimal inhibitory concentration (MIC) records were read using the Sensititre Vizion System (Trek, UK).

Most of the isolates were resistant to oxacilin (132; 90.4%), followed by clindamycin (79; 54.1%) and ceftriaxone (72; 49.3%). Only few strains showed resistance to linezolid (5; 3.4%) and ciprofloxacin, gatifloxacin, gentamycin and tetracycline (1; 0.7% of each). In addition, intermediate resistance to ceftriaxone and clindamycin was demonstrated in several isolates (70; 47.9% and 56; 38.4%, respectively). On the other hand, all strains were sensitive to ampicillin, erythromycin, gentamycin, penicillin, streptomycin, trimethoprim/sulfamethoxazole and vancomycin. Eleven antimicrobial resistance profiles were defined, including 4 strains (2.7%) that were susceptible and intermediate resistant to all substances tested. A total of 38 strains (26.0%) were resistant to one antimicrobial only, whereas the remaining 104 (71.2%) isolates were resistant to more than one antimicrobial. Among them, 40 (27.4%) strains displayed the multiresistance pattern.

Antimicrobial resistance, especially identification of multiresistant isolates, suggests that food of animal origin contaminated with *L. monocytogenes* may present a risk for public health.

Keywords: L. monocytogenes, food, antimicrobial resistance

Microbiological spotlights

P5.50

Regulation of subtilase cytotoxin expression by the global regulators Hfq and H-NS in *Escherichia coli*

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are a major cause of foodborne diseases such as haemorrhagic colitis and the haemolytic-uremic syndrome (HUS). Recent studies described that the production of subtilase cytotoxin (SubAB) contributes to the virulence of STEC. SubAB belongs to the family of AB_5-toxins. The homopentamer B-subunit mediates binding to the eukaryotic cell receptor N-glycolylneuraminic acid. The A-subunit exhibits the catalytic activity by cleaving the BiP/GRP78 chaperone in the endoplasmic reticulum leading to cell apoptosis. Whereas the cytotoxicity and other cellular mechanisms caused by SubAB have been widely investigated, the regulation of expression of this virulence factor remains unclear. This study addressed this issue focusing on the impact of the global regulator proteins Hfq and H-NS on the regulation pathway of the subtilase cytotoxin production in *E. coli* DH5a carrying a recombinant luciferase reporter as a model strain.

Methods: The influence of global regulators Hfq and H-NS on subtilase cytotoxin gene expression was analysed by creation of deletion mutants and application of luciferase reporter gene assays. Experiments were conducted in laboratory strain *E. coli* DH5α. The promoter activity was measured during five hours of growth by reporter gene assays.

Results: Deletion mutants of genes *hfq* and *hns* were created. Application of reporter gene plasmid harbouring subtilase cytotoxin promoter ($PsubAB_1$) in luciferase assays have indicated the influence of both, Hfq and H-NS in *E. coli* DH5α. Deletion of *hfq* and *hns* led to significant increase of $PsubAB_1$ promoter activity showing the greatest effect in the early growth phase for *hns* deletion mutant and in the log-phase for *hfq* deletion mutant.

Conclusion: The increased promoter activity of $PsubAB_1$ in *E. coli* DH5α Δ*hfq* and *E. coli* DH5α Δ*hns* in luciferase assay studies indicates the influence of Hfq and H-NS in the regulation pathway of subtilase cytotoxin. Further investigations will be performed in STEC wild type strains to indicate the role of global regulator proteins in virulence regulation.

Keywords: Subtilase cytotoxin, Hfq, H-NS, E. coli, luciferase reporter gene assay

Microbiological spotlights

P5.51

Influence of processing steps on subsequent inactivation of *Campylobacter jejuni* during cold storage under modified atmosphere

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Campylobacter infections are the main cause of bacterial enteritis over the world in humans and broiler meat is considered as the most important source of human campylobacteriosis. Some mitigation strategies for *Campylobacter* focus on slaughtering and processing steps. Slaughter includes several steps such as scalding and chilling which are likely to induce thermal and cold stress in *Campylobacter.*

The purpose of this study was to determine if the application of consecutive stresses could influence the subsequent inactivation of *Campylobacter jejuni* during cold storage under different modified atmospheres.

In a full experimental design, strains of *C. jejuni* of poultry origin were submitted to consecutive thermal and cold stresses, similar as those encountered during scalding and chilling in slaughter. Thermal stress consisted in immersing *C. jejuni* cultures in baths at 46 - 50 or 54°C for 4 min. Cold stress was applied immediately after thermal stress by plunging the previous cultures into baths at -4 or 3°C for 2 h. At last, cultures were stored at 6°C during seven days under two modified atmospheres (70% O2-30% CO2 and 50% CO2-50% N2). Three different strains of *C. jejuni* were studied independently. The three experimental designs were repeated four times. Viable *C. jejuni* cells were enumerated on Columbia agar after each stressful step and at the end of the cold storage. Results were analysed by ANOVA.

Inactivation of *C. jejuni* induced by cold storage was shown to depend significantly ($P < 0.0001$) upon the thermal stress it had previously encountered. The highest temperature of 54°C was associated with the highest inactivation of *C. jejuni* during cold storage. The strain and the gas mixture in the modified atmosphere were also shown to influence significantly the inactivation of *C. jejuni* during storage (main and interaction effect). Strain inactivation variability was characterized by 1- to almost 2- mean log reduction of *C. jejuni* during storage. The atmosphere (70% O2 - 30%CO2) induced a mean log-reduction of 1.7 log higher than the atmosphere (50% CO2 - 50% N2).

These results show that processing steps and associated stress may condition further inactivation of *C. jejuni* during storage and influence the contamination level at the consumer's plate. Better understand the adaptation strategies of *C. jejuni* during slaughtering may help adjusting control measures to reduce the contamination level of *C. jejuni* and hence limit consumer exposure.

Keywords: campylobacter, poultry, slaughter, stress, inactivation, adaptation

Microbiological spotlights

P5.52

Reliable and easy differentiation of the new genera *Franconibacter* spp. and *Siccibacter* spp. from *Cronobacter* spp. with MALDI-TOF MS

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Background: Taxonomical changes recently occurred to differentiate *Franconibacter* and *Siccibacter* isolates from *Cronobacter* genus. Indeed, only *Cronobacter* is recognized as a foodborne pathogen, affecting mainly premature neonates. According to the European Regulation 2073/2005, this pathogen should not be detected in infant formula and "no detection" result is required to release the product. False positive identifications lead in wasting infant formula batches. In this context, it is essential to differentiate *Cronobacter* spp from its closely related genus. This is of course more critical and challenging when former *Cronobacter* species have been elevated to *Franconibacter* and *Siccibacter* genus.

Materials and methods: DSMZ strains were cultivated on agar plates, and biomass was processed with the extraction method. MALDI-TOF MS reference mass spectra were obtained using a MALDI Biotyper (MBT) system (Bruker Daltonik). The quality control of the generated mass spectra was assessed according to the Bruker standard procedure for library content creation. MBT Compass software was used to create Mass Spectral Projections (MSPs). The MBT library was updated from 7311 MSPs to 7854 MSPs, and reference spectra for *Franconibacter helveticus, Franconibacter pulveris* and *Siccibacter turicensis* were added.

Results: Each reference spectrum (MSP) in the MBT library originates from a single strain. Therefore, a species is represented by several strains, generally representing the natural inherent MALDI-TOF MS variability. When comparing the MSPs of the studied genera, the following maximal score values are observed:
- 1.39 between *Franconibacter* spp and *Siccibacter turicensis*
- 1.40 between *Siccibacter turicensis* and *Cronobacter* spp
- 1.78 between *Franconibacter* spp and *Cronobacter* spp

Consequently, the critical differentiation between *Franconibacter* spp, *Siccibacter turinencis* and *Cronobacter* spp is achieved with the MBT system. No interference of the *Cronobacter* spp (n = 15), *Franconibacter* (n = 4), *Siccibacter* (n = 1) is observed with the other *Enterobacteriaceae* members (n = 591, including 29 *Enterobacter* spp).

Conclusion: The MALDI Biotyper with the updated Library with 7854 MSPs provides reliable and easy identification of *Franconibacter helveticus*, *Franconibacter pulveris*, *Siccibacter turincensis* and *Cronobacter* isolates. The MALDI Biotyper provides reliable identifications for 95 samples in less than 2 hours after cultivation.

Keywords: Franconibacter, Siccibacter, Cronobacter, Differentiation, MALDI-TOF MS

Microbiological spotlights

P5.53

The sporulation master regulator Spo0A controls neurotoxin production in group I *Clostridium botulinum* type A

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The species *Clostridium botulinum* comprises phylogenetically and ecologically diverse spore-forming and neurotoxin-producing strains. It consists of different Groups (I-IV) with distinct natural habitats and physiological properties. The peak botulinum neurotoxin production in *C. botulinum* cultures coincides with initiation of sporulation, which suggests that the two processes are under common regulatory mechanisms. Further understanding of the association between neurotoxin production and sporulation may bring important novel insights into the ecology and evolution of *C. botulinum*.

Spo0A is the master regulator of sporulation in all spore-forming bacteria. We recently demonstrated that Spo0A directly regulates botulinum neurotoxin gene expression in the ecologically distinct *C. botulinum* Group II type E (Mascher et al., Environ Microbiol, 2017). It is unclear whether this regulation is conserved in other botulinum neurotoxin-producing organisms.

Using various genome editing tools, we disrupted the *spo0A* gene in Group I *C. botulinum* type A strains. To determine if Spo0A contributes to neurotoxin regulation, we analyzed the phenotype and transcriptome of the *spo0A* mutants and compared to the corresponding wild-type strains. Insertional inactivation or clean deletion of *spo0A* practically abolished sporulation, but also markedly impaired BoNT expression compared to wild-type levels. Bioinformatic data analysis and biochemical assays are on-going and will determine how Spo0A controls the neurotoxin gene cluster.

The present study identified the Spo0A sporulation master regulator as a novel regulatory player in toxinogenesis in Group I *C. botulinum* type A strains, confirming the tight interconnection between BoNT production and sporulation. Similar to Group II *C. botulinum* type E strains, this suggests that Spo0A mediation of neurotoxin production is conserved among *C. botulinum* strains.

Keywords: Clostridium botulinum, sporulation, neurotoxin

Microbiological spotlights

P5.54

Easy and reliable identification of filamentous fungi by MALDI-TOF MS

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Background: MALDI-TOF MS has considerably changed the laboratory workflow for microbial identification, and is nowadays recognized as a reference method in this field. Libraries were implemented first for bacteria and yeast species. The technology is as well perceived as the most promising alternative for moulds identification, as it can definitely help in solving existing issues: lack of robustness of time-consuming phenotypic procedures, and very few experts in the field. Moreover, molecular testing is not established for routine purposes. MALDI-TOF MS provides reliable identifications from culture for 95 samples in less than 2 hours, and current efforts are targeted to further expand the available library.

Materials and methods: Fungal strains were obtained from culture collections or from MALDI Biotyper (MBT) users. Species identity was assessed by sequencing for most of the strains. All strains were cultivated in liquid Sabouraud medium, and sample processing was done according to the MBT protocol for the creation of reference spectra for the MBT Filamentous Fungi Library.

Results: One hundred seven new reference spectra were added to the library, with a focus on *Aspergillus* and *Fusarium* species. The names of several species have been updated according to the current nomenclature. However, synonyms can be found in the metadata of reference spectra to facilitate the access to taxonomical information. The recently implemented MBT Filamentous Fungi Library comprises 58 genera and 153 species, which cover the most important taxa in food mycology, *e.g*. *Alternaria* (n = 5 strains), *Aspergillus* (n = 120 strains), *Fusarium* (n= 52 strains), *Mucor* (n = 5 strains), *Penicillium* (n = 65 strains), and *Rhizopus* (n = 16 strains).
The described sample preparation is optimal for reference creation. For routine analyses, the fastest and easiest sample preparation, *i.e*. Direct Transfer (DT), provides identification results for most of the samples. MVZ Dr. Eberhard & Partner reported DT to be sufficient for 95.8 % (n = 2814) of their clinical filamentous fungi isolates tested over the past six years.

Conclusion: The MBT Filamentous Fungi Library 2.0 is suitable for both industrial and clinical applications, covering 58 genera and 153 species. Continuous efforts are ongoing to further expand species coverage. Reliable identification results can be obtained with the quick and easy Direct Transfer sample preparation for many species.

Keywords: Filamentous fungi, moulds, Identification, MALDI-TOF MS

Microbiological spotlights

P5.55

Occurrence and location of *L. monocytogenes* contamination on bovine carcasses in Belgian slaughterhouses

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Introduction: Despite the efforts already made by the sector, slaughterhouses are still confronted with the foodborne pathogen *Listeria monocytogenes* in the production environment and on carcasses. The presence of the pathogen on carcasses may result in contamination of the meat which may lead to economic losses and a risk for public health.

Purpose: This study on the prevalence and location of *L. monocytogenes* on beef carcasses after slaughter should be the basis for a better understanding of the complex introduction, persistence and variable contamination sources and routes of this pathogen. In addition, the possibility of using an indicator organism for *L. monocytogenes* contamination was investigated.

Methods: A total of 720 cattle carcass samples were taken just before cooling. *Listeria* spp. and *Listeria monocytogenes* were detected and enumerated according to ISO11290. Moreover, total aerobic count (TAC) was determined by plating on plate count agar (PCA; CM0325) and incubating at 30°C for three days. The sampling of 90 carcasses was carried out in three beef slaughterhouses. Carcass locations assumed to have an increased risk for contamination were swabbed. The eight following sites (400 cm^2 each) were sampled: pelvic duct, split surface of neck, inside throat region, hind leg (medial side), flank (medial side), brisket, inside foreleg and shoulder region.

Results:*Listeria* spp. and *L. monocytogenes* were detected in 28% (205/720) and 10% (71/720) of the swab samples respectively. Overall 47% (95% confidence interval from 36% to 57%) of the carcasses with a variation from 23% to 73% for the three slaughterhouses were found to be positive for *L. monocytogenes* for at least one of the eight sites. Among the different locations, the inside hind leg and the inside foreleg represented the highest contamination frequencies (13% and 12% respectively) for *L. monocytogenes* contamination. However, the contamination rate of the different sampling sites was not significantly different ($P>0.05$). Across all samples, only five samples exceeded the lower limit of enumeration (above -1.3 $\log_{10}$ CFU/cm^2). Statistical analysis showed that detection of *L. monocytogenes* was correlated significantly to the total aerobic count ($P< 0.05$).

Significance: The high prevalence of this pathogen highlights the need for identifying contamination sources and routes. The knowledge of the most contaminated carcass sites is useful in this context.

Keywords: Listeria monocytogenes; cattle; carcass

Microbiological spotlights

P5.56

Acquisition of an IncA/C2 blaNDM-1-carrying plasmid pRH-1238 in *Salmonella enterica* subsp. *enterica* serovar Infantis during a broiler chicken infection study – A potential threat for human exposure?

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Background: During 2012, a NDM-1-producing *Salmonella* Corvallis (*S*. Corvallis) harboring a bla_{NDM-1}-*fosA3*-IncA/C plasmid (pRH-1238) was isolated from a wild bird (*Milvus migrans*). This raised concerns on possible pRH-1238 introduction and transfer to other *Salmonella* recipient strains occuring in commercial broiler production. In order to evaluate the transferability of pRH-1238 plasmid *in vivo* without antibiotic pressure (reflecting non-use of carbapenems) a broiler chicken infection study was initiated.

Materials/methods: As donor, NDM-1 producing *S*. Corvallis and as recipients, nalidixic acid resistant *S*. Paratyphi B (*d*Ta+) and *S*. Infantis were selected. Three groups, each containing ten chicks were designated. On day 7th day of life, ~5x10^6 CFU of *S*. Paratyphi B (*d*Ta+) (G2) and *S*. Infantis (G3) were inoculated, whereas on 10th day of life, in all groups, ~5x10^6 CFU of donor strain, was administered. In Group 1 (G1) only donor strain was administered. Acquisition of pRH-1238 was investigated after fecal material overnight enrichment at 37°C in buffered peptone water supplemented with 1 mg/L cefotaxime, 0,125 mg/L meropenem and 50 mg/L nalidixic acid followed by plating on specific XLD plates. The *Salmonella* transconjugant strains were serotyped, submitted to PCR amplification of bla_{NDM-1} and bla_{CMY-16} genes and S1-PFGE restriction. Selection of transconjugant strains was whole-genome sequenced using MiSeq technology.

Results: The E.coli and *S*. Infantis transconjugants were detected on 13th day of life [G1 (n=4) and G3 (n=2)] followed by 15th [G1 (n=8) and G3 (n=3)], 17th [G1 (n=7) and G3 (n=1)], 21st [G1 (n=10) and G3 (n=1)], 24th [G1 (n=8) and G3 (n=1)], and 29th [Group 1 (n=7) and Group 3 (n=2)]. During the study, *S*. Paratyphi B (*d*Ta+) transconjugants were not detected. Using WGS we observed high percent (>95%) of sequence identity of pRH-1238 variants from transconjugants to reference pRH-1238 plasmid (GenBank Accession number KR091911.1).

Conclusions: Our study revealed that the transfer of multiresistance bla_{NDM-1-}carrying pRH-1238 plasmid to different E. coli and *S*. Infantis is possible, in the absence of antibiotic pressure. This acquisition is more frequent in gut Enterobacteriaceae than in *Salmonella*, possibly linked to their ecology and fitness within gut microflora. Still, pRH-1238 acquisition in broiler-associated and public health relevant *S.* Infantis is possible, posing potential threat for human exposure, downstream the production chain.

Keywords: NDM-1 carbapenemase, Salmonella Corvallis, plasmid transfer, Salmonella Infantis

Microbiological spotlights

P5.58

Growth and cereulide production comparison of emetic *Bacillus cereus* strains in milk

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Bacillus cereus is an opportunistic and ubiquitous pathogen. Consequently strains are commonly isolated in raw materials and occasionally in processed foods, such as rice, milk and dairy products, meats, pasteurized liquid egg, ready-to-eat vegetables, and spices. Due to the formation of toxins it can cause two types of food poisoning, emesis and diarrhoea. The emesis inducing toxin cereulide that is produced by a specific class of *B. cereus* (Group III), represents the most serious food safety risk linked to this pathogen as it has been associated with life-threatening conditions, such as acute liver failure, resulting to the death to several patient. Cereulide is, a cyclic dodecadepsipeptide, pH and heat stable. Its elimination from food products is literally impossible once pre-formed in the food. The growth of emetic *B. cereus* and cereulide production can occur during inappropriate time/temperature storage conditions during processing steps in the production chain or more commonly household abuse of pre-heated foods at the consumer stage. Intermediate products stored in the tanks under abused temperatures for extended times or product kept by the consumer under abused conditions could lead to cereulide production and subsequent food poisoning through intoxication. Very few is known on growth and cereulide production of emetic strains in food matrices. The goal of this study was to compare the growth and cereulide production of different emetic strains in milk at different temperatures (12°C, 15°C, 18°C, 20°C, 25°C, 30°C). Four emetic strains were compared: The reference strain F4810/72 and three strains isolated from processed wheat flour (B594), finished product cereals (B596) and raw wheat flour (B626).The results showed that growth and cereulide production are temperature and strain dependent. B596 demonstrated a slow growth, in particularly at 12°C, 18°C and 20°C. Whereas faster growth was observed with strain B626 at all temperatures, except at 25°C. Concerning cereulide, first results indicated that at 12°C, B626 produced 1 log (ng/ml) of cereulide, while B577 stand at the detection limit. At 30°C both strains produced around 3 log (ng/ml) of cereulide. The acquisition of these type of data is important to better understand the emetic strains variability of growth and cereulide production in food matrix. These data will also support food safety evaluations and give guidelines for the choice of emetic strains dedicated to challenge tests.

Keywords: Cereulide, toxin, emetic, Bacillus cereus, growth

Microbiological spotlights

P5.59

Antimicrobial activity of microcin J25 and reuterin against multidrug resistant *Salmonella*

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Background and Objective: In the livestock sector, which is one of the most promising economic sectors, low doses of antibiotics are used to promote animal growth, increase profitability and to control against microbial infections. Unfortunately, the improper and overuse of antibiotics have led to the dramatic emergence of multidrug-resistant bacteria (MDR) having the ability to adapt and develop a wide variety of resistance mechanisms essential to their survival. Our objective is to determine the antimicrobial activity of Microcin J25 and reuterin against MDR *Salmonella Enterica* serovars.

Material and methods: Seventy-six strains of *Salmonella Enterica* serovars including *S. Newport* (n=38), *S. Typhimirium* (n=13), *S. Senftenberg* (n=13), *S. Enteriditis* (n=6), *S. Choloerasuis* (n=4), *S. kentucky* (n=1) and *S. Heidelberg* (n=1) were tested in this study. The antimicrobial susceptibility test was conducted by disk diffusion method while the whole genome sequencing was employed for the detection of antibiotic-resistant genes. A microplate dilution assay was used to determine the Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values of microcin J25 and reuterin against salmonella strains.

Results: Eighteen *Salmonella Newport* shared low susceptibility to antimicrobials of the penta-resistance phenotype (ACSSuT) including ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline. The ACSSuT profile was found closely linked to int1-associated gene cassettes. All strains were also resistant to cephalosporins and harbored the bla_{CMY-2} gene. The remaining strains of *S. Newport* and all *S. Typhimirium*, *S. Senftenberg*, *S. Enteriditis*, *S. Choloerasuis, S. kentucky* and *S. Heidelberg* were susceptible to all the tested antibiotics. Interestingly, all of these MDR strains were shown to be susceptible to reuterin and microcin J25. The MIC values for Microcin J25 against these strains varied between 0.06 µg/ml and 0.4 mg/ml, and for reuterin between 0.03 µg/ml and 0.1 µg/ml. MBC values for microcin J25 and reuterin were four, eight or sixteen times higher than MIC value indicating a bacteriostatic effect.

Conclusion: Microcin J25 and reuterin demonstrated an important antimicrobial activity against MDR Salmonella suggesting a high potential as an alternative to antibiotics.

Keywords: Microcin J25, reuterin, Salmonella Enterica serovars, antimicrobial activity

Microbiological spotlights

P5.60

Shiga toxin-producing *Escherichia coli* (STEC) isolated from chicken meat in different regions of Brazil

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Shiga toxin-producing *E. coli* (STEC) is one of the most important foodborne pathogens involved in outbreaks, being frequently reported in food of animal origin. Although considered of great importance to public health, there are insufficient reports regarding the presence of STEC in chicken meat in Brazil, the first exporter country. Therefore, the aim of this study was to investigate the presence of STEC in chicken meat samples from poultry slaughterhouse in Brazil. Sixty-seven chicken meat samples collected from slaughterhouses located in southern, southeastern and midwestern regions of Brazil were analyzed for monitoring the presence of Shiga toxin-producing *E. coli* by using PCR according to ISO/TS13136 and USDA MLG 5B.05 methods, both with modifications. Among the chicken meat samples analyzed for STEC strains, one was positive for Shiga toxin-producing genes *stx2* and *eae* and thirty-three were positive only for *eae*. Our study detected STEC strains in chicken meat samples, indicating a risk of infection due to the consumption of these products when not properly cooked, or even through cross-contamination. Nevertheless, more research is required with a larger number of samples, since there are few studies regarding STEC in poultry meat reported in Brazil.

Keywords: microbiology food safety escherichia coli poultry foodborne pathogens

Microbiological spotlights

P5.61

Use of whole genome sequencing and FT-IR analysis for detection of virulence factors in the zoonotic pathogen *Arcobacter butzleri*

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Background: The genus Arcobacter (A.) was previously known as aero-tolerant Campylobacter. Today more than 20 species are known, but only a few cause infections in humans. A. butzleri is described as the most pathogenic one, but even within this species not all isolates seems to be pathogenic. We performed a study looking for A. in 1650 fecal samples using genus specific PCR and culture. The detection rate was about 1%, comparable to the incidence of Campylobacter in the cohort. In addition we collected A. isolates from stool samples and rectal swabs and performed antibiotic susceptibility tests.

Materials/methods: To investigate the correlation between putative virulence genes and clinical symptoms, whole genome sequencing of 50 Arcobacter strains was performed using HISeq Illumina sequencing. Briefly, after sequencing reads were trimmed for good quality and assemble with SPAdes. The assembled contigs were annotated using RAST (Rapid Annotation using Subsystem technology). Rapid large scale pan genome analysis was performed using ROARY pipeline. The presence of antibiotic resistance genes was verified by phenotypic susceptibility testing. We used metabolic fingerprinting by Fourier transform infrared (FTIR) spectroscopy to phenotypically analyze changes in metabolic profiles and surface patterns of the bacteria.

Results: Whole genome sequencing analysis revealed various patterns of different metabolic genes (amino acids, respiration). Environmental genes (regulating LPS, flagellum and chemotaxis) also showed some variability. We focused on virulence factors, e.g. cadF, ciaB or hec to correlate these to the clinical findings of the patients. Analysis of resistance genes resulted in a more frequent detection of fluoroquinolones resistance while macrolides resembled are higher susceptibility.
The FT-IR analysis grouped the A. isolates in several distinct subgroups. Yet, an unweighted analysis of the complete FT-IR spectrum did not parallel the clinical patterns of the hosts.

Conclusions: *Arcobacter butzleri* isolates depict not a homogenous genetic profile. Significant differences were found in metabolic gene clusters as well as in antibiotic resistance markers. In an ongoing research approach we did not yet found a single marker defining pathogenicity within the A. group.
FT-IR is a promising tool for the clustering of Arcobacter and analysis of metabolic profiles, but further studies are necessary to develop more standardized protocols.

Keywords: Arcobacter butzleri, Whole genome sequencing, FT-IR

Microbiological spotlights

P5.62

Campylobacter jejuni and *Campylobacter coli* in skin and intestine of turkey carcasses at time of slaughter

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The aim of our study was to determine the prevalence of *Campylobacter* spp. in turkey slaughterhouses located in the middle area of Algeria (Algiers, Boumerdes and Bouira). 200 samples were collected in 4 poultry slaughterhouses (neck skins and ceca). After research and identification of thermotolerant *Campylobacter*, species were detected (ISO 10272, 1995; OMS, 2003; OIE, 2005). Of the 200 tested samples, 145 thermotolerant *Campylobacter* strains were isolated, which represents an overall prevalence of 72.5%. Furthermore, our results showed that the highest contamination rate was recorded among cecal content (90.0%) followed by neck skin (55.0%) samples ($P < 0.05$). *Campylobacter jejuni* and *Campylobacter coli* were found in all the positive samples (100.0%). In conclusion, turkey industry can be considered as a significant threat to public health, because it participates wildly in the spread of pathogenic strains of *Campylobacter* species.

Keywords: thermotolerant Campylobacter, Campylobacter jejuni, Campylobacter coli, turkey, slaughterhouses

Microbiological spotlights

P5.63

Assessment of dietary exposure to aflatoxin B1 from maize based products in Serbia

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With an average annual production of 6.9 million tonnes of maize, Serbia is one of the prominent grain producers and exporters in Europe, with approximately 0.3 million tonnes used for human consumption at domestic market, annually. The maize is used as food 1. Directly through several food commodities, namely maize flour, polenta, corn flakes, maize snacks and other maize based foods, and 2. Indirectly through animal based products, originating from animals that were fed on maize feed. Worldwide, aflatoxin contamination of maize is a recognized food safety hazard. However, until a few years ago, aflatoxins have not been signalled as a key hazard of concern for primary agricultural production in Serbia and Europe. Recent changes in patterns of aflatoxins occurrence in maize due to extreme weather conditions, being a part of ongoing climate change, are a matter of concern entailing a high public health risk of acute and (sub) chronic exposure to aflatoxin B1. Thus has been witnessed in the 2013 aflatoxin crisis in Western Balkan. Screening of processed maize products and maize products in ready to eat form has shown aflatoxin B1 prevalence of 61.7% (n=60), with a mean value of aflatoxin B1 concentration in positive samples of 4.07 μg kg^{-1}. The range reported was from 1.01 to 21.7 μg kg^{-1}. Food consumption survey of maize based products, based on seven day recall method (n=1000), has shown aflatoxin B1 average intake of 0.83 and 1.04 ng kg^{-1} bw/day, for lower and upper bound aflatoxin B1 concentrations, respectively. High consumer's scenario showed aflatoxin B1 intake of 3.98 and 5.16 ng kg^{-1} bw/day, for lower and upper bound aflatoxin B1 concentrations, respectively.

Keywords: aflatoxin B1, maize, health risk, dietary exposure

Microbiological spotlights

P5.64

Inhibitory Efficacy of *Camellia sinensis* extract on Helicobacter *pylori*

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Helicobacter pylori bacterial infection can cause painful lesion in stomach, small intestine and esophagus in human body. The infection has been known to associate with stomach ulcer. *H. pylori* can multiply in the acidic stomach by regulating urease enzyme activity and releasing toxin to damage tissue. Tea leaf extracts are interesting as sources of effective and safe alternative natural agents for the treatment of *H. pylori* infection. Different types of tea; green tea, oolong tea and black tea are processed from leaves of *Camellia sinensis* var. *sinensis* and they are widely consumed as plant beverage. The phytochemical compounds such as phenolic and flavonoid compounds are presented in different types of tea. The aqueous extracts of green tea, oolong tea and black tea were determined for their inhibitory activity against *H. pylori* DMST20165, *H. pylori* isolate 31 and *H. pylori* isolate 36. The result showed that the extracts of tea leaves could inhibit all tested *H. pylori* isolates. Green tea extract showed the highest anti-bacterial activity against all *H. pylori* isolates with the diameters of inhibition zone ranging from 15.3 mm to 19.3 mm by agar disc diffusion method. Extracts of green tea, oolong tea and black tea could also inhibit all *H. pylori* isolates with the minimal inhibitory concentrations/minimal bactericidal concentrations of 3.91, 15.63 and 31.25 mg/ml, respectively. Moreover, oolong extract inhibited urease enzyme of all *H. pylori* isolates within 3 hours followed by green tea extract that could inhibit urease enzyme of all *H. pylori* isolates within 4 hours. In addition, extracts of green tea, oolong tea and black tea leaves contained catechin of 8.531, 25.125 and 5.047 mg/g extract when determined by high performance liquid chromatography. The extract of green tea leaves also showed the highest antioxidant activity and phenolic compound of 170.51 and 315.09 mg gallic acid equivalent/g extract. Therefore, all tea leaf extracts demonstrated antibacterial against *H. pylori* and antioxidant activities.

Keywords: Antibacteria, H. pylori, Tea leaf extract, Antioxidant, Urease inhibition

Microbiological spotlights

P5.65

Antimicrobial efficiency of edible electrospun chitosan/cellulose acetate/ gelatin nanofiber incorporated eugenol

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The antimicrobial nanofiber mats were successfully prepared by electrospinning process from natural polymer solution including chitosan, cellulose acetate and gelatin. The polymer solutions were blended at the volume ratio of 4:1:5, respectively. Various amounts of eugenol 0, 0.75, 1.5, 3.0 and 5.0 %v/v were directly incorporated into the polymer solutions. Electrospinning process was performed at 23 kV, flow rate at 0.7 mL/h and distance between needle and collector was 10 cm. The average diameters of Eugenol-incorporated edible electrospun ranged from 152.32 to 246.05 nm. In addition, the larger fiber diameters with junction was observed when the concentration of eugenol was increased, while the smooth and continuous structure were obtained without eugenol formulated. The release studies of eugenol, determined by a UV-vis spectrophotometer at the wavelength of 280 nm, presented that the cumulative release of eugenol of 0.75, 1.5, 3.0 and 5.0 wt.% incorporate electrospun nanofiber mats at 60 min were $1.34x10^{-3}$µg, $1.41x10^{-3}$ µg, $2.67x10^{-3}$ µg and $2.68x10^{-3}$ µg respectively. For the burst release of eugenol of 0.75 and 1.5 wt.% reached a plateau after 60 min while the burst release of eugenol of 3.0 and 5.0 wt.% continues to gradually increase. Antimicrobial properties of edible electrospun incorporated with eugenol were evaluated by dynamic shake test using four strains of foodborne pathogenic bacteria including *Escherichia coli* O157:H7 (VTEC)DMST12743, *Salmonella* Typhimurium ATCC 13311, *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 25923. All results showed that the Eugenol-incorporated edible nanofiber mats retarded the growth of bacteria. The population of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Bacillus cereus* and *Staphylococcus aureus* were increased as 7.74, 7.32, 8.52 and 7.90 Log_{10} CFU/mL respectively, within 24 h. under the application of Eugenol-incorporated electrospun nanofiber mats containing 5.0%v/v of Eugenol while more than 10 Log_{10} CFU/mL of population increasing were observed under the control condition. From these results, it is expected that the Eugenol-incorporated edible electrospun chitosan/cellulose acetate/gelatin nanofiber demonstrated the antimicrobial properties and presented the possibility use in food relate application.

Keywords: Nanofibers, electrospinning, antimicrobial, Eugenol-incorporated, edible electrospun

Microbiological spotlights

P5.66

Reproducibility of MALDI-TOF MS for pathogen confirmation and identification of non-pathogenic bacterial isolates

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Background: MALDI-TOF MS is nowadays recognized as a method to identify microbial isolates. 4 inter-laboratory studies were recently run, in order to assess the reproducibility of the MALDI Biotyper (MBT) for pathogen confirmation and identification of non-pathogenic isolates. Different variable conditions were tested: instruments, operators, types of target plates, culture media.

Materials and methods: The inter-laboratory studies were designed according to the new ISO 16140-part 6 standard (DIS, 2017). 4 studies were conducted, 24 to 36 isolates were evaluated per study. Each set of strains consisted of 16 pathogens including *Salmonella* spp, *Listeria monocytogenes*, *Cronobacter* spp and *Campylobacter* spp, as well as 8 to 20 relevant non-pathogenic strains. Each study was organized by an independent laboratory: two studies were run in Europe, the two others in North America. 7 to 17 laboratories were involved depending on the study, with 14 to 17 collaborators. The MALDI Biotyper workflow was directly tested from isolates cultured on 2 to 3 selective culture media. 16 standard formulations and chromogenics were tested. A non-selective agar was always used as a quality control. Two types of targets were tested: the reusable MBT steel target and the disposable MBT 96 Biotarget. The appropriate ISO procedures were run in parallel to confirm the pathogen and identify the other isolates. The data showing cross-contaminations with the ISO protocols were excluded.

Results: 170 sets of data and 4652 identification results were used for interpretation. Isolated colonies were selected for analysis with the MBT. No impact of the selective culture media was observed, even with mCCDA containing charcoal that might decrease the quality of the generated mass spectra. All the collaborators were therefore able to handle the method properly. No influence of the tested target plates was noticed. 64 foodborne isolates were tested under reproducibility conditions, the MALDI Biotyper shows 100% correct confirmation rate of foodborne pathogens. 44 various non- pathogenic micro-organisms were as well tested, the MALDI Biotyper provided more than 99% correct identification rate at the group or species level.

Conclusion: The MALDI Biotyper provides reliable and reproducible results to confirm the pathogen after a first presumptive screening step, and to identify the other bacteria. These collaborative studies demonstrate that the technique is easily implemented in routine testing.

Keywords: MALDI-TOF MS, reproducibility, pathogen confirmation, isolate identification

Microbiological spotlights

P5.67

Structure-function relationship of antifungal hydroxy unsaturated fatty acids, their mode of action and potential application as food preservatives

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Pathogenic and spoilage fungi are a challenge to the food industry worldwide. Antifungal hydroxy unsaturated fatty acids (HUFA) are metabolites produced both by food-grade bacteria and by plants. Plants produce HUFA often as responses of defence; however, some plants also accumulate HUFA in their seed oils. HUFA compounds from various sources are structurally diverse and to date, knowledge is limited on their biological activities. This study therefore aimed to elucidate the structure-function relationship of HUFA compounds extracted from plant seed oils and bacteria fermentation. Their mode of action and the potential applications as antifungal agents in food were also evaluated.

Fatty acids mixtures containing 6 different HUFA species were extracted from *Lactobacillus* fermentations and plant seed oils, including *Coriaria nepalensis*, *Dimorphotheca sinuata*, *Mallotus philippensis* and *Thymus vulgaris*. HUFA were purified by high-speed counter-current chromatography (HSCCC) and the structure and purity of HUFA were analysed by LC-MS/MS. The minimum inhibitory concentration (MIC) of HUFA was tested against food-related fungi, including 1 human pathogen (*Candida albicans*), 5 spoilage fungi (*Pichia membranaefaciens*, *C. valida*, *Wickerhamomyces anomalus*, *Aspergillus niger* and *Penicillium roqueforti*) and 2 food fermentation strains (*Saccharomyces cerevisiae* and *C. humilis*). To investigate the mode of action, we compared the predicted fungal metabolites and the membrane compositions of HUFA-sensitive and HUFA-resistant fungal strains.

The presence of -OH group and C=C bonds in the center of an 18 carbon fatty acid contributed to HUFA antifungal activity (min. MIC=0.23±0.03g/L). Active antifungal HUFA specifically targeted filamentous fungi while yeasts were resistant. Yeast resistance to HUFA was not associated with metabolism of HUFA, such as to lactones. Most but not all fungi that were sensitive to HUFA had a lower ergosterol content in their cytoplasmic membrane when compared to the resistant yeasts. This indicates the possible interaction of HUFA with membranes and the alteration of the fluidity of membrane low in sterol.

In conclusion, this study elucidated structural factors of HUFA that relate to their antifungal activity and proposed mechanisms of fungal resistance to HUFA. This study contributes to the understanding of HUFA's biological significance, especially as self-defense compounds, and their potential use to complement antifungal preservatives in food.

Keywords: Food antifungal,hydroxy unsaturated fatty acid,structure-function relationship,plant seed oil,sterol

Microbiological spotlights

P5.68

Efficacy of mulberry leaf extracts for inhibition of pathogenic enteric bacteria

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Diverse communities of microbiota in human gastrointestinal tract play important roles in health benefit. Moreover, some pathogens can infect gastrointestinal tract and caused diseases. Although, bacterial infections can be treated with antibiotics, drug resistant pathogens are increasing. To mediate the problem of antibiotic resistance, plant extracts are alternative agents for treatment of bacterial infection. *Morus alba* L. or mulberry plant is mainly cultivated as silkworm food source. Therefore, the aims of this study were to investigate biological properties of mulberry leaf extracts against pathogenic enteric bacteria and anti-free radical activity. Mulberry leaves from two cultivars; Buriram and Sakon Nakhon were extracted by distilled water, 95% ethanol and methanol. Antibacterial activity of mulberry leaf extracts was tested against five pathogenic enteric bacteria; *Escherichia coli*, *Salmonella enterica* subsp. *enterica* serovar Typhi, *Shigella dysenteriae, Staphylococcus aureus* and *Vibrio cholerae* by agar well diffusion and broth dilution assay. The result showed that the methanolic extract of mulberry leaves; Buriram and Sakon Nakhon cultivars could inhibit all tested bacteria with diameters of inhibition zones ranging between 12.00 - 18.67 mm. Aqueous extract of mulberry leaves, Buriram cultivar showed the highest activity with minimum inhibitory concentration and minimum bactericidal concentration of 7.81 and 31.25 mg/ml against *Shi. dysenteriae.* In addition, ethanolic extract of mulberry leaves, Sakon Nakhon cultivar demonstrated the highest total phenolic content of 49.68 mg gallic/g extract. Ethanolic extract of mulberry leaves, Buriram cultivar showed the highest total flavonoid content of 1.46 mg quercetin/g extract. The anti-free radical activity also was measured by 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Ferric reducing antioxidant power (FRAP) methods. Aqueous extract of mulberry leaves, Buriram cultivar had the highest anti-free radical activity of 5.89 mg TEAC/g extract using ABTS method. Moreover, aqueous extract of mulberry leaves, Sakon Nakhon cultivar showed the highest anti-free radical activity of 8.63 mg gallic/g extract and 112.00 mg $FeSO_4$/g extract using DPPH and FRAP methods. Therefore, mulberry leaf extracts should be utilized as natural antibacterial and anti-free radical agents.

Keywords: Antibacterial activity, Anti-free radical, Mulberry leaves, Pathogenic enteric bacteria

Microbiological spotlights

P5.69

Systematic literature review of antimicrobial resistance in bacteria isolated from retail food as a prerequisite for consumer exposure assessment

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Antimicrobial resistance (AMR) in bacteria is an increasing health concern. The spread of AMR bacteria (AMRB) between animals and humans and the exchange of AMR genes requires holistic approaches for risk mitigation. The food chain contributes to the transmission of AMRB between animals, the environment and humans. The extent of AMRB exposure of humans via food is currently only poorly understood leaving an important gap for intervention design.
This study aimed to assess AMRB prevalence in retail food and subsequent exposure of Swiss consumers in a systematic literature review of data published between 1996 and 2016 covering the Swiss agriculture sector and relevant imported food.
Data from 314 out of 9'473 collected studies were extracted yielding 122'488 food samples and 38'371 bacteria isolates of which 30'092 samples and 8'800 isolates were AMR positive. A median AMRB prevalence of >50% was observed for meat and seafood harboring *Campylobacter, Enterococcus, Salmonella, E. coli, Listeria* and *Vibrio* spp. and to a lesser prevalence for milk products harboring starter culture bacteria. Predominant AMRB were found in descending order against tetracyclines, penicillins and macrolides. In combination with Swiss food consumption patterns, AMR exposures scores (min=0, max=2) of levels 1 (medium) and 2 (high) were calculated for *Campylobacter, Salmonella, E. coli, Staphylococcus* and *Enterococcus* in meat of pork, poultry and beef origin; *Vibrio, E. coli* and *Staphylococcus* in seafood; and *Enterococcus* and technologically important bacteria (incl. starters) in fermented or processed dairy products.
Transmission of AMRB in raw meat can be controlled through proper kitchen hygiene and cooking procedures. Food ready for consumption such as fermented meat and dairy products results, however, in direct AMRB transfer to the consumer yielding a different risk profile and adapted mitigation strategies. Furthermore, observed knowledge gaps particularly for AMR prevalence in dairy, plants, fermented meat and novel food products as well as the role of specific indicator bacteria (*Staphylococcus*, *Enterococcus*), starter culture bacteria and their genetic background in AMR gene transfer should be better integrated in systematic food-related AMR surveillance programs. Food seems to be a partially neglected vector to complement One Health-based AMR mitigation strategies.

Keywords: Antimicrobial resistance; retail foods; food safety; exposure assessment;

Microbiological spotlights

P5.70

Growth potential of *Listeria monocytogenes* 1 in twelve different types of RTE salads: Impact of food matrix, storage temperature and shelf life

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Listeriosis is a food borne disease associated with high hospitalization and fatality rates; in 2014, EU member states reported 2194 cases with 98.9% hospitalization rates and 210 fatalities. Proper risk analysis and the development of effective food safety strategies critically depend on the knowledge of the growth characteristics of *L. monocytogenes* on the product in question. Ready-to-eat (RTE) salads present a challenge in this context due to the absence of a heat treatment step before consumption and the interaction of pathogens with the plant microbial microbiota.

This study provides challenge-test based data of the growth characteristics of *L. monocytogenes* on twelve RTE salads. Three strains of *L. monocytogenes* (serotype 1/2a, 1/2b, 4b) used in this study were all isolated from a RTE salad production facility. The strains were cold adapted and used as strain pools for the challenge-test experiments.

A total of 12 different RTE salad products were used for the challenge tests. Three RTE salad products were packaged under modified atmosphere. To avoid sampling effects due to uneven distribution of *L. monocytogenes* within a package, the whole content of a bag was used for analysis. For this purpose, 30 g salad portions were produced specifically for this study. The packaging foil and, where appropriate, the modified atmosphere was identical to the larger packages that were produced for retail. The RTE salad products were stored at 5 °C and 8 °C, for 4, 5, 6, 7 and 8 days. Temperature in both cold rooms was continuously controlled and recorded with temperature loggers.

The food matrix, storage time and storage temperature were factors with a significant impact on the growth of *L. monocytogenes*. While most tested salads permitted a significant increase of *L. monocytogenes* in at least one of the tested conditions, no growth was observed on celeriac, carrot and corn salad products. There was a considerable increase in growth at 8 °C compared to 5 °C.

Keywords: Listeria monocytogenes; challenge test; cold growth; salad; RTE

Microbiological spotlights

P5.71

The effect of slow cooking on survival of potentially pathogenic *Escherichia coli* O157: H7 and *Clostridium perfringens* in meat

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Foodborne pathogens are the main concern for public health and can be present in hazardous level in meat. Therefore, inadequate cooking of meat may lead to survival of vegetative pathogens, such as *Escherichia coli* O157:H7 and *Clostridium perfringens* resulting in unsafe meat. Recently, slow cooking of meat has gained popularity as using low temperature over prolonged time enhance organoleptic properties and preserve nutritional content. However, due to reduced temperatures, slow cooking could present food-safety risks in case the thermal processes are insufficient to achieve safe meat, especially pathogens that are heat resistant. Also, using a strong acid-based marinade (e.g. vinegar) to tenderize the meat is a common practice, however, if meat is contaminated, this can induce cross-stress protection in bacteria and therefore may become resistant to cooking temperatures. *E. coli* and *C. perfringens* are a major cause of food poisoning outbreaks involving meat and meat products, thus it is critical to examine their survival after slow cooking method which is applied by caterers. The aim of this study was to test thermal inactivation of *E. coli* K-12 and *C. perfringens* cultures at temperatures used by caterers (48 and 53°C) to compare with standard process at 55 and 60°C. Flow cytometry was used to detect viable but nonculturable (VBNC) bacteria that can form under stressful conditions and are undetectable by conventional plating methods. The survival of *E. coli* and *C. perfringens* with or without pH stress adaptation in BHI broth and fresh mincemeat after thermal treatment at 48, 53, 55, 60 and 70°C for 6 hours was assessed by plate counts and flow cytometric analysis staining with DiBAC4(3) and propidium iodide (PI) for injured and dead cell quantification, respectively. At 60°C the viability of bacteria was reduced sharply after an hour but remained constant until the end. At 53 and 55°C the viability of bacteria was reduced sharply (5-log reduction) after an hour followed by fluctuations in viability from no growth and up to 1-4-log increase at time points during cooking. Also, at 48°C the viability of bacteria was not affected and remained constant throughout cooking. These results indicate that slow cooking at 48, 53, 55 and 60°C does not suppress the growth of bacteria which was shown to be present in high amounts that can be pathogenic during or at the end of cooking.

Keywords: Meat Safety, Slow Cooking, Flow Cytometry, Escherichia coli, Clostridium perfringens

Microbiological spotlights

P5.72

Anti-ochratoxigenic activity of phenyllactic acid on *A. carbonarius* grape isolates

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Aspergillus carbonarius stands among the dominant toxigenic producers in a
variety of foodstuffs, including grape berries, producing usually high amounts of Ochratoxin A (OTA). In this work the fungicidal and anti-ochratoxigenic effect of phenyllactic acid (PLA) was investigated against two high OTA producer grape isolates of *A. carbonarius*. The effect of 2 different concentrations of PLA (5 mg and 10 mg) was determined *in vitro* by evaluating inhibition of growth and toxin production on MEA solid cultures at 25°C. Mycelium dry weight was used to determine fungal kinetics (growth rate and lag phase) along with OTA production after 7 days of acid exposure.
A clear growth rate reduction of 22.6% and 39.13% was observed for each fungal isolate at 5 mg PLA. Higher reduction was presented at 10 mg of PLA reaching 39.1% and 63.3% for the two *A. carbonarius* isolates, respectively. Regarding lag phase, an increase was revealed for all PLA concentrations studied. Specifically, 25.4% and 11.2% increase in fungal lag phase was observed at 5 mg and 32.7% and 31.7% at 10 mg of PLA, respectively. HPLC analysis of OTA revealed inter-strain differences among grape isolates and the efficacy of PLA for toxin reduction. OTA production was decreased by 52.9% and 3.9% at 5 mg of PLA and 85.1% and 68.4% for 10 mg of PLA.
Our results, highlighting the presence of intra-spesific variability, report that PLA can successfully result in growth inhibition and OTA reduction of *A. carbonarius*.

Keywords: fungi, toxins, grapes, Aspergillus carbonarius, phenyllactic acid

Microbiological spotlights

P5.73

Applications of antimicrobial substances effective against fresh-cut produce contamination

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Fresh-cut produce market has grown rapidly in recent years because of demand for convenient and healthy vegetables. Fresh-cut products are especially vulnerable microbial attack because of tissue damage during processing such as cutting and peeling. Damaged tissues could harbor contaminants including spoilage bacteria and foodborne pathogens. Therefore, control of pathogens is important for quality and food-safety of fresh-cut produce during entire process, from farm to table. As a sanitizer, chlorine has been used to decontaminate fresh produce. However, because of increasing concern about food safety, natural products such as bacteriocins and bacteriophages have been suggested as alternative antimicrobial substances. In this study, the carocin D (bacteriocin) and PP2 (bacteriophage) were used as natural antimicrobial substance to control *Pectobacterium carotovorum*. Three kinds of antimicrobial substance isolated from each different CNS (Coagulase-Negative Staphylococci) were also used as natural products to control *Staphylococcus aureus* including MRSA. Antimicrobial substances were treated at different steps and different methods: spraying at pre-harvest step, washing at post-harvest processing step, and aerosol spraying at transportation and selling step. Spraying carocin D and PP2 at pre-harvest step prevented soft rot in lettuce over three days. Viable cell number of *P. carotovorum* was decreased over 3 log CFU/g after treatment of carocin D and PP2 at post-harvest washing step. Aerosol spraying of carocin D and PP2 with humidifier reduced the viable cell number of *P. carotovorum* and prevented spoilage of fresh produce. Mixture of 3 antimicrobial substances isolated from *S. pasteuri, S. epidermidis* and *S. gallinarium* were also tested at application steps. Spraying mixture of antimicrobial substances at pre-harvest step reduced number of *S. aureus* about 2 log CFU/g on lettuce. Viable cell number of *S. aureus* was decreased over 2 log CFU/g after treatment of the mixture at post-harvest washing step. Aerosol spraying of the mixture with humidifier reduced the number of *S. aureus* over 2 log CFU/g on lettuce. These results indicate that mixture of antimicrobial substances used in this study could contribute to control quality of fresh-cut produce in the consequence of preventing growth of pathogenic bacteria.

Keywords: Fresh-cut produce, decontamination, biological control, antimirobial substance, pathogens

Microbiological spotlights

P5.74

Contamination characteristics of *Listeria monocytogenes* on radish sprouts during seed germination

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Nowadays, sprouts consumption are popular because of the essential nutritional contain such as phenolic compounds, vitamins, and antioxidants. However, Foodborne pathogenic bacteria can easily contaminate on sprout due to the optimum environment during seed germination. The aim of this study was evaluated the characteristic of *Listeria monocytogenes* contamination on Radish sprout. The sprout germination in different conditions were simulated. Soil, water and seed were contaminated with *L. monocytogenes* at three levels (Non-contamination, 7.00 Log_{10} CFU/g and 4.00 Log_{10} CFU/g). The germination simulated model of all treatment was performed in a acrylic box. All samples were harvests after 5 days. Microflora and *L. monocytogenes* was determined. In the natural sprouting model, the number of total bacteria was higher than the other treatments with significantly different ($p \leq 0.05$). In The contamination model, the results showed that the population of *L. monocytogenes* at high level of contamination in water was higher than others, but there was no clear difference ($p \leq 0.05$). For the sterile one, the amount of bacteria count was the lowest among all treatments with significant differences ($p \leq 0.05$). For germination rate of Radish sprout in all treatment, no significant differences ($p \leq 0.05$) was observed around 80%. The contamination distances of *L. monocytogenes* on Radish sprout w0ere also determined. The sprout at the average range for 10 cm. in height over the root was cut into a piece as 1 cm. Several parts of sprout were analyzed for the population of microorganism. The results showed that, the number of bacteria on each section of sprouts in control treatment was higher than sterile treatment and at the distance for 1 cm. over the root presents the highest number of bacterial contamination. However, there was no significant difference were observed in the number of bacteria on cotyledon. In the combination treatments (soil, water and seed were contaminated at different level), the characteristics of contamination of *L. monocytogenes* was similar to control treatment. The number *of L. monocytogenes* was around 3.00 - 5.00 Log_{10} CFU/g on the stalk and 5.00 - 6.00 Log_{10} CFU/g on cotyledon. This study can conclude that the microorganism can contaminate on sprouts during cultivation despite under control environment. The contamination was resulted from the microorganism contaminated in air. In addition, the contamination can find in all parts of sprouts.

Keywords: Listeria monocytogenes, Radish sprouts, contamination, germination model

Microbiological spotlights

P5.75

Whole genome sequencing and PFGE analyses of *Listeria monocytogenes* 1/2b and 4b serotypes strains isolated from ready-to-eat (RTE) food and food processing plants

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Listeriosis is one of the most common foodborne zoonoses caused the ubiquitous Gram-positive bacteria, *Listeria monocytogenes.* In this study, 36 strains from collection National Veterinary Research Institute in Poland were selected. The strains were isolated form RTE food of animal origin (raw or heat-treated) and from the meat processing environment. All isolates belonged to serotypes 1/2b (n=14) and 4b (n=22).

PFGE was run according to the European Union Reference Laboratory for *L. monocytogenes* protocol. The strains were clustered in 5 groups with the similarity higher than 82%. DNA for the Whole Genome Sequencing analysis was isolated with the commercial Genomic Mini isolation kit with modifications. All probes were sequenced by the Illumina MiSeq technique and analysed by online tools (CGE, BIGSdb-Lm, BLAST). *L. monocytogenes* of serotype 1/2b were classified to ST3 (3 strains), ST5 (10 isolates) and ST191 (1 strain) sequence types whereas strains of serotype 4b were of ST1 (4 isolates), ST2 (8 strains), ST6 (4 isolates) and ST145 (6 strains). A total of 65 virulence factor genes were examined, including pathogenicity islands (PAIs). The PAI LIPI-1 with *prfA*, *plcA*, *hly*, *mpl*, *actA*, *plcB* virulence genes was identified in 36 isolates. Pathogenicity island LIPI-3 with *llsA, llsB, llsD, llsG, llsH, llsP*, *llsX*, *llsY* genes was detected in 12 strains which belonged to ST1, ST3, ST6 and ST191. In none of the tested *L. monocytogenes* LIPI-4 pathogenicity island was present. Identification of the internalin genes reveled that *inlA*, *inlB*, *inlC*, *inlE*, *inlF*, *inlH*, *inlK* markers were detected in the strains whereas the *inlG*, gene was found only in 4 isolates. There were 7 *L. monocytogenes* with premature stop codon in the *inlA* gene and all of them were of 1/2b serotype and ST5. Presence of antibiotic resistance genes was also examined and the *fosX*, *lmo*0919, *sul*, *lmo*0441, and *norB* markers were demonstrated in all of the tested strains, while the *aacA4* gene was present in 25% of strains.

It can be concluded that *L. monocytogenes* strains belonging to serotypes 1/2b and 4b, isolated from RTE food and production sites may be a potential risk for public health.

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Keywords: Listeria monocytogenes, RTE, food, meat, NGS, PFGE, virulence, antimicrobial resistance

Microbiological spotlights

P5.76

Escherichia coli antibiotic resistance patterns in livestock and wildlife farms in South Africa

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Antibiotic resistance among wild animals is a growing public health issue, due to increased wildlife contact between humans, livestock and other animals, as well as transmission through the food chain and the rising trend of consumption of game meat. The aim of this study was to determine the levels of antibiotic resistance of livestock and wildlife on different types of extensive farms in South Africa. Fecal samples were collected from *Taurotragus oryx* (n=25), *Connochaetes taurinus* (n=25), *Connochaetes gnou* (n=25), *Damaliscus pygargus phillipsi* (n=25), *Dama dama* (n=25), *Antidorcas marsupialis* (n=25), *Bos taurus* (n=50) and *Ovis aries* (n=75) from five different farms in South Africa. The Kirby-Bauer disk diffusion method was used to determine antibiotic resistance of *Escherichia coli* to ampicillin, chloramphenicol, sulphonamide, tetracycline, streptomycin and nalixidic acid. Overall, the *E. coli* isolates were 32% (livestock) and 41% (wildlife) non- susceptible to the antibiotics. More specifically, *E. coli* antibiotic resistance was the highest towards sulphonamide (40%), streptomycin (28%) and tetracycline (9%). The wildlife had an average resistance significantly ($p \leq 0.05$) higher than the livestock species. Free range wildlife had lower levels of antibiotic resistance than those receiving a commercial diet on a regular basis. Polymerase chain reaction was used to detect the resistant genes *sul*2 (n=60), *bla*TEM (n=8) and *tet*B (n=63). The resistant genes were detected in 12% (*sul*2), 83% (*bla*TEM), 40% (*tet*B) of the phenotypically resistant isolates. It is speculated that these resistant genes are picked up from the soil and the surrounding environment and are spread throughout the ecosystem by the animals as well as by other natural vectors. Wild animals can be considered as a reservoir of antibiotic resistance genes, providing a vector for the transfer of resistance genes to other species, environments, and even humans via the food chain. Antibiotic resistant zoonotic food-borne pathogens is now considered by the World Health Organisation to be a major global health challenge. Antibiotic resistant cases in the farming industry are commonly documented in intensive animal production. However, there is limited research on antibiotic resistance of extensively produced food animals and wildlife in South Africa.

Keywords: Antibiotic resistance, wildlife, livestock, Escherichia coli, animal production

Microbiological spotlights

P5.77

Comparative trials of two half fraser products for detection of *Listeria monocytogenes*

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Food labs are more and more interested in time and cost saving aspects. Due to these aspects ready-to-use culture media become more popular. These media are often pre-weighed, dehydrated and irradiated. To avoid autoclaving a gamma-irradiation at 10-20 kGy is necessary. In this study growth promotion and stability of Half Fraser broth of dried culture media prepared by autoclaving was compared to a granulated ready-to-use and gamma-irradiated Half Fraser broth.
To demonstrate that irradiation has no influence on selectivity, stability and growth promotion of *Listeria (L.) monocytogenes* gamma-irradiated, not autoclaved Readybag® Half Fraser broth (Merck KGaA) and autoclaved GranuCult™ Half Fraser broth (Merck KGaA) were used in a comparative study. To demonstrate that different food matrices do not influence the Half Fraser broth, the study was done with the high prevalence food: prawns, cheese and raw sausages.
Examination of *L. monocytogenes* was done according to DIN EN ISO 11290-1:2017. Growth promotion tests were performed with four *L. monocytogenes* strains with shortest (22h) and longest (26h) incubation time plus 72h cold room storage of pre-enrichment media. Two food matrices (prawns and cheese) were spiked at low levels with two *L. monocytogenes* food isolates. 141 raw sausages were examined in a field trial. For stability study Readybag® Half Fraser was stored up to 96 h at 5°C and 25°C and productivity and stability were tested at different time points.
All presumptive *L. monocytogenes* colonies were confirmed by using Singlepath® L'mono immunoassay (Merck KGaA).

Results: Readybag® Half Fraser and GranuCult™ Half Fraser broths provided similar growth rates with all four *L. monocytogenes* strains. Food trials at low level inoculation (1 cfu/25 g) and the field trial with a high prevalence but a low level of enumerable cfu showed comparable results, too. Stability tests showed that prepared Readybag® Half Fraser broth has an acceptable productivity and selectivity even with a storage up to 96 h at 5°C and 25°C.

Conclusion: Readybag® Half Fraser broth is comparable to GranuCult™ Half Fraser broth. There is no significant difference in growth promotion, food trials and in the field trial. Prepared Readybag® is storable up to 96h.

Keywords: Listeria monocytogenes Half Fraser Diagnostics Labs ready-to-use culture media gamma-irradiation

Microbiological spotlights

P5.78

Prevalence and pathogenic potential of *Arcobacter butzleri*

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Arcobacter (*A.*) *butzleri* are gram-negative, mobile, spiral-shaped bacteria that belong to the family *Campylobacteraceae* and classified as potentially pathogenic microorganisms. However, in animals *A. butzleri* rather regarded as a commensal. *Arcobacter* spp. have been detected in foodstuffs of animal origin and in water.

The most sporadic infections of humans caused by *Arcobacter* are accompanied by abdominal cramps, prolonged watery diarrhoea and fever. So far, the human incidence is unknown, since *Arcobacter* is not included as standard in routine diagnostics. However, some studies have shown that *Arcobacter* is the fourth most frequently detected pathogen in enteritis patients.

In the genome of *A. butzleri,* 10 genes with homologies to virulence factors of other bacteria were detected. Nevertheless, the pathogenicity mechanisms and potential virulence factors of *A. butzleri* still need to be verified.

We determined the prevalence of *Arcobacter* spp. in several retail samples, e.g. in chicken meat (27%), minced meat (12%), fish (34%), bivalves (17%), shrimps (9%), cephalopods (27%), RTE salads (13%) as well as in the environment, like water (30%) and sand (18%).

We could demonstrate adhesive, invasive and cytotoxic effects of *A. butzleri* on different cell lines *in vitro*. The pathogenic potential depends on both, the cell line used and the bacterial isolate. However, the occurrence of the 10 putative virulence genes could not be correlated to the pathogenic potential observed by our studies. The reduction of the intestinal barrier function observed *in vitro* could be the pathomechanism described for human symptoms. Our *in vivo* infection experiments demonstrated a stable colonization of *A. butzleri* in the ileum and jejunum as well as induction of an immune response in a gnotobiotic IL-$10^{-/-}$ mouse model. In order to better asses the importance of *A. butzleri* for humans, it is necessary to investigate the pathomechanisms of *A. butzleri* as well as the human incidence.

Keywords: Arcobacter, prevalence, pathogenicity

Microbiological spotlights

P5.79

Escherichia coli and *Staphylococcus aureus* antibiotic resistance patterns in livestock and wildlife farms in South Africa

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Antibiotic resistance among wild animals is a growing public health issue, due to increased wildlife contact between humans, livestock and other animals, as well as transmission through the food chain and the rising trend of consumption of game meat. The aim of this study was to determine the levels of antibiotic resistance of livestock and wildlife on different types of extensive farms in South Africa. Fecal samples were collected from *Taurotragus oryx* (n=25), *Connochaetes taurinus* (n=25), *Connochaetes gnou* (n=25), *Damaliscus pygargus phillipsi* (n=25), *Dama dama* (n=25), *Antidorcas marsupialis* (n=25), *Bos taurus* (n=50) and *Ovis aries* (n=75) from five different farms in South Africa. The Kirby-Bauer disk diffusion method was used to determine antibiotic resistance of *Escherichia coli* to ampicillin, chloramphenicol, sulphonamide, tetracycline, streptomycin and nalixidic acid; and *Staphylococcus aureus* to tetracycline, erythromycin, vancomycin, penicillin and oxacillin. *E. coli* antibiotic resistance was the highest towards sulphonamide (40%), streptomycin (28%) and tetracycline (9%). *S. aureus* antibiotic resistance was the highest towards oxacillin (97%) and pencillin (96%). Streptomycin and β-lactam antibiotics are commonly found in nature. The sulphonamides and tetracyclines are the oldest commercially available antibiotics, which have been used since the 1940s and 1950s, respectively. The wildlife had an average resistance higher than the livestock species. Free range wildlife and livestock had lower levels of antibiotic resistance than those receiving a commercial diet on a regular basis. Of the *E. coli* isolates, 36% were non- susceptible to the antibiotics and 60% of *S. aureus* isolates were non- susceptible to the antibiotics. Wild animals can be considered as a reservoir of antibiotic resistance genes, providing a vector for the transfer of resistance genes to other species, environments, and even humans via the food chain. Antibiotic resistant zoonotic food-borne pathogens is now considered by the World Health Organisation to be a major global health challenge. Antibiotic resistant cases in the farming industry are commonly documented in intensive animal production. However, there is limited research on antibiotic resistance of extensively produced food animals and wildlife in South Africa.

Keywords: Antibiotic resistance. wildlife, livestock, zoonotic, food-bourne, pathogens

Microbiological spotlights

P5.80

Recreation of pink in cheese with different strains of *Thermus*

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Background of the Study: Pink discoloration defect impacts a range of ripened cheeses, including Swiss, Cheddar, and Italian-type cheeses. The problem manifests either at the surface of the cheese block as a uniform pink border, or distributed sporadically within the cheese block. The pink colour does not occur in the cheese until it has been packaged, at least 2 months after manufacture and can lead to product downgrade. Potential losses due to pinking from companies analysed are estimated at between €7,000 and €20,000 per year for companies with minor issues and increase to millions of Euros for those more affected by the problem Although much international research has focused on trying to elucidate the causes of this defect, recent research within this group has focused on the presence of Thermus thermophilus and its potential to cause the defect.

Objective: Current work is focusing on the potential for different strains of Thermus to develop the pink defect.

Methodology: Four Swiss-type cheeses was prepared at pilot scale in vats, each containing 380 kgs of milk using thermophillic cultures (Streptococcus thermophilus and Lactobacillus helveticus) and with a standard make procedure. The first vat was manufactured as a control while one of 3 different strains of Thermus was added to the remaining vats. The cheeses were ripened at 10 °C for 10 days, 22 °C for 6 weeks and subsequently stored at 4 °C. Cheeses were analysed for composition, microbial counts, and levels of proteolysis, short-chain volatile acids, free amino acids and instrumental colour measurement for 145 day of ripening.

Results: A study to identify the capacity of different species of Thermus bacteria to produce pink in cheese was undertaken at pilot scale. Triplicate trials of Swiss cheese manufacture were undertaken using standard starter and secondary starter cultures along with different species of Thermus. No significant difference was noted between samples in Composition, for levels of proteolysis and for release of short chain volatile acids. Colorimeter analysis of ripening cheese samples showed significantly greater redness in cheeses manufactured with only one of the Thermus species. Conclusions were drawn from the trials that Thermus has a role in creating pink defect but other factors are also influential, and a specific set of conditions along with the presence of Thermus bacteria are required for this defect to occur.

Keywords: Cheese,Thermus,Pink Defect

Microbiological spotlights

P5.81

Occurrence of *Listeria monocytogenes* in raw sausages from craft manufacturing butcher stores

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Raw sausages are fermented meat products which were not cooked or subjected to other heat treatment. The microbiology stability is caused by a strict selection of raw materials and by aging of the sausages. During the process of aging the sausages were dried and the a_W-value sank. Based on fermentation by starter cultures the pH-value becomes more acid. The aged sausages become dryer, more acid and more stable against microbiological spoilage and outgrowth of pathogenic microorganisms.
Listeria (L.) monocytogenes is a ubiquitous pathogenic microbe which could cause severe illnesses, notably the YOPIS-group (young, old, pregnant, immunocompromised segments of the public) is at risk. Spreadable raw sausages like “Mettwurst” or “Teewurst” is often eaten by children and by older people.
141 raw sausages from craft manufacturing butcher stores in Hessen, a county of Germany, were examined for *L. monocytogenes* and furthermore for Enterobacteriaceae as hygienic parameter. pH- and a_W-measurement was recorded for every sausage.
The qualitative and quantitative examination of *L. monocytogenes* was done according to DIN EN ISO 11290:2005 using reduced incubation times for the Fraser broth according to DIN EN ISO 11290:2017. Confirmation of presumptive *L. monocytogenes* colonies was done with SinglePath® L’mono (Merck KGaA). The detection limit of the quantitative examination was reduced to 10 cfu/ml. Each raw sausage was examined in duplicate samples of 25g each.
Examination of Enterobacteriaceae was done according to ASU L 06.00-24:1987-11. Interpretation according to “Standard values for the orientation of microbial contamination in food” (Draft, Feb. 2017) from “Deutsche Gesellschaft für Hygiene und Mikrobiologie” (DGHM).

Results:

1. Qualitative results
   In 69 of 141 raw sausages (49%) *L. monocytogenes* could be enriched.
2. Quantitative results
   In 14 of these 69 positive samples a quantitative confirmation was possible. Two samples contained ≥100 cfu/g *L. monocytogenes*. 12 samples contained between 10-50 cfu/g.
   Counting was not possible in 55 cases, which were qualitative positive for *L. monocytogenes*.
3. Quantitative examination of Enterobacteriaceae
   13 samples (9,2%) contained more than 10^3 cfu/g Enterobacteriaceae

Conclusion: In almost every second raw sausage a qualitative confirmation of *L. monocytogenes* was possible, but less than 2% of the samples contained ≥100 cfu/g. Nevertheless, people belonging to the YOPIS-Group still should be careful with consuming raw sausages.

Keywords: Listeria monocytogenes raw sausages ready-to-eat

Microbiological spotlights

P5.82

Infrared spectroscopy and multispectral imaging as means of assessing the microbiological spoilage of minced pork stored under modified atmosphere packaging

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Rapid and non-invasive methods, including vibrational and imaging spectroscopies, have been increasingly studied for their utilization in food quality assessment. The objective of this work was the evaluation of Fourier transform infrared (FTIR) spectroscopy and multispectral imaging (MSI) as means of assessing the microbiological quality of minced pork. For this purpose, portions of minced pork were stored in modified atmosphere packaging (80% O_2-20% CO_2) under isothermal (4, 8 and 12°C) and dynamic temperature (periodic temperature changes from 4 to 12°C) conditions for a maximum time period of 15 days. At regular time intervals during storage, duplicate samples were subjected to (i) microbiological analysis for the determination of total viable counts (TVC); (ii) FTIR spectroscopy measurements; and (iii) MSI acquisition. Two independent experimental replicates were conducted and a total of 231 meat samples were analysed. The collected FTIR and MSI data were subjected to pre-processing, i.e. smoothing based on the Savitzky Golay algorithm and SNV transformation, respectively. Partial least squares discriminant analysis (PLS-DA) was employed for the designation of minced pork samples to three microbiological quality classes, based on sensory evaluation and the EU legislation on microbiological criteria: satisfactory quality for TVC lower than 5.7 logCFU/g; acceptable quality for TVC between 5.7 and 6.7 log CFU/g; and unacceptable quality for TVC higher than 6.7 log CFU/g. Calibration of PLS-DA models was performed using the data collected during storage at isothermal conditions (n=177), whereas the data corresponding to dynamic temperature storage were used for the purpose of model prediction (n=54). The PLS-DA models exhibited good performance, with the overall correct classification of FTIR data for calibration and prediction being 81.3 and 85.2%, respectively, while the corresponding values for the MSI data were 85.3 and 79.6%. According to the results of the present study, FTIR spectroscopy and MSI hold an important potential for the qualitative assessment of the microbiological spoilage of minced pork.
This work has been supported by the project “PhasmaFOOD”.

Keywords: Microbiological spoilage; Minced pork; FTIR; Multispectral imaging

Microbiological spotlights

P5.83

Effect of environmental factors on enterorotoxin D production by *Staphylococcus aureus*

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The staphylococcal enterotoxin (SE) production, as a result of previous growth of toxinogenic strains, is the most crucial problem which may lead to the staphylococcal food poisoning outbreaks in human. The study of environmental conditions effect on the growth of pathogen organisms is necessary to controlling and limiting their potential risk and evaluation of microbiological safety and quality of foods. Therefore, the aim of our work was to characterize environmental conditions under *S. aureus* isolate is able/unable to produce SED.

The effect of temperature (15, 18, 21, 25, 30 and 37 °C), water activity (in the range from 1.0 to 0.84) and pH value (6.0, 5.5, 5.0, 4.5 and 4.0 adjusted by lactic acid) was studied on the *S. aureus* 14733 growth and SED production. Also, the mutual effect of temperature, water activity (0.99 and 0.97), and the pH value (6.0 and 5.5) was described. Finally, also the effect of lactic acid bacteria of Fresco culture addition (in 3 or 6 log CFU/ml) on the *S. aureus* growth and SED production was described.

Based on the cultivation experiments performed in nutrient broth it can be concluded that the isolate was able to produce SED at 18 °C and a_w = 0.877, or at 21 °C and a_w = 0.899 and at 37 °C and a_w = 0.842. The SED was detected at 37 °C already after 6 h of incubation in all tested combination of pH values and water activity (even if the cell concentration was lower than 5 log CFU/ml) and in pH value of 5.0 when the concentration of cells was 3.3 log CFU/ml. During co-cultivation of 14733 isolate with Fresco culture, the SED was produced only after reaching *S. aureus* late stationary phase, however only if its concentration was higher than 5 log CFU/ml. The minimal starter culture addition needed to *S. aureus* growth and SEs production inhibition at temperatures related to raw milk cheese manufacture should be at least 4 log CFU/ml. Regarding EU Regulation No. 1441/2007, *S. aureus* 14733 was able to produce SED in 8.7 % of cases at cell concentrations lower than 4 log counts and in 21.7 % of cases at cell concentrations lower than 5 log counts.

The work was supported by VEGA project No. 1/00532/18 and contract APVV-15-006.

Keywords: enterotoxin D, environmental factors, predictive microbiology

Microbiological spotlights

P5.84

Prevalence, characterization and antibiotic resistance of *Listeria monocytogenes* isolated from raw goat milk and milk products

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Listeria monocytogenes is an important food-borne pathogen responsible for listeriosis. The consumption of goat milk and goat milk products has been growing and the data on the occurrence of *L. monocytogenes* in these products in Poland is limited.
The aim of the study was to assess the prevalence and to characterize
L. monocytogenes isolated from raw goat milk and ready for consumption dairy products in Poland.
A total of 404 samples were collected from 2014 to 2017 in dairy farms and in retail shops, including raw goat milk (297), different types of cheese (78) and goat milk powder (29). The presence of *L. monocytogenes* was analyzed using the ELFA method (VIDAS LMO2, bioMerieux) and the ISO 11290-2 standard. The species identification of the isolated strains was performed by biochemical tests and PCR, whereas *L. monocytogenes* was serotyped with multiplex PCR. Antimicrobial resistance of the isolates was determined using Minimal Inhibitory Concentration (MIC) test.
L. monocytogenes was detected in 29 of 404 (7.2%) samples using the ELFA method and the reference method confirmed the bacteria in 23 (5.7%) samples, including 19 (6.4%) raw milk and 4 (5.1%) cheese made from unpasteurized milk produced in small scale dairy farms samples, respectively. *L. monocytogenes* was not found in cheeses and goat milk powder from the retail trade. The confirmed *L. monocytogenes* were classified into 1/2a, 1/2b and 4b serotypes. Serogroup 1/2a (60.9%) was the most prevalent followed by serogroups 4b (34.8%) and 1/2b (4.3%). Antimicrobial resistance analysis showed that all *L. monocytogenes* strains were susceptible to most of the agents used in this study: ampicillin, erythromycin, gatifloxacin, gentamycin, levofloxacin, linezolid, penicillin, rifampin, streptomycin, tetracycline, trimethoprim/sulfamethoxazole, vancomycin, and quinupristin/dalfopristin. Resistance was observed only to oxacillin (82.6% isolates), ceftriaxone (78.3%), ciprofloxacin (13.0%) and clindamycin (8.7%).
The results obtained in this study indicate the presence of *L. monocytogenes* in raw goat milk and cheese made from unpasteurized goat milk. The consumption of raw milk and traditional dairy products produced in small scale dairy farms may pose a potential risk of infection caused by these bacteria. Therefore, *L. monocytogenes* as an important foodborne pathogen should be monitored.

Keywords: Listeria monocytogenes, antibiotic resistance, goat milk and milk products

Microbiological spotlights

P5.85

Characterization and antibacterial activity evaluation of newly isolated *Lactococcus lactis* strains intended to be used for technological and safety improvement of cheese

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The aim of the study was to characterize antibacterial activity, technological and safety features of newly isolated *Lactococcus lactis* bacteria that could be used for technological and safety improvement of cheese. *L. lactis* isolates (n=166) from goat and cow milk samples were identified to the species level and for the presence of nisin and lactococcin B production encoding genes by PCR. Three strains were found to harbour nisin encoding gene and one strain was found to harbour lactococcin B encoding gene. All strains were characterized for safety aspects evaluating hemolytic activity, harmful enzymes like β-glucosidase or β-glucuronidase activity and antibiotic resistance towards seven antibiotics tested like chloramphenicol, clindamycin, streptomycin, gentamicin, tetracycline, erythromycin and ampicillin. Strains that were found to produce hemolytic activity expressed α-hemolysis and strains that were found to be resistant to antibiotics expressed resistance to tetracycline.

Strains that were evaluated as safe were examined for antimicrobial activity and technological properties such as ability to rapidly produce acid, salt tolerance, diacetyl production and caseinolytic activity. Tested strains showed good antimicrobial activity against tested food spoilage and pathogenic bacteria including *Listeria monocytogenes*, *Escherichia coli*, *Brochotix thermosphacta* and others. Thirty strains showed high acidifying activity as were able to induce pH drop by more than 2 units after 24 h.

Selected strains were examined in the cheese matrix for evaluation of their ability to improve safety and technological characteristics of cheese.

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Keywords: Lactococcus lactis, cheese, safety, antibiotics, pathogenic bacteria

Microbiological spotlights

P5.86

Reduced enterotoxin D formation on boiled ham in *Staphylococcus aureus* Δagr mutant

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Staphylococcal food poisoning (SFP) is a common cause of foodborne illness worldwide, and enterotoxin D (SED) is one of the most frequent *Staphylococcus aureus* enterotoxins associated with it. It has been reported that the expression and formation of SED in *S. aureus* is regulated by the quorum sensing Agr system. In this study, the effect of *agr* deletion on *sed* expression in *S. aureus* grown on boiled ham was investigated. Growth, *sed* mRNA and SED protein levels in an *S. aureus* wild type strain and its isogenic Δ*agr* mutant were monitored for 14 days at 22°C. The results showed that although deletion of the *agr* gene did not affect the growth rate or maximum cell density of *S. aureus* on boiled ham, it had a pronounced effect on SED formation during the first 5 days of incubation. The SED concentration was not reflected in the amount of preceding *sed* transcripts, suggesting that *sed* transcription levels may not always reflect SED formation. The expression of RNAIII transcript, the regulatory signal of the Agr system, was also monitored. Similar transcription patterns were observed for RNAIII and *sed*. Surprisingly, in the Δ*agr* mutant, *sed* expression was comparable to that in the wild type strain, and was thus unaffected by deletion of the Agr system. These results demonstrate that the Agr system appears to only partially affect SED formation, even in a real food environment.

Keywords: Staphylococcus aureus; staphylococcal food poisoning; SED formation; Agr quorum sensing system

Microbiological spotlights

P5.87

Spectroscopy-based sensors under a unified feature selection approach for microbial contamination and storage time prediction of ready-to-eat rocket

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The objective of the present study was the comparative evaluation of spectroscopy-based sensors, from visual to infrared, for the non-invasive estimation of microbial contamination and storage time (i.e. time-on-shelf) of ready-to-eat rocket salad, through the development and implementation of a unified feature selection approach. For this purpose, rocket salads were stored aerobically in their original packages under isothermal (4, 8 and 12°C) and dynamic temperature conditions for a maximum time period of 11 days. At regular time intervals during storage, duplicate rocket samples were subjected to (i) microbiological analysis for the determination of total viable counts; and (ii) Fourier transform infrared (FTIR), near infrared (NIR) and visible (VIS) spectroscopy measurements. Two independent experimental replicates were conducted and a total of 232 rocket samples were analysed. The acquired spectra were normalized under the SNV normalization scheme. A feature selection step was then introduced on the basis of random forest (RF) regression ensemble, followed by partial least squares (PLS) regression for microbial contamination and storage time estimation using the features (wavelengths/wavenumbers) selected by the RF ensemble. The data corresponding to isothermal and dynamic temperature storage were used as training and external test set, respectively. The number of selected features for FTIR, NIR and VIS spectral data were 94, 92 and 79, respectively. With regard to PLS regression model performance, FTIR spectroscopy appeared to outperform NIR and VIS spectroscopies in the estimation of both microbial contamination and storage time, with the coefficient of determination (R^2) value being 0.511 and 0.958, respectively. Since the final reduced sets of features are considered de-correlated with minimum redundant information, regression is more robust than using the full sets of features. In addition, the creation/usage of limited size features sets could be very useful in food-specific and low-cost sensor development.
This work has been supported by the project “PhasmaFOOD”.

Keywords: Feature selection; Microbial contamination; Rocket; Spectroscopy; Storage time

Microbiological spotlights

P5.88

Characterization of *Bacillus cereus* group isolates from powdered food products

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Mashed potato powder as well as powdered infant formula (PIF) are frequently contaminated with *Bacillus cereus sensu lato (B. cereus s.l.)*, mainly with its spores. These products have also been implicated in foodborne illnesses. Here, we characterized *B. cereus s.l.* isolates originating from powdered products based on sporulation assays, toxin gene profiling, *panC,* and *spoIIIAB* typing combined with a SplitsTree analysis. Furthermore, cytotoxicity assays with *B. cytotoxicus* isolates were performed. A total of 78% of PIFs tested positive for *B. cereus s.l.*, whereas 92% of all mashed potato powders were positive. In total, 43 isolates were further characterized. The *nhe* and *cytK2* genes were most frequently detected. Moreover, a cereulide-producer was detected from PIF. Most isolates were assigned to *panC* group III, but members of group II, IV, V, and VII could also be found. Nine *B. cytotoxicus* were isolated out of nine mashed potato powders. All of them were assigned to *panC* group VII. Cytotoxicity assays of these nine isolates revealed one highly cytotoxic strain, while all other isolates exhibited no detectable cytotoxicity, underpinning that cytotoxicity of a certain *B. cereus* group strain cannot be deduced from the sole presence or absence of toxin genes.

Keywords: Bacillus cytotoxicus; Bacillus cereus group; Vero cell assay; mashed potato; powdered infant formula

Microbiological spotlights

P5.90

High strain variability in stress tolerance of *Listeria monocytogenes*

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Listeria monocytogenes, a severe foodborne pathogen, causes life-threatening listeriosis. *L. monocytogenes* surmounts many harsh stress conditions routinely used in food-processing premises for controlling bacterial contamination. Genetic variants underlying the strain variability in growth of *L. monocytogenes* at stressful conditions are yet to be elucidated. To identify challenging contaminant strains in the food-processing chain, it is pivotal to learn the genetic mechanisms rendering certain *L. monocytogenes* strains particularly stress tolerant. We studied the growth diversity of 389 *L. monocytogenes* strains at cold (4°C), heat (42.5°C), and osmotic (9% NaCl) stress, and at acid (pH 5.6) and alkali (pH 9.0) conditions. The strains represented the most common human, food, and environmental serotypes 1/2a (n=244), 1/2b (n=36), 1/2c (n=33), 3a (n=5), and 4b (n=71). Growth parameters were computed using model-free splining. To classify growth patterns into discrete phenotypes, strains were hierarchically clustered using Ward's method. Significant differences were observed in growth parameters between clustered strains at all stresses (Kruskall-Wallis). Genomes of all strains were sequenced using Illumina HiSeq PE250 platform (aimed coverage of 100x). Sequence element enrichment (SEER), a genome-wide association study (GWAS) method using *kmers*, is harnessed to explore genetic variants, like single nucleotide polymorphisms (SNPs), genes, and mobile genetic elements, associated with particularly good or poor growth under given stresses. Serotype 4b strains proved to be fairly tolerant to all stresses. Serotype 1/2a presented high diversity in growth at temperature stresses and grew well at low pH but moderately to poorly in high salt concentration. Serotype 1/2b strains were fairly good growers at high temperature and pH conditions and in high salt concentration but only moderately tolerated low temperature. Serotype 1/2c strains grew well at 4°C, while growing poorer at other stresses. The results demonstrate that *L. monocytogenes* manifests markedly high strain diversity in stress tolerance and that some serotypes are associated with a certain type of stress tolerance pattern. GWAS analyses to identify genotypes potentially causal to differences in stress tolerance are in progress. Based on the GWAS results, diagnostic measures may be designed for identifying potentially tolerant and thus problematic *L. monocytogenes* strains in the food-processing environment.

Keywords: Listeria monocytogenes, stress tolerance, strain variability, GWAS

Microbiological spotlights

P5.91

Determining the recovery rates of *Mycobacterium avium* ssp. *paratuberculosis* from spiked milk by culture and qPCR*

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The notoriously slow growing *Mycobacterium avium ssp. paratuberculosis* (MAP) is the causative agent of Johne's disease - a chronic intestinal inflammation in ruminants. Infected animals shed MAP via feces and milk during the subclinical stage of disease. The reported detection of MAP in raw and pasteurized milk points to a potential exposure of the consumer by dairy products.
Due to its slow growth, the isolation and contamination-free culture of MAP from foods is complicated. It is also challenging to prepare the MAP DNA from biological samples. We therefore assayed the recovery rates of MAP from artificially contaminated milk and certified raw milk samples on HEYM agar-slants. A decontamination protocol was established to avoid an overgrowth by the accompanying bacterial flora. Furthermore, several isolation methods were tested to optimize the retrieval of MAP from spiked milk in conjunction with the subsequent DNA preparation. By the means of single copy gene *f57*-based, quantitative real-time PCR the loss of DNA during the sample preparation was determined and detection limits were assayed.
Based on the optimized decontamination protocol 10^2 MAP in 50 ml milk and certified raw milk were detectable on HEYM agar after 12 weeks of incubation. However, up to 20 % of the original inoculum was lost during the sample processing. Spiking with higher amounts of MAP (> 10^5 per 50 ml) resulted in occasional overgrowth by the accompanying bacterial flora despite a rigorous decontamination. The DNA blood and tissue Kit® with a slightly modified lysis procedure was best suited to extract MAP DNA from M7H9 culture resulting in 70 % recovery. In contrast, different pre-treatments of spiked milk prior to DNA isolation revealed a loss of MAP input of up to 95 %. As a result thePCR detection limit was 1 x10^3 MAP genome equivalents per 50 ml. Thus, the classical microbiological detection of MAP in milk is still superior over PCR detection. This phenomenon is mainly caused by the massive depletion of MAP during the pre-treatment of milk.
In 40 milk and certified raw milk samples from retail MAP could neither be detected by culture nor by PCR as established in the present study.
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Keywords: Mycobacterium avium ssp. paratuberculosis, milk, HEYM agar, PCR

Microbiological spotlights

P5.92

Microbiological quality of raw milk sold as farm-gate milk from raw milk vending machines

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Following § 17 of the German Tier-LMHV and § 5, subparagraph 1 of the Austrian raw milk Regulation respectively raw milk may only be sold to consumers under specific conditions.
An increasing number of this farm-gate milk disposal is being distributed through farm-gate raw milk vending machines. Many consumers feel attracted to this offer and decide to buy raw milk from these milk filling stations. Although the vending machines need to carry an easily readable sign saying “heat raw milk before consumption”, it cannot be excluded that several consumers in fact drink the potentially with zoonotic pathogens contaminated raw milk without previous heating.
According to a BfR (German Federal Institute for Risk Assessment, April 2016*) statement, there might be a connection between the increase in farm-gate raw milk vending machines and the parallel increase in the occurrence of diseases caused by Campylobacter.
In October 2015 a major Campylobacter onset was reported in Germany, Lower Saxony, where 100 people fell sick and an epidemiological link could be made to farm-gate raw milk from a vending machine.
Hygiene parameters were examined parallel to the pathogen analyses, as problems with cooling as well cleaning and disinfection can most likely occur in the raw milk vending machines.
A project initiated by Lower Saxony called “microbiological status of raw milk from farm-gate raw milk vending machines” was realized within the frame of the German BÜp 2016 - a nationwide monitoring.
The poster presents the all-German data of the BÜp as well as the data from Lower Saxony and Austria for 2017. In summary it can be stated that in 9% of the German samples and in 4% of the Austrian samples pathogens could be detected.
* Stellungnahme Nr. 008/2016 des BfR vom 13. April 2016
„Rohmilch: Abkochen schützt vor Infektion mit Campylobacter“

Keywords: raw milk vending machine, pathogens, hygiene

Microbiological spotlights

P5.93

Effect of food-related stress conditions and loss of *agr* and *sigB* on *seb* promoter activity in S. *aureus*

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Staphylococcal enterotoxin B (SEB) causes staphylococcal food poisoning and is produced in up to ten times higher quantities than other major enterotoxins. While *Staphylococcus aureus* growth is often repressed by competing flora, the organism exhibits a decisive growth advantage under some stress conditions. So far, data on the influence of food-related stressors and regulatory mutations on *seb* expression is limited and largely based on laboratory strains, which were later reported to harbor mutations. Therefore, the aim of this study was to investigate the influence of stress and regulatory mutations on *seb* promoter activity. To this end, transcriptional fusions were created in two strains, USA300 and HG003, carrying different *seb* upstream sequences fused to a *blaZ* reporter. NaCl, nitrite, and glucose stress led to significantly decreased *seb* promoter activity, while lactic acid stress resulted in significantly increased *seb* promoter activity. Loss of *agr* decreased *seb* promoter activity and loss of *sigB* increased promoter activity, with the magnitude of change depending on the strain. These results demonstrate that mild stress conditions encountered during food production and preservation can induce significant changes in *seb* promoter activity.

Keywords: Staphylococcus aureus, staphylococcal enterotoxin B, stress response, agr, sigB

Microbiological spotlights

P5.94

Monitoring the effect of food spoilage bacteria on *Salmonella* Enteritidis biofilm formation

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Salmonella has been associated with major foodborne outbreaks, while *Salmonella enterica* ser. Enteritidis is a major pathogen associated with poultry and egg processing industry. The importance of *Salmonella* for food industry has been linked to its ability to form biofilms, however the presence of other species and their effect on *Salmonella* biofilm formation should be considered. To this respect, the influence of food spoilage bacteria on the ability of *S.* Enteritidis to form biofilms on a stainless steel (SS) surfaces, was evaluated. *S.* Enteritidis was left to form biofilm in mono-cultures or co-cultures with meat spoilage bacteria on SS immerged in TSB at 20°C for 6 days. Specifically, spoilage bacteria that belong to the genera *Leuconostoc*, *Lactobacillus*, *Serratia*, *Citrobacter*, *Hafnia*, *Proteus*, *Pseudomonas* and *Brochothrix* were included in this study**.** Biofilm population was enumerated by bead vortexing-plate counting method.
According to the obtained results, all microorganisms tested were able to produce biofilm on SS coupons after 6 days incubation at 20°C, while biofilm formation seemed to be influenced by the bacterial species and/or strains. In particular, *S.* Enteritidis reached biofilm population of approximately 5.2 log CFU/cm^2. High levels of biofilm population were enumerated for the most of the rest bacteria tested except one of *Br. thermosphacta* strain (less than 3 log CFU/cm^2). Dual species conditions seemed to affect *S.* Enteritidis biofilm formation in most of the cases. In brief, when *S*. Enteritidis co-cultured with a *Br. thermosphacta* strain, *S. liquefaciens*, *H. alvei*, and *P. vulgaris*, a slight reduction in the number of *S*. Enteritidis sessile cells (approximately 0.5 log CFU/cm^2) was observed compared to mono-cultures studies. In addition, the number of *S*. Enteritidis sessile cells was increased (approximately 0.5 log CFU/cm^2) when the pathogen was co-cultured with *C. freundii* and *Ps. fragi*. This research expands our knowledge on the physiology of multi-species biofilms formed by food relevant microorganisms under food related conditions.

Keywords: Salmonella, biofilm, food spoilage bacteria, co-cultures

Microbiological spotlights

P5.95

Detection and characterization of *Clostridium difficile* in German retail food products

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Clostridium (C.) difficile is a strictly anaerobic spore-former and well-known cause of healthcare-associated infections. Hospitalized, elderly people, especially those who have comorbidities, represent the classical risk population. Consequently, efforts have been made with focus on health care procedures and prevention measures to reduce outbreaks and prevalence rates in humans. In contrast to this, in recent years an increasing number of publications report that up to one third of hospitalized cases of *C. difficile* infections (CDI) are community-acquired. These cases often differ from the classical risk population as even younger people are affected or infections are independent from an antibiotic treatment. Infection sources and risk factors are rarely known, but zoonotic transmissions have been suspected based on phylogenetic relationships between human and animal isolates. Furthermore, *C. difficile* has been isolated from different food products worldwide but prevalence data for Germany are still missing.

To estimate the risk of foodborne colonization of humans with *C. difficile*, we established and validated a detection method for *C. difficile* spores in food. The method enables to screen food samples using real-time PCR directly from the enrichment culture and isolate *C. difficile* from PCR-positive samples. Artificial contamination studies with salad and pork meat proved this method to be highly sensitive and specific. The detection limit was sufficient to detect even contaminations below one spore/gramm food. From 2016 to 2018, we analyzed animal (pork ground meat and chicken meat) and non-animal food products (leaf salads) from German retail. The results ranged from low but apparent *C. difficile* contamination rates in leaf salads (3%) to higher rates in chicken meat (15%). Most of the isolates were toxigenic strains carrying *tcdA* and *tcdB* genes, but also binary toxin gene *cdtA/B*-positive RT078 strains have been found. Further characterization resulted in PCR-ribotypes which are well-known for human CDI in Europe (e.g. RT002, RT014).

In conclusion, this is the first description of *C. difficile* contamination of food products in Germany. Characterization of these isolates reveals their similarity with endemic hospital-associated *C. difficile* strains and point towards their potential to induce a toxin-mediated diarrhea in humans. Further investigations have to be implemented to elucidate transmission pathways and the resulting relevance for human CDI.

Keywords: Clostridium difficile, food, prevalence, Germany

Microbiological spotlights

P5.96

Formation of modified ocratoxin in wine grapes in different maturation stages

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Differences in physico-chemical parameters of grapes varieties and their changes during grape maturation has a direct influence on the properties of wines. In addition, these differences may also impact on the growth and metabolite production by fungal species. In wine grapes, the fungi of higher incidence and responsible for the production of ochratoxin A are *A. carbonarius* and *A. niger*. In addition to the parent mycotoxin (ochratoxin A), the formation of modified forms of this mycotoxin can take place due to the defence mechanisms of the plant or as well as due to fungi metabolism. Modified mycotoxins are metabolites that normally remain undetected during conventional analysis for the parent toxin. In this sense, this study aims to evaluate the influence of grape variety and their maturation stage on the formation of modified ochratoxin. A conidia suspension of *A. niger* (10443) was inoculated on grapes of three varieties (Syrah, Touriga Nacional and Muscat Italia), at different maturation stages: veraison, 15 days after veraison begins and harvest. A mass spectrometer ESI-LTQ-XL Orbitrap Discovery was used for the detection of modified ochratoxin. Data was acquired in the survey scan mode and were obtained in positive mode using mass range of 200-750 m/z, five times. The multivariate regression method, partial least squares-discriminant analysis (PLS-DA), was used as a tool to identify modified ochratoxin through their exact mass, with a maximum error of 2 ppm. Among the targets sought, we identified 14-de-carboxy-ochratoxin A ($[M + Na]^+$: 382.0815) in the Muscat Italia variety at the maturing stages: veraison and 15 days after veraison begins; and ethylamide-ochratoxin A ($[M + K]^+$: 469.0939) in the Syrah variety at the harvest. These results indicate that modified ochratoxin can be formed depending on the grape variety and maturation stage. The findings of this study are of great relevance in the context of risk analysis and in the adoption of suitable agricultural practices to prevent both the presence of Ochratoxin A and the formation of modified mycotoxins. The presence of these modified forms of ochratoxin raises concerns regarding their potential toxicity and further possibility of underreporting.

Keywords: Conjugated. Masked mycotoxin. Mass spectrometry. Risk analysis.

Microbiological spotlights

P5.97

Whole genome MLST as a tool to screen for potential outbreaks quickly and easily, applied to publicly available *Listeria monocytogenes* isolates

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Listeria monocytogenes (Lmo) is an important foodborne pathogen, especially in pregnant patients, neonates, elderly or immuno-compromised individuals. Following considerable cost reductions, complete Lmo genome sequencing has dramatically increased the number of publically available genomes on the Sequence Read Archive (SRA) of NCBI. Rapid and automated processing of whole genome sequencing data ensures a reliable and easy to follow workflow in routine molecular surveillance, reducing the time needed to detect and contain an outbreak. Whole genome or core genome MLST (wgMLST and cgMLST) are particularly useful for this application, as the results are stable and comparable within a species and therefore suitable for compiling a database for outbreak screening.

In this study, we demonstrate that wgMLST can be used to screen for possible outbreaks quickly and without prior knowledge of possible epidemiogical links.

A database containing all isolates submitted to SRA (appr. 15,000) before June 2017 had been previously constructed, containing de novo assemblies and wgMLST allele IDs. Strains newly collected in 2018 (103 in total) were screened against this database. Inclusion criteria were within 20 core alleles difference of one of the query isolates and a core percentage of higher than 95%.

The primary analysis of all 103 isolates (de novo assembly and allele calling) was run overnight in less than 8 hours. The isolates with sufficient quality (101) were screened against the database in less than 3 minutes. A total of 931 hits were found. We observed 17 clonal complexes at a core allele distance of 20, the largest cluster contained 472 isolates, the smallest 2. For 6 query isolates, no matches were found. When investigating the largest cluster in more detail, we found that the oldest isolates were from 2000 and many of the isolates obtained from food had been found on some type of deli meat or fish. Several subclusters with a tighter temporal or geographical link were found. This can indicate a persistence of Lmo somewhere in the chain of these products and warrants further investigation into common sources and suppliers of these products.

We demonstrate that wgMLST is suitable for the detection of so called slow outbreaks that are the result of a persistence of Lmo within a food production environment with only rare contamination of the final product, resulting in spaced clinical cases. This makes it an appropriate technique for outbreak surveillance.

Keywords: whole genome MLST, outbreak, slow outbreak, surveillance

Microbiological spotlights

P5.98

Variability of growth and ochratoxin a production by *Aspergillus carbonarius* in wine grape-based culture medium

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A. carbonarius is one of the main contaminants of grapes, being responsible for the production of ochratoxin A (OTA) that can be found in wines and grape juices. The variability of growth and metabolites production are inherent to each microorganism, occurring even at similar conditions. In this sense, this study aims to determine the variability of growth and OTA production by *A. carbonarius* strains in wine grape-based culture medium. Five strains were inoculated in grape-based culture medium, following incubation at 25 °C for 21 days. The diameter of the colonies was measured daily basis. The growth data were adjusted to the Baranyi model. The OTA detection was performed by High Performance Liquid Chromatography. The experimental (E), biological (B) and strain (S) variabilities were evaluated for growth and OTA production. An excellent fit of Baranyi model for *A. carbonarius* strains was observed. Growth rate values ranged from 5.84 - 6.75mm.day^{-1}. The levels of OTA production ranged from 4.80 - 7.02µg/g on day 3; 21.46 - 29.52µg/g on day 6; 37.33 - 63.79µg/g on day 15 and 27.37 - 40.32µg/g on day 21.The lowest levels of OTA were observed at day 3 for all strains. The highest production of this mycotoxin was detected on day 15, and a decline on day 21 was observed. The reduction of ochratoxin A levels may be related to the degradation or modification of this toxin over time by the fungus itself. The strain and biological variabilities of the growth rate was similar and greater than the experimental variability (S = B > E). For OTA production, strain variability was higher over all evaluated days, except on day 3 (E = B = S). The existence of intra-species variability demonstrates the need for selection of representative strains for predictive studies, which can cover a greater range of conditions, considering their different kinetic behaviour. Such selection would allow the use of valuable information in risk analysis. Although mycotoxins do not always correlate directly with growth, the most effective control of the OTA presence in food is through the control of fungus growth.

Keywords: Risk assessment. Secondary metabolites. Mycotoxin detection.

Microbiological spotlights

P5.99

A noval mathematical model for studying synergism againt *Campylobacter jejuni*

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No single validated method for studying antimicrobial interactions is available. Over 60% of the dual antimicrobial studies used the FICI method and 36% applied the time-killing method during the past decade. Each method has advantages and disadvantages, and generates different outcomes that might not be comparable with each other. Most importantly, synergy between antimicrobials can be over- or under-estimated resulting in the misleading decision in its further research and development. Mathematical modeling can offer a better accuracy and be used not only to identify synergism, but also to evaluate interactions at different level of antimicrobial potency (MIC or MBC), which provides more precise and meaningful outcomes for the application, stress response, or mechanism studies of the synergistic combinations.

The aim of this study is to discover an antimicrobial synergistic effect against *Campylobacter jejuni*, a leading foodborne pathogen that causes human gastroenteritis, by cinnamon oil, encapsulated curcumin, and zinc oxide nanoparticles (ZnO NPs).

We compared three approaches to study the antimicrobial interactions including time-killing method, fractional inhibitory concentration index (FICI) method, and a mathematical concentration-effect model. Nonlinear isobologram analysis was performed to evaluate the synergy in different combinations, and a median-effect equation was applied to identify the combinations of synergistic effects at median, bacteriostatic and bactericidal reduction levels.

The time-killing method overestimated the synergistic interaction between antimicrobials while FICI method failed to detect an existing synergistic phenomenon. This lack of accuracy and sensitivity was manly due to combining antimicrobials based on their MICs or sub-MICs without comprehensive understanding of their concentration-effect curves. Our results showed that each targeted antimicrobial had a unique concentration-effect relationship. Specifically, encapsulated curcumin showed a sharp sigmoidal curve while cinnamon oil and ZnO NPs had a hyperbolic curve. A mathematical model was successfully constructed to study the interaction between antimicrobials with different shape of concentration-effect curve.

This novel mathematical model could accurately study antimicrobial interactions against different foodborne pathogens and provide an alternative method to develop new effective combinations.

Keywords: Synergism, FICI, isobologram, Median-effect equation, Food safety, Campylobacter jejuni

Microbiological spotlights

P5.100

Antifungal activity of *Saccharomyces cerevisiae* against different ochratoxin A producing *Aspergillus* spp. and *Penicillium verrucosum*

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Aspergillus and *Penicillium* genera include some of the main Ochratoxin A (OTA) producing species associated with food quality and safety. In cereals, dried fruits, etc, these fungi are common members of the indigenous microbiota able to cause spoilage or produce mycotoxins such as Ochratoxin A depending on species characteristics. The aim of the present study was to assess the potential of *Saccharomyces cerevisiae* Y33 as a bio-control agent against fungal growth and toxin production. Competition experiments were undertaken using 15 different fungal species belonging to *Aspergillus* and *Penicillium* genera. Yeast and fungal purity was checked on Yeast Malt Agar (YMA) and Malt Extract Agar (MEA) cultured at 25°C for 2 and 7 days, respectively. *S. cerevisiae* was inoculated by spreading on MEA Petri dishes (*ca.* 10^5 CFU/mL) and fungal species were spot inoculated at *ca.* 10^5 conidia/mL. Kinetic parameters were estimated using the primary model of Baranyi and Roberts. Control plates (i.e., fungal growth in the absence of yeasts) were also inoculated in every case. The plates were incubated at 25°C for 14 days in the dark. Samples for OTA detection were taken at 7 and 14 days and determined by HPLC.
Results showed that for *Aspergillus carbonarius* F71, *Aspergillus tubigensis* F31, *Aspergillus niger* F157, and *Aspergillus ochraceus* F33, the growth rate was estimated to 7.3, 6.1, 12.1, and 9.9 cm^2/day, respectively. Concerning OTA production, the highest amount of toxin concentration was determined at 7 days and was 1579.3, 31.5, 24.4, and 7.5 ng/g, respectively. *Penicillium verrucosum* F36 growth rate was 1.4 cm^2/day and OTA concentration was 10.3 ng/g. Under *S. cerevisiae* Y33 interaction effect, no growth was observed for all fungal species and toxin production was lower than 5 ng/g, with the exception of *A. carbonarius* F71 where OTA was estimated to 11.4 ng/g. *S. cerevisiae* Y33 can be considered as a promising bio-control agent, able to limit toxin production of many different fungal species, colonizing food matrices which are considered as food spoilers or pathogens.

Keywords: Saccharomyces cerevisiae, Aspergillus spp., Penicillium verrucosum inhibition, toxin production, nat

Microbiological spotlights

P5.101

Physicochemical stress exposures influence *Escherichia coli* O157:H7 and *Listeria monocytogenes* resistance in UV-C-irradiated coconut liquid endosperm and apple juice

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This study determined the effects of prior exposures to physicochemical stresses on the subsequent UV-C resistance of *E. coli* O157:H7 in coconut liquid endosperm (pH 5.01, 5.10 °Brix, 0.075% malic acid) and apple juice (pH 3.33, 12.20 °Brix, 0.49% malic acid). Five strains of *E. coli* O157:H7 and two strains of *Listeria monocytogenes* were individually exposed to all single and combination of heat (40°C), gradual acidification (1% glucose, final pH= 4.5) and abrupt desiccation (7% NaCl, a_w= 0.96) stresses prior same-genus cocktail inoculum preparation and to UV-C inactivation. UV-C inactivation curves in coconut liquid endosperm and apple juice showed log-linear inactivation behavior of both test organisms, indicative of homogeneity in UV-C resistance/susceptibility of individual strains. Regardless of previous stress exposure, both test organisms exhibited greater UV-C resistance when suspended in apple juice than in coconut liquid endosperm. Regardless of physiological state, *E. coli* O157:H7 exhibited greater resistance than *L. monocytogenes* in both suspending media. In coconut liquid endosperm, the greatest UV-C resistance was observed when cells were previously exposed to combined acid and heat stresses (D_{UV-C} 209.81 mJ/cm²). The least UV-C resistance was observed in cells subjected to the combination of acid, desiccation and heat stresses (D_{UV-C} 152.17 mJ/cm²). The greatest UV-C resistance was observed *L. monocytogenes* previously exposed to combined acid and heat stress (D_{UV-C} 37.32 mJ/cm²), while the least resistance was observed after exposure to heat-stress (D_{UV-C} 25.40 mJ/cm²). When suspended in apple juice, *E. coli* O157:H7 exhibited greatest resistance to UV-C after exposure to combined desiccation and heat stress (D_{UV-C} 3784.81 mJ/cm²). The least resistance towards UV-C was observed in non-stressed cells (D_{UV-C} 1776.23 mJ/cm²). *L. monocytogenes* previously exposed to combined acid and desiccation stress exhibited the greatest resistance to UV-C in apple juice (D_{UV-C} 726.76 mJ/cm²), while the least resistance was observed in cells previously exposed to combined acid and heat stress (D_{UV-C} 298.81 mJ/cm²). These results underscore the importance of considering implicit microbial physiology, and intrinsic suspending medium in the establishment of UV-C process schedules for fruit-based beverages.

Keywords: Physicochemical Stresses, E. coli O157:H7, Listeria monocytogenes, Ultraviolet Processing

Microbiological spotlights

P5.102

Surveillance and monitoring of foodborne pathogens in the Brazilian meat production chain

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Brazil has a leading role in the world scenario of meat production as one of the main exporting countries of poultry, beef and pork. Considering the relevance of these meat production chains in the international trade, the Brazilian Ministry of Agriculture (MAPA) has several control programs to ascertain the quality and safety of these products, in compliance with international standards and requirements. Among the main programs, the following must be highlighted: 1) Control and surveillance of *Salmonella* in poultry and turkey (Instrução Normativa 20, 2016 Oct 21); 2) *Salmonella* and Shiga toxin producing *Escherichia coli* (STEC) in beef (Norma Interna DIPOA/SDA 1, 2015 Jun 17); and 3) *Listeria monocytogenes* control in ready-to-eat (RTE) food of animal origin (Instrução Normativa 9, 2009 Apr 8). These programs are conducted by the Federal Food Inspection Service (SIF), an official department of MAPA, the responsible for monitoring the slaughtering and the industrial processing as well. Based on a number of academic studies, prevalence of *Salmonella* in poultry has decreased in the last years, but its control remains a challenge. *Salmonella* and STEC are rarely detected in beef, but cattle is described as reservoirs of these pathogens, indicating proper control of contamination during slaughtering. *L. monocytogenes* is frequently found in the processing environment of beef and pork industries, demanding proper control. In addition to these programs, MAPA has established back in 1999 strict requirements for GMP and HACCP for quality and safety monitoring in food industries (Resolução DIPOA 01, 1999 Jan 21). An updated edition of the Brazilian Regulation of Industrial and Sanitary Inspection of Products of Animal Origin (RIISPOA) was recently published (2017), in which the main guidelines for the SIF activities were updated based in a contemporary scenario. In addition to the control done by the MAPA in the food industries, the Ministry of Health is responsible for monitoring the safety of foods available to consumers in retail sale, what is done by the Agência Nacional de Vigilância Sanitária (ANVISA). To sum it up, the surveillance programs currently in place in Brazil control the contamination of key foodborne pathogens in the Brazilian meat production chain in a farm to fork approach.

Keywords: Brazil; meat; foodborne pathogens; inspection

Microbiological spotlights

P5.103

Inhibitory effects of medicinal plant extracts on pathogenic bacteria and antioxidant activity

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Nowadays, people use medicinal plants for treatment the disease because medicinal plants have many biological properties. Contamination of food by pathogenic bacteria causing enteric diseases results in diarrhea, nausea, vomiting and gastroenteritis. Using synthetic drugs for treatment the diseases may cause antibiotic resistant bacteria. Thus, application of natural extracts for treatment of pathogenic bacteria causing enteric diseases is an alternative choice for treatment of diseases instead of synthetic drugs. In this study, fourteen medicinal plants; *Amomum verum*, *Chrysanthemum indicum*, *Curcuma longa*, *Rhinacanthus nasutus*, *Erythina subumbrans*, *Centella asiatica*, *Phyllanthus emblica*, *Moringa oleifera*, *Thunbergia laurifolia*, *Acacia concinna*, *Terminalia chebula*, *Terminalia bellerica*, *Chromolaena odorata* and *Cinnamomum burmannii* were selected to investigate anti-bacterial and antioxidant properties. The plants were extracted by ethanol and water. Ethanolic extract of *Phyllanthus emblica* and aqueous extract of *Crysanthemum indicum* gave the highest percentage yield of 33.09 % (w/w) and 33.00 % (w/w) among fourteen medicinal plant extracts. The efficacy of fourteen medicinal plant extracts were tested against *Staphylococcus aureus, Shigella dysenteriae, Salmonella* Typhi and *Escherichia coli* strain O157:H7 using agar disc diffusion method. The ethanolic and aqueous extracts of *P. emblica* were the most effective extracts and could inhibit all tested bacteria with inhibition zones ranging from 8.8 - 20.7 mm. The extract also showed minimal inhibitory concentration and minimal bactericidal concentration against *S. aureus* with the lowest concentrations of 7.81 and 31.25 mg/mg. Moreover, antioxidant activity of medicinal plant extracts was determined by DPPH radical scavenging assay and the highest antioxidant activity of 724.099 mg gallic acid/g extract was found from ethanolic extract of *Moringa oleifera*. Phenolic compounds were also measured by Folin-Ciocalteu method and aqueous extract of *Cinnamomum burmannii* showed the highest total phenolic compound by 487.754 mg gallic acid/g extract. Therefore, the medicinal plants can be used as antibacterial and antioxidant agents.

Keywords: Medicinal Plant, Extracts, Pathogenic Bacteria, Antioxidant activity

Microbiological spotlights

P5.104

Prevalence and characterization of antibiotic resistant *Salmonella* serovars isolated from fresh vegetables and poultry processing environments in Malaysia

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Salmonella is among the most important bacterial foodborne pathogens worldwide with fresh vegetables and poultry as a major route of transmission to man. In this research, the prevalence, antibiotic resistance and resistance genes in *Salmonella* serovars isolated from green leafy vegetables, poultry and their related processing environment were studied. A total of 642 samples comprised of vegetables, poultry and their related environmental obtained from different selected wet markets were examined. Using the ISO 6579:2002 Horizontal Method (ISO, 2002), 187 (29.1%) samples were determined to be positive for *Salmonella* spp., with 87 (21.5%) isolates from vegetables, 17 (48.0%) from chicken carcasses and 83 (41.0%) from environment samples, respectively. Thirty-seven distinct serovars were identified and *S*. Corvallis, *S*. Brancaster, *S*. Albany, *S*. Weltevredent, *S*. Hvittingfoss, *S*. Paratyphi B and *S*. Typhimurium were the most prevalent serovars. Among the 187 isolates identified, 19 isolates (10.1%) were resistant to at least one antimicrobial, while 103 isolates (55.1%) were resistant to more than three antimicrobials. High rates of resistance were observed for streptomycin (66.6%), tetracycline (44.4%), sulfonamides (44.4%), ampicillin (26.7%), chloramphenicol (29.1%), trimethoprim-sulfamethoxazole (11.6%), nalidixic acid (12.8%) and kanamycin (11.2%). Resistance determinants, against tetracycline (*tetA* and *tetB*), β-lactamase ampicillin ($bla_{\text{TEM-1,}}$ and *temB*), streptomycin (*strA*, *strB and aadA),* sulphonamides *(sull and sulII) and* chloramphenicol *(floR* and *cmlA)* were detected by PCR among antibiotic resistance *Salmonella* isolates. This study provides information about the occurrence of foodborne *Salmonella* in Malaysia, alarming the emergence of multi-drug resistant *Salmonella* strains, and present useful data on the resistance determinants.

Keywords: Salmonella spp, Serotyping, Antibiotic resistance, Resistance genes

Microbiological spotlights

P5.105

Incidence, serotypes and pulsotypes of *Listeria monocytogenes* in popsicles and ice cream acquired from retail shops in Campinas, Brazil

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Listeria monocytogenes has been recently associated with foodborne diseases linked to dairy products, such as ice cream. For instance, between 2010-2014, listeriosis cases associated with ice cream resulted in 10 hospitalizations and 3 deaths in the USA. In Brazil, there is a lack of data on the incidence and characterization of *L. monocytogenes* in ice cream and popsicles. Thus, the purpose of this work was to evaluate the incidence and characteristics of *L. monocytogenes* in ice cream and popsicles available in the retail shops of Campinas, Brazil. A total of 100 samples (58 popsicles and 42 soft ice cream) from 13 different brands were analyzed. A total of 75% of the samples presented milk in their composition. Detection of *L. monocytogenes* was realized according to ISO 11290-1:1996, and 2 to 5 presumptive colonies were selected for subsequent identification. The identification of presumptive colonies was performed by PCR assay using primers for 23S rRNA and *hly* genes. For serotyping, a multiplex PCR assay was utilized to separate the four major serovars (1/2a, 1/2b, 1/2c, and 4b). PFGE was performed according to PulseNet™ (Standardized Laboratory Protocol for Molecular Subtyping) protocol for *L. monocytogenes*, using the restriction enzyme *Apa*I. A total of 28 presumptive colonies were isolated from 16 samples analyzed. After PCR assay, 14 isolates were identified as *L. monocytogenes* recovered from one sample of ice cream and two of popsicle, indicating an incidence of 3.4% and 2.4%, respectively. These isolates were found to belong to serovar 1/2b. PFGE typing indicated the occurrence of three pulsotypes, i.e., i) the first pulsotype consists of strains from soft ice cream of Chocolate flavor, while the second and third pulsotypes were strains isolated from popsicles of Corn and Chocolate bar covered with vanilla ice cream flavors. As conclusion, low incidence of *L. monocytogenes* was observed in the samples analyzed. Also, the fact that serovar 1/2b was found in different samples, indicates the occurrence of strains with pathogenic potential in these foods.

Keywords: Milk products, foodborne pathogen, PFGE

Microbiological spotlights

P5.106

Occurrence and Antibiogram of *Escherichia coli* O157:H7 in locally-fermented milk (nono) sold under market conditions in Nasarawa State, Nigeria

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Escherichia coli O157:H7 is an emerging pathogen frequently associated with the consumption of food of bovine origin globally. The present study evaluated the occurrence of *E. coli* O157:H7 in locally fermented milk (*nono*) sold under market conditions in Nasarawa State, Nigeria and the patterns of their antibiotic susceptibility. A total of 420 *nono* samples were purchased across Nasarawa State. The samples were bacteriologically analyzed in the laboratory for the presence of *E. coli* O157:H7 by means of cultural techniques (involving enrichment and selective plating on CT-SMA), biochemical assay (Microbact 12E) and serological confirmation (latex R30959601). Confirmed isolates were further subjected to antimicrobial susceptibility test using the agar disc diffusion technique. The results of the study showed that out of 420 *nono* samples examined, 19 (4.5%) were contaminated with *E. coli* O157:H7. The highest occurrence rate (5.7%) was recorded in samples obtained from Akwanga, Wamba and Doma Local Government Areas, while Lafia and Keffi had the least occurrence rate (2.9%). With respect to the senatorial zones, Nasarawa North had the highest occurrence rate of 5.7% while the Southern zone had the least (3.6%). There was no significant difference (P>0.05) in the occurrence of *E. coli* O157:H7 isolated from *nono* samples with respect to the various Local Government Areas. Antibiotic susceptibility profiles showed that all the isolates were resistant to multiple antibiotics, except ciprofloxacin and gentamicin, resulting in nine different resistant patterns. All the 19 (100%) isolates were resistant to penicillin and tetracycline, 18 (94.7%) to erythromycin, 16 (84.2%) to amoxicillin, oxacillin and sulphamethoxazole/ trimethoprim, 13 (68.4%) to chloramphenicol and 8 (42.1%) to streptomycin; 15 (78.9%) and 17 (89.5%) of the isolates were sensitive to ciprofloxacin and gentamicin respectively. The predominant antimicrobial resistance pattern was penicillin-tetracycline-chloramphenicol-amoxicilin-erythromycin-oxacillin-sulphamethoxazole/ trimethoprim with the occurrence rate of 36.8% among the 19 isolates tested. *Nono* consumption has potential health risks to consumers not just in Nasarawa State but possibly to the nation at large. Hence proper hygiene in the processing and marketing of *nono* is recommended. The multiple antimicrobial resistance exhibited by *E. coli* O157:H7 strains in this study is an indication of possible antibiotic abuse.

Keywords: emerging, pathogen, nono, resistance, Nigeria

Microbiological spotlights

P5.107

Comparative efficacies of two local adsorbents: Activated charcoal and Nzu (Calabash Clay) in the reduction of Afl atoxin M1 in cow's milk

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Numerous health risks associated with Aflatoxin M_1 (AFM_1), expensive and non-availability of adsorbent for its reduction in Nigeria drives the demand for local readily available materials for its control. Adsorption studies of AFM_1 were performed using activated charcoal (AC) and Nzu (calabash clay) (0.5, 1, or 2%) at aflatoxin contamination rates of 9, 231 or 456ng/l for 5hs at 4, 16, 28 and 32 °C. The aflatoxin-adsorbing capabilities depend on the adsorbent concentrations, contact time and treatment temperature. At 4, 16 and 28° C treatment temperatures, AC demonstrated very mild adsorption activity; while at 32° C a significant reduction ($p< 0.05$) were observed at the highest contamination rate and adsorbent concentration . However, at all the treatment temperatures calabash clay keeps increasing the aflatoxin concentrations in the milk by 10% .with the highest increase at 32° C with 2% calabash clay. Results from this study indicate that activated charcoal demonstrates a potential for aflatoxin reduction at high temperatures and calabash clay is very unsafe either as an adsorbent for aflatoxin reduction or for consumption. The use Activated charcoal should be encouraged and calabash clay should be banned from use and consumption if public health must be protected.

Keywords: Aflatoxin M1, Cow's milk, Adsorbents, Activated Charcoal, Calabash Clay.

Microbiological spotlights

P5.108

Antimicrobial activity of bacteriophage for the chicken meat production

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The most important pathogens involved in the foodborne diseases arer Salmonella y Campylobacter, which are transmited to human by poultry and the chicken meat. To avoid this, the industry has implemented several actions incluying protocols of productions, use of desinfectants and vaccines for the animals. However, this set of actions do not be successful, and we have yet outbreaks of Salmonella and a lot of cases of Campylobacteriosis. Even more, campylobacter is present in the 76% and 60% of the chicken carcasses in Europe and Chile respetively. On the other side, the regulation of use of antibiotic in the primary production, valid from the 2005, show the urgent the search of new alternative for the pathogens control. In this sense, the phages has been an attractive alternative, since are recognized as “GRAS” and, in this moment, exists commercial products based in this technology. In this job we show the isolation of 14 strains of phages of Salmonella and 5 phages of Campylobacter, which were characterized in the host range, adsorption time and viral progiene. Among them the phages of Salmonella F6 and F7 shown a shorter adsorption time (30 min y 60 min, respectively) and a high viral progenie (4 fold y 200 fold respectively). On the other hand, the phages F1 and F3 of Campylobacter shown shorter adsorption time (20 min for both) and a high viral progenie (300 and 3000 fold respectively). Finally we evaluated the antimicrobian activity in vitro of the phage. The phage F3 decrease the bacterial survival a 91% at 1 hour and 71% at 24 hours. On the other side, the phage of Salmonella, F6 and F7 decrease the bacterial survival at 1,7% at 24 hours. This results indicates that the phages could be an alternative for the microbiological control of Salmonella and Campyobacter. In this moments we are evaluated the antimicrobial activity of the phages in alimentarial matrices. This results are important to evaluate the posibility of aplications of phage in the chicken meat industry as a solution for two of more important pathogens associated to this food.

Keywords: Phage, antimicrobial, salmonella

Microbiological spotlights

P5.109

Presence of *Salmonella* and some indicator microorganisms in shell eggs produced in different housing systems in Turkey

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Shell egg can contaminate with some pathogens and microorganisms during their formation in reproductive system or after laying. Contaminated shell eggs can cause some foodborne illnesses, such as Salmonellosis, in humans after their consumption. Also, level of microbial contamination plays an important role on shelf life of the eggs.The aim of this study to determine the presence of *Salmonella* and some indicator microorganisms such as coliform, Enterobacteriaceae, and *E. coli* in the shell eggs produced in conventional cage, organic, cage free, and free range housing systems in Turkey. A total of 80 conventional cage, organic, cage free, and free range shell eggs were collected from supermarkets in Diyarbakir, Turkey. Presence of *Salmonella* in the eggshells and the egg internal contents was determined using standard selective enrichment, selective plating, and biochemical tests. The enumeration of coliform, Enterobacteriaceae and *E. coli* in the eggshells were performed using standard pour plate method as previously reported in the literature.

Conventional battery cage, organic, cage free, and free range eggshells was found to be contaminated with coliform bacteria as 45%, 45%, 5%, 30%, respectively. *E. coli* was found in 30% of the conventional battery cage, 10% of the organic, 5% of the cage free, 30% of the free range, and most eggs had counts ranging from 1,3 to 2,40 $\log_{10}$ CFU/ml of rinsate. Organic shell eggs had higher Enterobacteriaceae load (1,85 ± 0,54 $\log_{10}$ CFU/ml of rinsate) than conventional cage, cage free, and free range. None of the shell eggs was positive for *Salmonella*.

The results of this preliminary study showed that shell eggs produced in different housing systems can be contaminated with some important indicator microorganisms.

Keywords: Shell egg, Salmonella, Enterobacteriaceae, E. coli, Coliform

Microbiological spotlights

P5.110

Genomic characterization and virulence typing of *Campylobacter jejuni* strains isolated from poultry

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Campylobacter jejuni is a major foodborne pathogen that causes severe gastroenteritis in humans. Chickens act as the host for *C. jejuni*, wherein the pathogen colonizes the ceca thereby leading to contamination of the carcass during slaughter and subsequent human infections. In the human gut, the pathogen attaches and invades the intestinal epithelium followed by toxin mediated cytopathy and enteritis. Little is known about how this pathogen is able to colonize multiple hosts whilst competing with specialist microbiota in the gut. Comprehensive characterization of *C. jejuni* strains could facilitate in better understanding of their pathophysiology and development of effective intervention strategies. Whole genome sequencing is a powerful technology that provides in-depth genetic information and is increasingly being utilized to study the evolution, epidemiology, virulence, and detection of foodborne pathogens. Herein, we report the complete genomic sequence of four wild-type *C. jejuni* strains (called, S1, S3, S4 and S8), isolated from poultry in the United States. In addition, mechanistic analysis was conducted to test the colonization potential and virulence attributes of the strains.

Whole genome sequencing, de novo assembly and genome annotation revealed a chromosome of 1,714,057, 1,671,321, 1,685,319 and 1,690,693 bp in isolates S1, S3, S4 and S8, respectively. The genome GC content of all strains was between 30.9 and 31.2% with 1718 to 1861 coding sequences and 44-49 RNAs. Multiple genes coding for virulence factors such as motility, toxin production, and stress tolerance were observed in all tested strains. In addition, genes imparting resistance to antibiotics such as beta-lactams and toxic compounds such as Copper and Arsenic were also found in all 4 strains. The *C. jejuni* genome of tested strains also revealed CRISPR sequences imparting adaptive immunity against phage invasion. Follow up mechanistic analysis showed that all strains were capable of motility at 42°C and 37°C and attaching to epithelial cells from chickens and humans. Taken together, the genomic data from these potentially virulent strains will facilitate in better understanding of the colonization and pathogenesis mechanisms of *C. jejuni* leading to better control strategies. Comparative analysis outside the conserved core genomes of these 4 strains to reveal any unique genomic features is currently underway.

Keywords: Campylobacter, whole genome sequencing, virulence, poultry, preharvest safety

Microbiological spotlights

P5.111

Antifungal activity of selected natural preservatives and their interaction with food components

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Food spoilage caused by moulds may represent a considerable economic loss for the food industry, in addition to being a health risk for consumers with respect to mycotoxin producing mould species. Despite the desire for safe food, there is an increasing consumer demand to avoid or diminish chemical food additives, resulting in a growing interest in new safe and biodegradable preservatives.

The present study examines the antifungal effect of the essential oil (EO) *Origanum vulgare*, its active components carvacrol and thymol, and a few active components of other EOs, namely, eugenol and *trans*-cinnamaldehyde, against the foodborne moulds *Penicillium verrucosum* and *Aspergillus westerdijkiae*. Therefore, initially the minimum inhibitory concentration (MIC) was determined as well as the minimum fungicidal concentration (MFC) by broth macrodilution of each antifungal agent. Furthermore, the influence of the inhibitors on morphological changes of hyphae and spores of the moulds, which grown on malt extract agar containing *trans*-cinnamaldehyde and *O. vulgare* EO, were investigated by scanning electron microscopy.

The effect of milieu conditions like food components and different pH and a_w values on the efficacy of the natural preservatives were assessed by determination of the MIC in food model media. For this purpose, matrices were used with different levels of carbohydrates (1, 4 and 6 %), protein (1, 5 and 10 %) or fat (1, 5 and 10 %) as well as different pH (pH 7.0, 5.6, 4.5 and 3.5) and a_w values (0.99, 0.92, 0.90 and 0.87 a_w).

Regarding the antifungal activity of the natural preservatives, the following ranking in order of decreasing inhibitory action is: *trans*-cinnamaldehyde > carvacrol = thymol > *O. vulgare* EO > eugenol. The two tested inhibitors *trans*-cinnamaldehyde and *O. vulgare* EO caused morphological alterations of the mycelium, such as decreasing pigmentation, shrunken and aberrantly grown hyphae, and loss of conidiophores and spores.

The antifungal efficacy of EOs depends on the ingredient modifications. Fat had a negative impact on the EO efficacy. On the contrary, the natural preservatives were more effective at higher concentrations of carbohydrates and protein (except *trans*-cinnamaldehyde) and at moderately acidic pH and lower a_w values.

Keywords: natural preservatives, foodborne moulds, morphological alteration, food components, pH, aw

Microbiological spotlights

P5.112

Automated ribotyping and cluster analysis of *Listeria monocytogenes* isolates from a South African ready-to-eat food factory

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Sixty four (64) isolates from a ready-to-eat food factory were ribotyped using DuPont RiboPrinter®. Cluster analysis was conducted to evaluate the relatedness of strains from different sources, aiming to establish possible contamination routes and mechanisms. From the 29 ribotypes obtained, nine different DuPont ID's were assigned, which was indicative of the variety of contamination sources within the RTE factory, on par with similar studies conducted. Lineage assignments of *Listeria monocytogenes* could be made using the DuPont ID's and the RTE factory studied was found to host both lineage I and II strains. The geographical distribution of similar strains indicated that work boots, trolleys and crates were vectors for *L. monocytogenes*. The Pearson correlation dendrogram also indicated the harbourage of strains and possible drain biofilms in both low and high-risk areas. Assigned DuPont ID's allowed for comparison of strains found with similar studies as well as comparison on international database, Food Microbe Tracker. DuPont ID 1038, 1041, 1042 and 18596 found in the factory have been previously implicated in food recalls and clinical listeriosis cases. DuPont ID 20243, that was isolated from the RTE factory, has not yet been logged on the global Food Microbe Tracker database The *L. monocytogenes* contamination trends identified in this RTE factory, correlated with current global trends.

Keywords: Listeria monocytogenes, South Afriva, Ready-to-eat food

Microbiological spotlights

P5.113

The absence of N-acetylglucosamine in aall teichoic acids of *Listeria monocytogenes* modifi es biofilm architecture and tolerance to cleaning and disinfection procedures

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Listeria monocytogenes is able to form biofilms composed of cells and extracellular matrix. In 2015, Brauge et al. demonstrated that the major polysaccharide present in the extracellular matrix of the *L. monocytogenes* biofilm was teichoic acid. Moreover, around 50% of *L. monocytogenes* strains (out of 93 strains) carried a mutation of the *lmo2550* gene involved in the GlcNAcylation of this teichoic acid. In order to better characterize biofilms *L. monocytogenes*, we evaluated the impact of the absence of the GlcNAc residue on adhesion and 48-h biofilm development, as well as biofilm-related phenotypes.

Methods: We studied the wild-type EGD-e strain and two mutants, EGDeΔ*lmo2549* and EGDeΔ*lmo2550*, inactivated respectively in the *lmo2549* and *lmo2550* genes encoding glycosyltransferases, involved in the GlcNAcylation of teichoic acid in *L. monocytogenes*. First, we evaluated the impact of these mutations on adhesion, formation of *L. monocytogenes* biofilms by epifluorescence microscopy, and by counting the viable cultivable population on agar media. Second, we studied their further detachment after mechanical and chemical actions by qPCR and PMA-qPCR assays.

Results: The mutation of the *lmo2549* or *lmo2550* genes caused a decrease in bacterial adhesion to stainless steel during the adhesion step. Bacterial population was not significantly different after 24-h biofilm formation. The biofilm architecture was different between the wild-type strain and the two mutants with the presence of bacterial micro-colonies for mutants, which were not observed in the wild-type EGD-e strain biofilm. Upon a water flow or cleaning procedure at a shear stress of 0.16 Pa, the mutant biofilms showed a higher detachment rate compared to wild-type strain. Meanwhile, an increase in the amount of residual viable but non-culturable population on stainless steel was recorded in the two mutants. Our data suggest that the GlcNAc residue of teichoic acid played a role in adhesion and biofilm formation of *L. monocytogenes*.

Keywords: foodborne pathogen, Listeria monocytogenes, biofilm extracellular matrix, biocide

Microbiological spotlights

P5.114

Mycotoxin diffusion in dry-cured meat products

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The environmental conditions reached during the ripening process of dry-cured meat products favour the growth of a mould population on their surface. These moulds may produce several secondary metabolites on the surface and diffuse into the food. The aims of this study were to: a) evaluate the validity and applicability of a multianalyte HPLC-MS/MS method in dry-cured meat products and b) investigate the production and diffusion of several secondary metabolites produced by *Penicillium* spp. into these products. Dry-fermented sausage and dry-cured ham pieces (4×4×3 cm) were inoculated with *P. nordicum*, *P. verrucosum*, and *P. griseofulvum* and incubated at 25 ºC for 15 days. Later, the samples were divided into 3 layers: A (0-1 cm), B (1-2 cm) and C (2-3 cm). Regarding the validity studies, the method was successfully in-house validated for the main analytes produced by the moulds most commonly found in these products. The apparent recoveries (R_A) values ranged between 80 and 106% and the matrix effects (SSE) values between 67 and 208%. Aflatoxin B_1, ochratoxin A, and griseofulvin were not affected by the SSE; however, cyclopiazonic acid and roquefortine C were slightly affected by the matrix. Finally, most of the limits of detection and quantification were lower than 1 µg/kg. On another side, *Penicillium* strains inoculated on both dry-cured meat products produced between 12 and 16 secondary metabolites on the surface of the meat products (layer A). Some of them were able to diffuse into the inner core of meat products (layers B and C); however, the concentration of metabolites decreased as they diffused inward. Besides, the same toxigenic strain synthesised different secondary metabolites depending on the product. In conclusion, the analytical method can be used for the analysis of secondary metabolites in dry-cured meat products. Besides, it was shown that the strains of *Penicillium* spp. produced various secondary metabolites at high concentrations, and these compounds may diffuse into the inner core of the foods. Therefore, it is necessary to look for efficient strategies to avoid toxigenic mould growth for minimising mycotoxin in dry-cured meat products.

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Keywords: dry-cured meat products, diffusion, Penicillium, mycotoxins

Microbiological spotlights

P5.115

Proteomic approach to the ochratoxigenic *Penicillium nordicum* inhibition by protective cultures

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During processing of dry-cured meat products the conditions of temperature and water activity (a_w) reached are appropriate for fungal colonisation. Uncontrolled mould can produce mycotoxins in these products, mainly Ochratoxin A (OTA). *Penicillium nordicum* (Pn) is the main OTA-producing species in dry-cured ham. The use of native microorganisms as protective cultures may prevent OTA contamination in dry-cured meat products. *Penicillium chrysogenum* (Pc), which produces the antifungal protein PgAFP, and *Debaryomyces hansenii* (Dh) have proved to be useful to fight OTA production. However, their mechanisms of action have not been completely elucidated. The aim of this work was to evaluate the effect of Pc, Dh and PgAFP, and their combinations in the ability to produce OTA by *P. nordicum* growing in dry-cured ham-based medium, as well as in the proteome of *P. nordicum*. Combinations of protective agents were co-inoculated with *P. nordicum* on dry-cured ham-based medium for 14 days at 25 ºC. OTA levels were measured by uHPLC-MS/MS for every treatment. LFQ proteomic analyses were carried out in Q-Exactive mass spectrometer for Pn. The presence of PgAFP was evaluated in the cultures.

Dh and Pc significantly reduced OTA production by Pn. Dh caused higher abundance of proteins related with redox reactions, due to a potential oxidation effect. Dh also reduced the abundance of proteins involved in the Cell Wall Integrity (CWI) pathway in Pn. Conversely, Pc decreased proteins involved in oxidoreduction reactions in Pn, while proteins related to CWI pathway were found both in higher and lower abundance, pointing a deep remodeling as response. On the other hand, the production of PgAFP by Pc was not detected.

Co-inoculation of Dh and Pc with Pn did not lower OTA production, likely due to opposed mechanisms described for each biocontrol agent.

PgAFP had limited impact on Pn proteome and OTA production. Finally, the combined use of PgAFP-Dh triggered a response trough CWI of Pn, which overcomes the effect caused by Dh alone.

This work paves the way to use these antifungal agents in the most appropriate way to reduce OTA content. However, the combined use of these agents is not advisable in dry-cured ham processing.

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Keywords: ochratoxin A, biocontrol, D. hansenii, P. chrysogenum, dry-cured ham

Microbiological spotlights

P5.116

Listeria monocytogenes in various readyto-eat foods over a five-year period in Estonia

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The present study focused to the prevalence and numbers of *Listeria monocytogenes* in various categories of readytoeat (RTE) food products. A total of 30,016 RTE food samples were analysed for prevalence from 2012 to 2016. We found that 3.6% of the RTE food samples were positive for *L. monocytogenes*. The highest prevalence (11.6%) was found for RTE fish and fish products. The overall prevalence of *L. monocytogenes* in other food categories was within the range of 0% to 3.9%. Also, 14,342 RTE food samples were analysed to determine the numbers of *L. monocytogenes*. A food safety criterion was exceeded for 0.3% of RTE food samples. Samples most often exceeding the legal safety limit were RTE salted and cold-smoked fish products. Very high prevalence, respectively 28.6% and 26.5%, and high numbers of *L. monocytogenes* was found for salted fish and cold-smoked fish products. It demonstrates the necessity to increase awareness among RTE fish producers, also food control institutions and for susceptible risk groups. Currently there are no sufficient data about contamination patterns of *L. monocytogenes* in RTE salted and cold-smoked fish processing. The present Estonian surveillance system of human listeriosis and state surveillance for *L. monocytogenes* prevalence in foods does not include molecular characterization of isolated strains. This strongly limits the capacity of epidemiological analyses, and makes effective foodborne outbreak detection impossible.

Keywords: Listeria monocytogenes, RTE food, prevalence and numbers

Microbiological spotlights

P5.117

Bacillus thermoamylovorans: First report of evaporated milk spoilage and method of detection

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Here we report the first documented incident of spoilage of canned evaporated milk by *Bacillus thermoamylovorans*, a spore forming, moderate thermophilic, facultive anaerobic bacterium.
Thermally stressed (55oC for 7 days) products with lower than usual pH, ranged from 4.8 to 5.3, were microbiologically examined. The isolated microorganism, responsible for the spoilage, was identified by 16S rDNA sequencing, as B. thermoamylovorans. Raw milk and additives were microbiologically examined for the presence of the microorganism. The point of entry was determined to be the raw milk coming from one specific milk collection centre.
A primer-probe qualitative Real Rime PCR method targeting 16S rDNA region of the bacterium was developed to detect the specific species. The inclusivity of the qPCR assay was tested with DNA extracted from 12 strains and 30 products positive for the presence of *B. thermoamylovorans*, while the exclusivity was assessed with 58 isolates belonging to 34 spore forming species and 44 isolates belonging to 37 gram positive and gram negative species most commonly isolated from raw milk and milk products. The detection limit (LOD_6) for *B. thermoamylovorans* was 10 genome copy numbers, the qPCR efficiency was 90%, the linearity (R^2) was 0,999 and the slope of regression was calculated at -3,575. Naturally contaminated samples of raw milk were tested for the presence of *B. thermoamylovorans* with a microbiological method and the new developed qPCR assay. Results showed that the qPCR method is suitable for the detection of strains of *B. thermoamylovorans* in raw milk samples with a 36 hours incubation step at 50°C.
To our knowledge, the developed qPCR assay is the first study about the detection of spoilage *B. thermoamylovorans* in raw and evaporated milk.

Keywords: Bacillus thermoamylovorans, evaporated milk, spoilage, real time PCR

Microbiological spotlights

P5.118

Survival of *Escherichia coli* and *Listeria innocua* on lettuce after irrigation with contaminated water

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The number of microbial disease outbreaks related to the consumption of fresh produce has increased in recent years. Leafy green vegetables are associated with a large number of disease cases and outbreaks. These foodstuffs are susceptible to microbial contamination via a number of different pathways, such as contaminated manure or irrigation water. Once present on the plant, these bacteria may persist for different periods of time, which represents a risk for consumer's health.
The objective of this study was to analyse the survival of *E. coli* and *L. innocua* in lettuce plants inoculated with contaminated irrigation water via a single overhead spray irrigation event, in Irish winter glasshouse conditions. Survival of both strains was also evaluated in stored irrigation water.
Three adjacent plots of seven by four lettuce plants (*Lactuca sativa* var. *capitata*) were inoculated with water spiked with marked *E. coli* FA7 Lys9 or *L. innocua* ATCC 51742. Each plant was inoculated with 300mL of water with 10^7 cfu/mL of either strain. Three plants from each plot were removed at eight sampling points, up to 28 days, when plants reached a harvestable state. 25g of each plant were analysed for the presence of either strain via colony enumeration, enrichment and qPCR. In parallel, individual 20L water microcosms were spiked with 10^7 cfu/mL of either strain and sampled at similar time points. Each microcosm was concentrated and the pellets were analysed as described above.
We observed the survival of both strains in lettuce plants up to 28 days after inoculation. Direct quantification showed a 4 log decrease in the concentration of *E. coli* 14 days after inoculation, and a 3 log decrease in the concentration of *L. innocua* 10 days after inoculation. *E. coli* was detected on water samples up to 3 days after inoculation and *L. innocua* was detected up to 28 days. These results demonstrate that *E. coli* and *Listeria* strains are able to persist in lettuce after a single contamination event up until the plants reach a harvestable state. Furthermore, the persistence of *L. innocua* in stored irrigation water up to 28 days after inoculation increases the potential for multiple plant contamination events from irrigation water, emphasising the importance of ensuring that irrigation water is of a high quality.

Keywords: Fresh produce microbial contamination, irrigation water, outbreaks, E. coli, L. innocua

Microbiological spotlights

P5.120

Investigating the ability of *Listeria monocytogenes* to form biofilm on surfaces relevant to the mushroom production environment

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Listeria monocytogenes poses a threat to all fresh fruits and vegetables, including mushrooms, due to its ubiquitous presence in the natural environment. Mushrooms (*Agaricus bisporus*) are Ireland's largest horticultural crop and although they have not been linked with listeriosis outbreaks, the organism still poses a threat to the industry due to its presence in the environment and its ability to form biofilms. This threat is highlighted by studies demonstrating that *L. monocytogenes* is present in the mushroom production environment. The aim of this study was to investigate the biofilm formation potential of *L. monocytogenes* strains, isolated from mushroom production environment, at temperatures and on surfaces that are relevant to the mushroom industry. Preliminary assessment of biofilm formation of 74 mushroom industry isolates of *L. monocytogenes* was carried out using a crystal violet assay on polystyrene microtitre plates at 18°C and 25°C for 72h. This assay showed that the mushroom industry isolates were able to form various levels (weak, moderate or strong) of biofilm on microtitre plates under industry relevant temperatures. Strains were then selected according to their biofilm forming ability and were assessed for their biofilm formation potential on different surfaces using the CDC biofilm reactor at 25°C for 72h. The surfaces tested were stainless steel, aluminium, glass, copper, rubber, polycarbonate, polypropylene, concrete and industry specific materials including two types of tarpaulin and mushroom growing net. All of these surfaces (excluding concrete and copper)
were found to be able to support biofilm levels ranging $\log_{10}$ 3-6.8 CFU/cm^2 for seven different *L. monocytogenes* strains. Copper was found to have low biofilm levels of $\log_{10}$ 2.18 CFU/cm^2 on average while concrete had high biofilm levels of $\log_{10}$ 7.82 CFU/cm^2 on average. Additionally, a commercially available concrete sealant was tested and on average was found to achieve a 2.34-log reduction of biofilms levels on concrete. These results indicate that *L. monocytogenes* can readily form biofilms on industry relevant surfaces, and additionally identifies areas of specific concern, where rigorous cleaning and disinfection is required.

Keywords: Listeria monocytogenes, biofilm, mushroom industry

Microbiological spotlights

P5.121

Performance assessment of the 3M™ Molecular Detection Assay 2 – *Cronobacter* according to ISO 16140-2 (2016) standard in infant formula, infant cereals, raw materials and environmental samples

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Introduction: *Cronobacter* species form a group of Gram-negative bacteria which may cause lethal illness in infants. The 3M™ Molecular Detection Assay 2 - *Cronobacter* is designed to detect *Cronobacter* spp. in food by means of loop-mediated isothermal amplification of specific DNA target sequences and detection by bioluminescence.

Purpose: An independent study was conducted to compare this new alternative method to the ISO 22964:2017 (reference method) according to the ISO 16140-2 (2016) standard for NF Validation approval.

Methods: Different enrichment protocols were tested: food and environmental samples were 1:10 diluted and incubated at 37 ± 1 °C during 18-24 h in Buffered Peptone Water (BPW; for 10 g samples) or pre-warmed BPW (for 300 g samples). 300 g samples containing probiotics were supplemented with vancomycin (10 mg/L) and incubated during 22-24 h. After lysis (15 ± 1 min at 100 ± 1 °C), DNA amplification was performed. The study compared the sensitivity, relative detection level (RLOD), inclusivity and exclusivity.

Results: Overall, 314 samples were analyzed by both methods. Depending on the enrichment protocol, the sensitivity ranged between 80.0 % and 96.0 % for the alternative method and between 66.7 % and 100 % for the reference method. The relative trueness ranged between 77.4 % and 97.9% and the false positive ratio for the alternative method ranged between 1.1 % and 3.3 %. Depending on the tested matrix, the RLOD ranged between 0.255 and 2.317 suggesting that both method have a similar level of detection. The 50 tested target-strains were detected and no cross reaction was observed with the 30 tested non-target strains.

Significance: The alternative method is reliable for the detection of *Cronobacter* spp. and the negative results are available 2 days earlier than reference method.

Keywords: Cronobacter spp., foodborne pathogen, Pathogen detection

Microbiological spotlights

P5.122

Cronobacter sakazakii, new emerging pathogen for elderly, interaction with lactic acid bacteria

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Cronobacter sakazakii is an emerging pathogen for infants, children from 3 days to 4 years of age as well as adults of over 70 years of age. Although it may cause diseases all-aged people, it is most effective on humans who have a weak immune system. In adults, *Cronobacter* spp. may also cause diseases, such as pneumonia, septicemia, osteomyelitis, splenic abscesses, and wound infections. The overall incident rates of *C. sakazakii* in adults was similar to that of vibriosis and yersiniosis. *C. sakazakii were* can be found in a variety of foods, food ingredients, raw vegetables, drinking water and food production environment. Thus, ready to eat foods as well as ingredients contaminated with this species used to prepare foods pose a public health risk to adults. In this study out of 59 milk based samples 3 were found to be *C. sakazakii.* Antimicrobial substances produced by lactic acid bacteria during fermentation, used for food preservation since ancient times. In this study, effect of different species of lactic acid bacteria isolated from foods were used against isolated *C. sakazakii* strains. These bacteria were belong to genus *Streptococcus , Weissella , Pediococcus , Lactococcus* and *Lactobacillus.* Our result show that lactic acid bacteria have bactericidal effect on *C. sakazakii.* Hence, the use of LABs or their antibacterial metabolites could be an alternative way to inhibit *C. sakazakii* in the diet for elderly as well as in other foods.

Keywords: Cronobacter sakazakii, elderly, emerging pathogen, lactic acid bacteria, Weissella

Microbiological spotlights

P5.123

Usage of cold hydrogen peroxide vapour for inactivation of murine norovirus on fruit and vegetable surfaces

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Human norovirus (hNV, family *Caliciviridae*) is responsible for gastroenteritis outbreaks worldwide. For research investigations, surrogates as murine norovirus (MNV) are utilized for studying norovirus infection due to the lack of a cell culture for hNV. Cold nebulized hydrogen peroxide vapour (H_2O_2) is a superfine dry fog which prevents harmful effects of humidity and is used until now especially for environment disinfection in hospitals and pharma industries. H_2O_2 damages bacterial and viral structures by oxidation. It is known that H_2O_2 can be utilized as cold fog (nebulization at room temperature) in order to inactivate MNV on surface areas. Although hNV contamination of fruits and vegetables is an ongoing problem the virucidal efficiency of this application regarding the inactivation of norovirus on different fresh produce is not characterized.

In this study, MNV (S99 P19) was used in order to illustrate if cold nebulized H_2O_2 inactivates the virus on different fruit and vegetable surfaces (smooth surface: apples, blueberries; rough surface: cucumber, strawberries). Two different application systems (DCXpert, DCX Technologies GmbH and DiosolGenerator MF, DIOP GmbH & Co. KG) were used for a cold fogging decontamination with H_2O_2 (60 min, max. 260 ppm H_2O_2). Plaque assay was performed after recovery of MNV from untreated and treated fresh produce to compare quantity of infective MNV.

Infective MNV were reduced on smooth surfaces (apples, blueberries) by approximately 4 $\log_{10}$ with cold nebulized H_2O_2. However, similar treatment of artificially contaminated cucumber resulted in lower virucidal efficiency of this application, whereas a median value of 1.9 $\log_{10}$ reduction can be determined. The treatment of inoculated strawberries resulted in no reliable reduction rates for MNV.

Nevertheless, this study presents first insights into the potential of cold nebulized H_2O_2 for treatment of fresh produce. However, further steps should improve and characterize this application system in relation to fresh produce more detailed in order to extend the scope of cold nebulized H_2O_2.

Reference: M. Weinstock, J. Pfannebecker, M. Dabisch-Ruthe, B. Becker (2018): Inactivation of murine norovirus on fruit and vegetable surfaces by cold nebulized hydrogen peroxide. Food and Environmental Virology. Under review.

Keywords: vaporized hydrogen peroxide, murine norovirus, fruits, vegetables, inactivation

Microbiological spotlights

P5.124

Interlaboratory study on the detection of human noroviruses in frozen strawberries in Germany and Austria 2017

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Contaminated soft fruits are considered as one of the main reasons for gastroenteritis caused by human norovirus. Since the end of 2013, a workflow procedure (part of the technical specification DIN CEN/ISO TS 15216-2) is available for the qualitative detection of norovirus and hepatitis A virus. The detection of human noroviruses (GGII) in frozen strawberries was examined in an interlaboratory study carried out by the working group for "Food-Associated Viruses" (ALV-Working Group, Germany). The aim of the study was to show whether the use of the technical specification in routine application provides reliable results. Each sample package contained 2 x 25 g frozen strawberries with 10^7 noroviruses, 2 x 25 g frozen strawberries with 10^5 as well as 2 x 25 g frozen strawberries without norovirus inoculant. The 18 participating laboratories applied their established materials and devices for virus extraction and detection according to DIN CEN/ISO TS 15216-2. Nine laboratories succeeded in the qualitative detection of all norovirus-contaminated and the non-contaminated samples. Eight laboratories were not able to detect the samples contaminated with 10^5 noroviruses. Two laboratories were incapable to detect samples with a titer of 10^7 noroviruses. Overall, the result shows that only very high norovirus titers (10^7 per 25 g sample) were reliably detected.

Reference: J. Pfannebecker, B. Becker (2018): Detection of human noroviruses in frozen strawberries: Comparative study of the working group for food-associated viruses (Arbeitsgruppe Lebensmittelassoziierte Viren - ALV) 2017. Journal of Food Safety and Food Quality. Under review.

Keywords: human norovirus, viruses in soft fruits, frozen strawberries, DIN CEN/ISO TS 15216-2

Microbiological spotlights

P5.125

Food safety as poultry meat consumers understand it: Investigation at shopping points in Slovenia

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Campylobacteriosis is the most frequently reported foodborne bacterial enteric infection in developed countries. Poultry meat represents the single most important source of infection. The prevalence of pathogenic campylobacters on poultry meat in retail outlets is high, so it is crucial for poultry consumers to know the measures that eliminate the risk of *Campylobacter* infection. The aim of our study was to investigate Slovenian consumers' knowledge and practices in poultry meat handling during purchase, transport and preparation in the home kitchen, and to assess the awareness of the microbiological risk associated with poultry meat, with an emphasis on *Campylobacter*. A cross-sectional study of consumer food safety knowledge and habits was conducted from March to April 2015 in front of supermarkets in all different statistical (geographic/demographic) regions of Slovenia. A convenience sample of 560 consumers was obtained. Gender and age distribution were controlled by 28 interviewers, each of whom distributed 20 questionnaires. The questionnaire included 33 questions divided in four parts. Descriptive statistical methods were performed for all the questions. For assessment of the association between consumers' characteristics and their knowledge, habits and attitude on microorganisms with emphasis on *Campylobacter* in poultry meat, the Chi-square test was done. Slovenian consumers are aware of food safety, but some critical violations are still made: use of same cutting board for the poultry meat and other foods, not assuring cold chain during transport, inconsistent storage of poultry meat, thawing of poultry meat at room temperature. One intriguing finding of the research is the insufficient knowledge of some basic food microbiology and *Campylobacter* as only 57.4% knew that retail poultry meat can be contaminated with potentially pathogenic bacteria and over 80% did not know about *Campylobacter* and its importance. Men, people with lower educational attainment, young adults and seniors showed less knowledge and awareness than others did. One of the important conclusions can be that only by knowing consumers' attitudes and behaviour and by detecting gaps in their knowledge changes can be made in the direction of education.

Keywords: food safety, consumer awareness, microbiological risks, poultry meat, Campylobacter

Microbiological spotlights

P5.126

Murine calicivirus inactivation during the process of fermented sausages

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Introduction: Italian salami is a raw fermented sausage that locally can be produced with pork meat, fat and liver. A preliminary study was performed to investigate the survival of murine calicivirus during the process of salami. This microorganism was used as a surrogate of the zoonotic hepatitis E virus (HEV).

Material and method: Continuous RAW264.7 cell line (mouse monocyte macrophage) was used in order to verify virus viability/infectivity. The titer ($TCID_{50}$/mL) was calculated according to Reed & Muench formula.
Batter was either inoculated with 1% v/w viral suspension (starting titre 7 Log $TCID_{50}$/mL) or not (negative control). 3 replicates from one batch were analyzed at each sampling time. Process followed company specification.
Samples (50 g) were blended and 2 g were lysed with glass beads, and centrifuged. The supernatants were filtered (0.22 µm) and inoculated on RAW264.7 cell line.
The viral titre was determined both by visualization on 24-well plates (100X optical microscope), and by end point-PCR on the samples criolysates. Batter/salami were homogenized in peptone water (1/10 v/w). Mesophilic lactic acid bacteria (LAB) were enumerated by plate count. Temperature, pH and a_w were monitored at each sampling time.
Virus concentration was reported as log $TCID_{50}$/mL; microbiological count (CFU/g) was reported as log CFU/g. The average and standard deviation values were determined on 3 samples. Significance was statistically analysed by Student *t*-test at a 95% confidence interval ($P< 0.05$).

Results: Results showed that after inoculum the viral titre was about 4 Log $TCID_{50}$/mL while, after 49 days, the concentration was 1 log $TCID_{50}$/mL.
LAB count increased in the first 3 days (fermentation) from 6.2 to 8.6 Log CFU/g and pH dropped from 5.65±0.02 to 4.95±0.03; while no significant differences were observed in LAB concentration, pH showed a linear increase (5.57±0.20) until the end of process. The a_w values decreased from 0.96±0.01 to 0.90±0.02.

Discussion: The obtained results in this preliminary study showed that the salami process inactivated murine calicivirus (HEV surrogate) significantly.

Keywords: virus inactivation, HEV surrogate, liver, salami

Microbiological spotlights

P5.127

Characterization of isolates of monophasic *Salmonella enterica* serovar Typhimurium (1,4,[5],12:i:-) in Estonia

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Salmonella spp. is one of the four key global causes of diarrhoeal diseases (WHO, 2017). According to the Health Board, the salmonellosis is the second leading bacterial enteric infection in Estonia with 279 reported cases (21.1 cases per 100,000 inhabitants) in year 2017 and the link with contaminated food can be assumed in 54 cases (Terviseamet, 2018).
The main aim of this study was to give an overview of the occurrence and serovar diversity of *Salmonella* spp. in food production chain in 2013 - 2017 in Estonia. The secondary aim was to characterize and compare the isolates of *Salmonella enterica* serovar Typhimurium monophasic variant originated from food and patients in 2015 - 2016 in Estonia.
During the years 2013 - 2017 the most prevailing *Salmonella* serovars isolated from food production chain were *S*. Derby, *S*. Typhimurium and *S*. Infantis with the prevalence of 34%, 11% and 8%, respectively. Monophasic *S*. Typhimurium represented the second most frequent *Salmonella* serovar in samples taken from meat processing plant or at retail level. During the same period the most often isolated *Salmonella* serovars in human patients were *S*. Enteritidis, *S*. Typhimurium and its monophasic variant 1,4,[5],12:i:- and *S*. Infantis. Similarly to many other countries the prevalence on monophasic *S*. Typhimurium has been emerged during last years both in food and among human population.
In present study, 38 *S. enterica* 1,4,[5],12:1:- isolates of human, animal and food origin were characterized. Isolates were confirmed as monophasic variant by polymerase chain reaction and further characterized by phenotypic (antimicrobial resistance) and molecular typing (pulsed-field gel electrophoresis) methods.
All monophasic *S*. Typhimurium isolates of human origin were simultaneously resistant against ampicillin, tetracycline and sulfamethoxasole. One isolate of them was multiresistant against nine antimicrobials. The isolates from food production chain showed similar resistance profile. Multiresistance was determined for 24 isolates out of the 31 isolates of food chain origin.
Twenty one XbaI-PFGE profiles among monophasic *S*. Typhimurium isolates were revealed. Altogether seven PFGE clusters based on 100% similarity were revealed among human and food chain monophasic *S*. Typhimurium isolates, and three clusters shared both human, swine and pork isolates. The latter finding can refer a role of animal sources in the spread of this pathogen in Estonian food chain.

Keywords: Salmonella, food production chain, Estonia

Microbiological spotlights

P5.129

UV-C inactivation of bacterial toxins

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The two most important toxin-producing bacteria implemented in food intoxication are *Bacillus cereus* and *Staphylococcus aureus*. *S. aureus* is found in 30-50% of the population in the nostrils and skin. Some strains can produce Staphyloccocal enterotoxins (SE) and cause food -borne intoxications. The toxin-producing *B. cereus* causes two types of food poisoning leading to the emetic syndrome or the diarrhoeal syndrome. The diarrhoel syndrome is predominantly linked to two multicomponent enterotoxins: the nonheamolytic enterotoxin (Nhe) and the haemolytic BL (HBL). These enterotoxins are heat sensitive, while the emetic syndrome is caused by cereulide, a peptide with remarkable resistance to heat, even at alkaline pH. An important technology to disinfect liquids, packaging materials and (food) contact surfaces is UV-C, which has germicidal effects at a wavelength of 254 nm. In literature some data is available on UV-C inactivation of aflatoxins, yet data is lacking on bacterial toxins.
Enterotoxins were produced by culturing toxin-producing strains 24h in TSB at 30°C or 37°C for *B. cereus* and *S. aureus*, respectively. Toxins were extracted by centrifugation and supernatants was diluted in distilled water before UV-C inactivation. Cereulide was produced by flooding BHI agar plates with 1mL of an overnight BHI culture and incubating the plates for 24h at 30°C. UV treatment was done in a UV chamber with 2mL of sample in petridishes and for two hours, which is equivalent to a dose of ca. 43.5 W/cm^2. *B. cereus* enterotoxins were analysed with Duopath (Merck), while *S. aureus* enterotoxins were analysed with VIDAS 2 (bioMérieux). Cereulide was extracted with methanol and detected using the boar semen assay.
Our results indicate that ca 43.5 W/cm^2 has no or little effect on inactivation of cereulide and Nhe. HBL was inactivated (to levels below LOD) in the diluted sample although not in the undiluted sample implying that the inactivation is only partial and dilution brought the remaining amount of HBL (detected component) below LOD, a possibility that cannot be ruled out for Nhe as well. For *S. aureus* toxins, this UV treatment resulted in a negative VIDAS 2 test results for toxins C and B. Increasing the dose to ca. 64 W/cm^2 resulted in inactivation of toxins A and D, but not E. These data should be confirmed with a quantitative assay, nevertheless they suggest that UV-C does inactivate SE's, except SEE, and might reduce the concentration of active Nhe and HBL.

Keywords: Bacterial toxins, UV-C inactivation, Bacillus cereus, Staphylococcus aureus

Microbiological spotlights

P5.130

Actual topics from horizontal standardization in food microbiology at DIN NAL working group "Microbiology in the food chain"

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The International standardization of methods in food microbiology are worked out in the International body ISO/TC 34/SC 9 „Food Products - Microbiology" in close cooperation with the European body CEN/TC 275/WG 6 „Microbiology of the food chain".
The scope of these bodies is the development and publication of horizontal methods in the field of microbiological analysis of the food chain from primary production stage to food and animal feed products, including the environment of food production and handling.
The European Union decided to base its harmonized European standards wherever possible on International Standards.
In the Vienna Agreement of June 1991, ISO and CEN formally committed themselves to basing their work on international standardization. In other words, whenever possible, CEN adopts International Standards as European standards which are, in turn, promulgated as national standards in each of the CEN member countries, e. g. for Germany as DIN EN ISO standards.
Food microbiology experts of both ISO/CEN standardization bodies meet in an annual plenary meeting, lastly during the week of 19-23 June 2017 in Tokyo, Japan. The next plenary meeting will take place in Lausanne, Switzerland, on 18-22 June 2018.
This International standardization work is mirrored by the German standardization body DIN NAL Working group "Microbiology of the Food Chain". Experts are delegated to participate to the International standardization work and the plenary meetings.
In the framework of a CEN mandate for food hygiene legislation addressed to CEN, since 2006 there were 15 of those standards elaborated or revised which are given as a reference method by EC Directive 2003/99/EC on microbiological criteria. Beside the elaboration and revision of these standards, a main part was the validation of these standards. This project has been finished by mid 2017 and the new or revised methods have been recently published by mid 2017.
Mostly all new or revised standards have now a horizontal scope and include at least a flow diagram of the method, the formulation, preparation and performance testing of the culture media and a summary of the method validation studies and performance characteristics including the used food samples.
Alongside classical conventional methods using, new elaborated standards cover molecular genetic methods.

Keywords: Standardisation, ISO, CEN, DIN, NAL, Microbiology in the Food Chain, ISO/TC34/SC9, CEN/TC275/WG6

Microbiological spotlights

P5.131

Environmental stress-induced bacterial lysis and extracellular DNA release contribute to *Campylobacter jejuni* biofilm formation

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Campylobacter jejuni is a microaerophilic bacterium and supposed to persist in a biofilm to antagonize the environmental stress. This study investigated the influence of environment on *C. jejuni* biofilm formation and the corresponding mechanisms. We report an extracellular DNA (eDNA)-mediated mechanism of biofilm formation in response to aerobic and starvation stresses. The eDNA was determined to be a major constitutional material of *C. jejuni* biofilm and released from bacterial lysis. The deletion mutation on the stress response genes *spoT* and *recA* enhanced the aerobic influence by stimulating lysis and increasing eDNA release. Flagella were also involved in biofilm formation, but mainly contributed to attachment rather than induction of lysis. The addition of genomic DNA from either *Campylobacter* or *Salmonella* resulted in a concentration-dependent stimulation effect on biofilm formation but not due to forming a pre-coating DNA layer. The degradation of DNA disrupted biofilm structure and dispersed the encased bacteria. In a dual-species biofilm, eDNA separated *Campylobacter* and *Salmonella* at distinct spatial locations that protect *Campylobacter* away from the oxygen stress. Our findings demonstrated an essential role and multi-functions of eDNA in biofilm formation of *C. jejuni*, including facilitating initial attachment, establishing and maintaining biofilm structure and allocating bacteria cells.

Keywords: Campylobacter jejuni, biofilms, extracellular DNA, stress response, environmental stress

Microbiological spotlights

P5.132

Evaluation of antimicrobial activities of plant aqueous extracts against different strains of *Salmonella* Typhimurium and their application to improve safety of pork meat

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Naturally derived compounds have always been used in food industry not only for their flavoring properties but also for their antimicrobial activities. However, the application of plant aqueous extracts as natural food preservatives has not been extensively studied.

The purpose of this study was to examine the antimicrobial activity of ten plant aqueous extracts against three strains of *Salmonella (S.)* Typhimurium and evaluate their application in food industry.

Oregano, thyme, calendula, basil, laurel, rosemary, spearmint, corn silk, and garlic aqueous extracts collected by hydrodistillation and a commercially acquired aqueous oregano extract were tested for their *in vitro* antimicrobial efficiency against three *S.* Typhimurium strains (4/74, FS8, FS115) at 4°C and 37°C. Subsequently, pork meat was inoculated with FS8 strain (~6logcfu/g), marinated for 3h in aqueous laboratory or commercial oregano (OLE or OCE) extract with or without oregano essential oil (0.2%), covered with sodium alginate edible coatings prepared of the above extracts and stored at 4°C for four days.

The antimicrobial effect of extracts was mainly plant and storage temperature-dependent. Oregano extract exhibited the strongest antimicrobial activity independent of the storage temperature; thyme and calendula extract were also active against the three pathogenic strains for both storage temperatures. At 4°C, pathogen populations were reduced for all plant extracts apart from that of garlic. Higher populations were enumerated for FS8 strain ($P < 0.05$) during 7th and 8th day of storage for calendula extract stored at 4°C. At 37°C oregano, calendula, thyme, and basil displayed a bactericidal effect, eliminating or reducing enumerated populations by approximately 4.0 log(cfu/ml) within 9-24hours. Garlic, spearmint, rosemary and corn silk permitted its growth. Contrary to the above tested plants, laurel had a strain-dependent effect on *S.* Typhimurium at 37°C; FS8 increased by 1.3 log(cfu/ml), while 4/74 and FS115 were reduced by 2.2 and 1.5 log(cfu/ml) respectively. Combination of marination and edible films containing essential oil enhanced antimicrobial action of the extracts in pork meat, especially when commercial aqueous oregano extract was used.

These findings suggest that plant aqueous extracts, derived as by-products of essential oil production, could serve as alternative natural food preservatives

Keywords: aqueous extracts, salmonella, edible films, pork meat

Microbiological spotlights

P5.133

Isolation and mycotoxigenic activities of some mycofl ora of dried red crayfish *(Procambarus clarkii)*

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Red crayfish(*Procambarus clarkii*) is a freshwater specie that can be found in all continents of the world usually as an invasive pest. The consumption of crayfish as food by humans and animals cuts across borders globally. Crayfish when cooked and consumed supplies vitamins, protein and minerals as nutrients. This study estimated the mycoflora and mycotoxin contamination of dried red-crayfish samples purchased randomly from five different markets in Ibadan, Nigeria. The moisture content of the samples was found to be less than 9%.The mycoflora were isolated and identified using standard microbiological methods. Some of the isolates were assayed for mycotoxin production using the thin layer chromatography(TLC) plug agar method. Four different fungal species were found associated with the crayfish samples sold. These were *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* specie and *Rhizopus* specie. *Aspergillus niger* has the highest frequency of occurrence(51.1%) followed by *Aspergillus flavus*(33.3%), *Rhizopus* sp.(14.4%) and *Penicillium* sp.(1.1%).Result of the mycotoxin analysis done showed that some of the isolated fungal species produced various mycotoxins. Findings from this study are useful in developing and establishing public health standards as consumption of this crayfish expose the consumers to the probable toxic effect of the mycotoxins produced by the fungi. A better understanding of the ecology of dried red crayfish distribution systems will facilitate the development of effective quality control strategies that will ensure safe and high-quality crayfish for consumers.

Keywords: crayfish; mycoflora; mycotoxin; Procambarus clarkii; thin layer-chromatography

Microbiological spotlights

P5.134

Comparison of the growth kinetics of *Chryseobacterium pennae* sp. nov., isolated from feather waste and *Chryseobacterium carnigallinarum* sp. nov., isolated from chicken portion

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Food spoilage accounts for a great loss in the food industry with microbial spoilage being the most common cause of spoilage. Temperature plays a crucial role in the growth and spoilage activities of microorganisms and influences enzymatic production and activities. It is an important indicator of pathogenicity and hence should be investigated for every novel strain. The effect of temperature on growth was investigated using two novel strains of Chryseobacteria (1_F178 [*Chryseobacterium pennae* sp. nov.] and 5_R23647 [*Chryseobacterium carnigallinarum* sp. nov.] isolated from feather waste and chicken portions respectively) and *Chryseobacterium carnipullorum* DSM 25581T with the results compared to that of *Pseudomonas fluorescens* DSM 4358 which is a major food spoilage organism in the food industry. The study was done on a temperature gradient incubator with temperatures ranging from 14 ˚C to 47 ˚C and also at 4 ˚C. Growth was monitored by measuring the optical density at 600 nm. The experiment was done in triplicate per bacteria. Linear regression analysis was used to determine the maximum specific growth rate (μmax) at each temperature while an Arrhenius model was used to describe the linear relationship between specific growth rate and temperature. The results showed *P. fluorescens* having the highest μ_{max} of 0.60 h^{-1} at an optimum temperature of 30.6 ˚C. *C. carnipullorum* had the highest μ_{max} (0.55 h^{-1}) for the Chryseobacteria at an optimum temperature of 32.5 ˚C followed by 1_F178 (0.44 h^{-1}) and 5_R23647 (0.37 h^{-1}) at optimum temperatures of 31.5 ˚C and 26.3 ˚C respectively. The high growth rates of *P. fluorescens* confirm why they outgrow Chryseobacteria in spoilt food samples. Growth observed at 4 ˚C for all strains indicate their ability to grow in refrigerated food products which is detrimental to the food industry. The optimum temperatures (below 37 ˚C) of these novel strains show them to be non-pathogenic. The Chryseobacteria strains had optimum growth at mesophilic temperatures. This will be beneficial in the feather disposal process which the poultry industry is facing. Keratinases degrade keratin (main component of chicken feathers) and are mainly produced at mesophilic temperatures with some *Chryseobacterium* species having keratin-degrading abilities. This can replace the chemical degrading processes which are environmentally unfriendly and require a lot of energy thus making the process expensive.

Keywords: Temperature, Growth rate, Chryseobacterium, Keratinases, Food spoilage, Poultry, Keratin

Microbiological spotlights

P5.135

The inhibitory effect of carvacrol, t-cinnamaldehyde, carrot seed essential oil and hop β-acids on spore germination of group II C. *botulinum* NCTC 11219

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Non-proteolytic (group II) *Clostridium botulinum* is an important concern for the safety of minimally processed chilled foods, as it can grow and produce botulinum neurotoxin under refrigeration. However, *C. botulinum* studies are cumbersome because of bio-safety and dual use restrictions. To overcome these barriers, we have previously constructed an atoxigenic gIICb strain by deletion of the botulinum neurotoxin gene *bont/E* [1].

In recent years, plant extracts, essential oils (EO) and their constituents, are gaining interest as natural food preservatives. It has been well established that several EO components have a broad antimicrobial activity against a wide range of foodborne pathogens and spoilage bacteria. However, limited data are available on their effects on endospores, particularly those of anaerobes like *C. botulinum*. In this study, we used the atoxigenic strain NCTC 11219 Δ*bontE::ermB* to assess the inhibitory effects of carvacrol, *trans*-cinnamaldehyde, carrot seed EO and hop β-acids on vegetative cell growth and on nutrient-induced spore germination.

The effect on vegetative cell growth was established by determining the minimal inhibitory concentration (MIC) of the compounds by the broth dilution method. The effect on spore germination was measured by determining the loss of heat resistance in an optimal germinant mixture (L-alanine, L-lactate and HCO_3^-). Furthermore, the release of Ca^{2+}-DPA was also determined as an additional indicator of the early stages of spore germination.

The MIC values of the compounds varied over several orders of magnitude, from 2.5 ppm (hop β-acids) to 5000 ppm (carrot seed EO). For all the tested compounds, with the exception of the hop acids, spore germination was inhibited >90% at MIC and >50% at 0.1xMIC. The hop β-acids did not inhibit spore germination at concentrations up to 4xMIC. These results suggest that hop β-acids have a different mode of action than the other compounds. The combination of compounds with a low MIC and compounds that strongly inhibit spore germination may lead to more effective natural antimicrobials against *C. botulinum* in chilled foods.

Keywords: Clostridium botulinum, spore germination, natural antimicrobials, MIC-values

Microbiological spotlights

P5.136

Antimicrobial resistance in *Staphylococcus* species and lactic acid bacteria from African fermented foods

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Antimicrobial resistance (AMR) is a current global public health threat and the food chain constitutes one of the dissemination routes.

Staphylococcus species from alkaline fermented foods (Bikalga, Soumbala, and Ntoba Mbodi) and lactic acid bacteria (LAB) from an acidic fermented product (Nono) were identified and screened for AMR phenotypic and genotypic characteristics. The susceptibility of the bacteria to 25 antimicrobials and the presence of 38 AMR genes were studied. Also, their ability to transfer AMR genes to other bacteria was investigated by conjugation experiments using *Enterococcus faecalis* JH2-2 and *E. faecium* BM 4105 as recipients.

The *Staphylococcus* isolates (90) included 15 coagulase negative and positive species and 24 rep-PCR/PFGE clusters. The dominant species were *S. sciuri* (in Bikalga and Ntoba Mbodi) and *S. simulans* (in Soumbala). All isolates were susceptible to gentamycin, kanamycin, streptomycin, and vancomycin, but for the other antimicrobials, their susceptibility was variable. For example, all clusters of *S. arlettae*, *S. cohnii*, *S. gallinarum*, *S. haemolyticus*, and *S. sciuri* were resistant to clindamycin whereas the other species were not. Out of six clusters of *S. sciuri*, only one was resistant to erythromycin. Genes encoding resistance to penicillin (blazA), chloramphenicol (*cat501*), trimethoprim [*dfr(A)*, dfr(G)], erythromycin (*erB*, *msrA*, *mphA*), methicillin (*mecA*), and tetracycline [*tet* (K)] were detected. Transferability screening of erythromycin, chloramphenicol and tetracycline genes generated *E. faecalis* JH2-2 transconjugants that became resistant to the antimicrobials, but none of the corresponding genes was detected by PCR.

The LAB (100) included seven species and 11 rep-PCR clusters with *Lactobacillus fermentum* being the dominant species. They were susceptible to ampicillin, ceftriaxone, oxacillin, trimethoprim/sulfamethoxazole and rifampicin, but the susceptibility was variable for other antimicrobials. For instance, *L. fermentum*, *L. senioris*, *Enterococcus thailandicus*, and *Streptococcus infanterius* were resistant to tetracycline and erythromycin while the other species were susceptible to the antimicrobials. Tetracycline [*tet*(M), *tet*(S)] and streptomycin (*aadE*) resistance genes were detected in the *E. thailandicus* and *Strep. Infanterius* bacteria. Conjugation experiments generated tetracycline-resistant *E. faecalis* JH2-2 transconjugants that have acquired the *tet*(S) and *aadE* genes through a plasmid-mediated transfer.

Keywords: African fermented foods, Staphylococcus, lactic acid bacteria, antimicrobial resistance

Microbiological spotlights

P5.137

Isolation and mycotoxin production of some mycofl ora of West African soft cheese ('Wara')

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'Wara' is a natural and an unripened-cheese consumed in Nigeria and several parts of West Africa.This cheese is prepared by coagulating fresh cow milk with a leaf extract of sodom- apple (*Calotropis procera*).'*Wara'* is a very good source of protein, fats and minerals such as calcium, iron and phosphorus, vitamins and essential amino acids, thus making it an important food in the diet of both old and young people.This study was carried out with the aims and objectives to: isolate and identify some mycoflora associated with the West-African soft cheese(wara); and then detect the mycotoxins produced by the isolated mycoflora. 27 samples of cheese('wara') obtained from three different points of collections within three different markets were sampled in Ibadan, Nigeria for fungal contaminations. Fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus oryzae*, *Penicillium expansum*, *Fusarium flocciferum*, *Candida valida* were isolated and identified using standard microbiological methods. *Aspergillus niger* has the highest percentage frequency of occurrence (33.87%) followed by *Aspergillus flavus* (27.14%) and *Penicillium expansum*(13.92%) then *Fusarium flocciferum*(5.80%), *Aspergillus oryzae (*18.56%) and *Candida valida* (0.23%).Three of the isolates were assayed for aflatoxin production using the enzyme linked immunosorbent serological assay(ELISA) technique and it was found that they all produced aflatoxins at the same proportion. An insight into the microbiological, environmental, processing and handling factors that facilitate contamination will allow development of evidence-based policies, technologies and procedures aimed at reducing the risk of contamination of this cheese.This study highlight some recommendations for policy-making.

Keywords: cheese; aflatoxin; fungi; ELISA; mycoflora; ´wara´

Microbiological spotlights

P5.138

Investigation on the antibacterial properties of *Ziziphora cliniopodiodes* and *Thymus daenensis* essential oils against some foodborne pathogenic bacteria

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Due to the side effects of chemical preservatives and the *increasing consumers´ demand* for herbal and natural compounds, the use of essential oils as a food preservative have been widely studied. The aim of the present study was to investigate the chemical *compound* of Ziziphora cliniopodiodes and Thymus daenensis essential oils and their antibacterial properties against some food borne pathogenic *bacteria.* The eessential oils were analysed by gas chromatography/mass spectrometry (*GC/MS*) .A micro-dilution method in 96-well plate was used to evaluate the minimum inhibitory concentration (MIC) of essential oils. According to the results of MIC, the evaluation of minimum bactericidal concentration (MBC) of essential oils were determined. All experiments were done triplicate and average of data were determines as results of MBC and MIC. Statistical analysis conducted by SPSS software edition 22. Among the detected compounds, Ziziphora clinipodiodes essential oils by gas chromatography coupled with mass spectrometry (GC/MS), Thymole (34.25%) Pulegoneand (14.41%), Carvacrol (10.91%) and in Thymus daenesis, Carvacrol (37.20%) and octadecenuec acid methyl ester (21.67%) were dedicated the highest values. The results of this research revealed that minimum inhibitory concentratin of ziziphora clinipodiodes essential oils and Thymus daenesis were 0.61-1.25 and 0.31-1.25 mg/ml, respectively. Generally, antibacterial effect of ziziphora clinipodiodes were greater than Thymus daenesis. In addition, sensitivity of gram positive bacteria in both essential oils were evaluated higher than gram negative bacteria.

Keywords: Ziziphora clinopodioides, Thymus daenensis, essential oils, Minimum inhibitory concentration, Antiba

Microbiological spotlights

P5.140

Occurrence and antibiotic resistant phenotypes of *Staphylococcus aureus* from locally-pasteurised cow milk (Kindirmo) sold in a community in central Nigeria

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Staphylococcus aureus is one of the leading causes of gastroenteritis acquired from contaminated foods such as milk and milk products. However, there is paucity of informing regarding the occurrence of this pathogen in milk products in central Nigeria. This cross sectional study investigated the occurrence and antibiogram of *S. aureus* from locally-pasteurised milk (kindirmo) sold in a community in Central Nigeria. A total of 123 samples were obtained from three different sampling points; Nasarawa Market (41), Tammah Area (41) and Gunki Settlement (41), and screened for the presence of *S. aureus using standard microbiological techniques.* Confirmed *S. aureus* isolates were further subjected to antimicrobial susceptibility test using the agar disc diffusion technique. The cumulative occurrence of *S. aureus from the samples examined was* 9.76% (n = 12); with 14.63% (n=6) from Nasarawa Market and 7.32% (n=3) from Tammah Area and Gunki Settlement respectively. High instances of antibiotic resistance by the isolates were shown to amoxicillin (100%), ampiclox (100%), norfloxacin (83.3%), rifampicin (83.3%) and streptomycin (66.7%). Ten (10) different resistant phenotypes were observed with varying combinations of 2, 3, 4, 5 and 6 antibiotics occurring at 8.3% (8) and 16.7% (2) respectively. All the isolates had multiple antibiotics resistant (MAR) index of 0.2 and above depicting availability and misused of antibiotics in the study area. The presence of *S. aureus* in locally-pasteurised milk (kindirmo) and their resistance to commonly used antibiotics pose a great health risk for consumers of this milk product. There is a need to implement appropriate control measures to reduce contamination as well as the spread of resistant *S. aureus* strains and the burden of disease in humans.

Keywords: Staphylococcus aureus, locally-pasteurised cow milk (kindirmo), Antibiotic Resistance, Nigeria

Microbiological spotlights

P5.141

Molecular detection of virulence and resistance genes in *Salmonella enterica* serovar Typhi and Paratyphi A, B and C isolated from human diarrhea samples and lettuce in Burkina Faso

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Objectives: In Burkina Faso (BF), dirty water, in particular those of the stoppings and the gutter are used for irrigation of vegetables. The aim of this study is to contribute to the knowledge on the molecular level of *Salmonella* Typhi and Paratyphi circulating in the hospitals and environment next to hospitals in BF.

Methods: *Salmonella* Typhi and Paratyphi strains isolated from patients between 2009 to 2015 and lettuce samples isolated in 2014 in BF were characterized by simple PCR using specific primers.

Results: Out of 100 *Salmonella* isolated, 53% were from human and 47% from lettuce samples. Globally, the highest prevalence was observed with *inv*A, *mis*L, *pip*D, *orf*L and *spv*R genes in 97%, 96%; 74%; and 21%. Forty of these isolates carried class 1 integron, 31 from clinical samples and 9 from lettuce samples. Sequencing showed seven different gene cassette arrangements, with *aadA1* in 13/15 strains, *aadA7* and *aac(3)-Id* in 2/15 strains. Eight percent (8/100) of *Salmonella* harbored *gyrB* and *parE* genes with 6 from clinical and 2 from lettuce isolates. Sequencing showed no mutation in these genes. Three distinct PFGE types were observed from clinical samples with 90-95% similarity in each case. All *Salmonella* from lettuce had similar pulsotypes.

Conclusion: This study showed the diversity virulence and resistance genes harbored of *S.* Typhi and Paratyphi from both clinical and lettuce samples in BF. Lettuce is a potential source of transmission of *Salmonella* causing diarrhea among human in BF.

Keywords: Salmonella Typhi, resistance genes, lettuce, clinical samples, Burkina Faso.

Microbiological spotlights

P5.142

Detection of *Aspergillus flavus* link on commercial feeds

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This study was conducted to determine the growth of *Aspergillus flavus* on commercially produced feeds after different lengths of exposure in the market.

Serial dilution method was used to determine the *Aspergillus flavus* load of the three brands of feeds. Selecta got the highest average fungal load of $4x10^3$ cfu/gram after three days of exposure, followed by Purina with $1x10^3$ cfu/gram and finally B-Meg with too few to count.

The environmental parameters gathered showed that favorable conditions were favorable for the growth of *Aspergillus flavus.* the average temperature of feeds was 30.67C° with moisture content of 13.27% and a pH of 6.34.

The test for aflatoxin revealed that the *Aspergillus flavus* strain isolated from Purina at two days exposure is positive of either aflatoxin B_1 or B_2. These metabolites are the most toxic and carcinogenic substance of biological origin.

Keywords: Aspergillus flavus, aflatoxin, aflatoxin B1, aflatoxin B2

Microbiological spotlights

P5.143

Evaluation of DNA extraction methods for PCR-based detection of bacterial pathogens in food of animal origin

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Several outbreaks caused by food-borne pathogenic bacteria have been associated with the consumption of animal origin food products. Among the alternative rapid detection methods, real-time PCR is considered to be the fast and accurate tool allowing quantitative detection of the pathogens. However, a crucial step for the success of PCR-based detection is an extraction of template DNA. In our study, two laboratory procedures - DNA extraction using phenol-chloroform-isoamylalcohol and modified CTAB protocol for animal samples and five commercial kits intended for DNA extraction from food were evaluated based on the analysis of total extracted bacterial DNA from food sample as well as from model food samples artificially contaminated by defined concentrations of *Listeria monocytogenes* and *Escherichia coli* as models for G+ and G- bacteria, respectively. Cheese samples were at first analysed by culture methods for total bacterial counts and *L. monocytogenes* and *E. coli* quantity or presence and by *L. monocytogenes*-specific real-time PCR targeting *act*A gene and *E. coli* specific real-time PCR targeting *sfm*D gene. Sample preparation using bead-based homogenization was optimized and applied for all seven evaluated methods. Evaluation of total extracted bacterial DNAs from cheese samples was performed by amplification of 1013 bp fragment of 16S rDNA by conventional PCR and electrophoresis, quantitative amplification of 215 bp fragment of 16S rDNA. DNAs extracted from artificially contaminated samples were evaluated by target-specific TaqMan real-time PCRs for *L. monocytogenes* and *E. coli*. The results of the study suggest that all seven tested procedures can be used for the extraction of intact and amplifiable bacterial DNA, as well as DNA from selected food-borne pathogens with defined variability relevant to certain purposes.
This work was supported by Slovak Research and Development Agency under the contract No. APVV-16-0119.

Keywords: DNA extraction, food-borne pathogens, food samples

Microbiological spotlights

P5.144

Rapid spectroscopic detection of *Cronobacter sakazakii* using anti-C. *sakazakii* functionalized gold nanoparticles

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Pathogenic bacteria contribute to various globally important diseases, *Cronobacter sakazakii* is an opportunistic foodborne pathogen that can infect newborns. There is a requirement for accurate techniques to rapidly detect and identify pathogenic bacteria, with the aim to prevent spreading of pathogenic bacteria and to assure food safety. In this study, we developed a rapid, sensitive, and label-free method for detection of bacteria pathogens using targeted colloidal nanoparticles (NPs) and applied it to *C. sakazakii*. The method is based on functionalization of poly (ethylene glycol) brush stabilized gold nanoparticles by biotinylated anti-*C sakazakii* antibodies via streptavidin binding. Results demonstrate that the nanoparticle stability and degree of functionalization with anti-*C sakazakii* could be controlled and that quantitative and specific targeting of *C. sakazakii* could be achieved. The rapid three-step method achieved a limit of detection of 1.8×10^2 colony forming units per mL using a common UV-Vis spectroscopy readout. In summary, our method offers the significant advantages of short assay time, high sensitivity and a simple operating procedure for pathogenic bacteria detection.

Keywords: Cronobacter sakazakii, Rapid detection, Gold Nanoparticle

Microbiological spotlights

P5.145

Development of a multiplex real-time PCR system for the detection of food-relevant *Listeria* species and identification of *Listeria monocytogenes* in a single reaction

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Listeria monocytogenes is considered to be one of the most important food-borne pathogens. It can cause severe disease in humans with mortality rates up to 33 %. Infections have been traced to the consumption of contaminated foods that have short shelf lives, emphasising the need for rapid detection methods. *L. monocytogenes* is often found in samples that contain other *Listeria* species. They are therefore used as an indicator for the presence of *L. monocytogenes* and general process hygiene.
We developed a rapid, accurate and sensitive real-time PCR method for the simultaneous detection of food-relevant *Listeria* spp. and specific identification of pathogenic *L. monocytogenes* in a single reaction. The lyophilized reagents included in the **food**proof *Listeria* plus *L. monocytogenes* Detection LyoKit ensure minimal contamination risk. The number of known species belonging to the genus *Listeria* has increased from 6 to currently 18 in less than ten years. Most of the newly described species, however, differ substantially from the originally described *Listeria*, rendering them unsuitable as indicator organisms for the pathogenic *L. monocytogenes*. Based on phenotypic and genomic characteristics, a subdivision into new genera and *Listeria* "sensu stricto" that includes the species most closely related to *L. monocytogenes,* has been proposed. Although genetically distinct from these species, *Listeria grayi* shares important characteristics like motility and growth at < 7°C. The PCR assay detects all six *Listeria* sensu stricto species plus *L. grayi* in one detection channel, and the presence of *L. monocytogenes* is additionally detected in a second channel. The **specificity** of the LyoKit was verified by testing more than 150 in- and exclusivity strains. As expected, the non-motile *Listeria* sensu lato species were not detected. It is a great challenge to detect *L. monocytogenes* in an excess of other *Listeria* species. We validated the **sensitivity** of the detection and identification by spiking both enrichment medium and a pure culture of *L. innocua* (approx. 10^9 colony forming units per millilitre) each with serial dilutions of *L. monocytogenes*. Sample preparation was conducted with the **food**proof StarPrep Two Kit, and 25 µl of the extracts were tested with the LyoKit. A minimum of 10^2 cfu/ml *L. monocytogenes* could be identified with the method, *i.e.* even an excess of 7 log levels of another *Listeria* species did not impair the sensitive detection of the pathogenic *L. monocytogenes*.

Keywords: Listeria, real-time PCR, qPCR, PCR, multiplex, rapid method, Listeria monocytogenes

Microbiological spotlights

P5.146

Screening of lactic acid bacteria from Nono, a naturally Nigerian fermented cow's milk, for antimicrobial resistance

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Aim: To investigate the antimicrobial resistance (AMR) patterns of lactic acid bacteria (LAB) involved in production of *Nono*, a traditional fermented milk, especially in relation to transferable AMR genes.

Methods and Results: The LAB were identified by phenotyping and genotyping using rep-PCR and sequencing of the 16S rRNA, *rpoA* and *pheS* genes. They were then screened for AMR phenotypic and genotypic characteristics. The susceptibility to 18 antimicrobials and the presence of 28 AMR genes were studied. Furthermore, the ability of the isolates to transfer AMR genes to other bacteria was investigated by conjugation experiments using *Enterococcus faecalis* JH2-2 as the recipient. The gene transfer was screened in the potential transconjugants by PCR using total and plasmid DNA extracts. Implication of transposons in the transfer process was also investigated.
The LAB (100 isolates) included seven species and 11 rep-PCR clusters with *Lactobacillus fermentum* being the dominant species. The isolates screened were all susceptible to ampicillin, ceftriaxone, quinupristin/dalfopristin, oxacillin+2%NaCl, trimethoprim/sulfamethoxazole and rifampicin. However, their susceptibility to the other antimicrobials was variable according to the isolate and the antimicrobial. For instance, *Streptococcus salivarius* subsp *thermophilus*, *Leuconostoc pseudomesenteriodes* and *Lactobacilus delbruckii* were susceptible to tetracycline and erythromycin while the others were resistant to the antimicrobials. Genes encoding resistance to tetracyline [*tet*(M), *tet*(S)] and streptomycin (*aadE*) were detected in the *Enterococcus thailandicus* and *Strep*. *Infantarius*. Conjugation experiments using filter and non-filter mating methods generated tetracycline-resistant *E. faecalis* JH2-2 transconjugants that have acquired the *tet*(S) and *aadE* genes. The transfer of the genes was evidenced using the plasmid DNAs only, suggesting that the isolates acquired the genes through a plasmid-mediated transfer. No transposon was detected.

Conclusion: LAB from *Nono* exhibit variable AMR phenotypes and genotypes. The number of AMR genes detected and that of isolates hosting the genes were limited. However, the genes were transferable to *E*. *faecalis* JH2-2 and this constitutes a food safety issue. Since the isolates of *E. thailandicus* and *Strep. infantarius* contain transferable AMR genes, their use as multifunctional starter cultures is not recommended.

Keywords: Nono, lactic acid bacteria and antimicrobial resistance

Microbiological spotlights

P5.147

Afl atoxins (B1, B2, G1 and G2) contamination of rice in the Association of Southeast Asian Nations (ASEAN) member states

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Rice (*Oryza sativa*) is the important staple food for more than half of the world's population. There are 10 member states in ASEAN: Brunei Darussalam, Cambodia, Indonesia, Lao PDR, Malaysia, Myanmar, Philippines, Singapore, Thailand and Vietnam. According to the FAO in 2017, Thailand and Vietnam are the world's leading rice exporter. Mycotoxin can contaminate agricultural commodities in both pre- and post-harvest steps. Due to their highly toxicity, aflatoxins have been the main focus on food safety. This study aims to evaluate the occurrence data of aflatoxins in rice in ASEAN member states over the last 5 years. The online database PubMed from National Library of Medicine, USA and Sciencedirect from Elsevier, the Netherlands were used to retrieve publications from the year 2013 to 2017 by using the following keywords: rice, aflatoxin and the name of the ASEAN member states. Interestingly, the search results showed only 4 articles from Singapore, Thailand and Vietnam. The study from Singapore showed that aflatoxin B_1 was not detected in 21 samples of imported broken rice from Myanmar. The study in Thailand included 240 rice samples from two harvesting periods (120 samples per season). 59% and 10% prevalence were found in samples from wet and dry season, respectively. The results showed that contamination of mycotoxins depends on environmental factors. 12 out of 240 rice samples exceeded the EU maximum level for AFB_1 of 2 µg/kg. The other 2 publications were the data from Vietnam. One study focused on dietary exposure and included only 3 samples of rice and products. The mean value of aflatoxin B_1 contaminated in the samples was 2.69 µg/kg. The other study included 111 rice samples. 24.3% were found to be contaminated with aflatoxins in the range 2.06-77.8 µg/kg. Small grains as rice has been mentioned that they are not the target for aflatoxin producing fungi. However extensively reports in the past indicated that aflatoxins are common found in rice. There are no regulatory limits of mycotoxin in Cambodia, Lao PDR and Myanmar. The regulatory limits of aflatoxin B_1 and total aflatoxins from 0 to 50 µg/kg were set up differently in the other 7 member states. The exported commodities have to meet the regulation of the importing countries. It is possible that a low quality commodity will be left for local consumption instead of destroying or diverting. Therefore annually aflatoxins contamination monitoring is needed for human health risk assessment.

Keywords: Aflatoxin, ASEAN, food safety, rice

Microbiological spotlights

P5.148

Species composition of toxigenic fungi isolated from sorghum kernels in Korea

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To assess the incidence and distribution of toxigenic fungi on sorghum in Korea, the samples were collected from 9 fields in Danyang and Yeongwol areas at two weeks intervals from heading to harvesting time in 2017. Fungal isolates were identified based on morphological characteristics and DNA sequence analyses using intenal transcribed spacer (ITS), translation elongation factor 1-α (TEF-1α) and β-tubulin (BT) genes. The most prevalent fungal genus was *Fusarium* followed by *Cladosporium*, *Actinomucor*, *Alternaria*, *Epicoccum*, *Bionectria*. *Fusarium* isolates were comprised of *F. graminearum* species complex (FGSC), *F. fujikuroi* species complex (FFSC) and *F. incarnatum-equiseti* species complex (FIESC). *F. graminearum* (150 isolates, 20.4%) and *F. thapsinum* (22.4%) were the most common on sorghum kernels, and *F. asiaticum*, *F. vorosii*, *F. proliferatum*, *F. incarnatum*, *F. tricinctum*, *F. chlamydosporum* were also recovered. Frequencies of *Fusarium* species increased rapidly from the milky stage to soft dough stage. Especially, frequencies of FGSC increased persistently across the growth stages (from 10.0% to 54.2%). These findings demonstrate the dominance of *Fusarium* species and growth stage-specific association of *Fusarium* species in sorghum agroecosystems.

Keywords: FGSC, FFSC, FIESC, fusarium, sorghum, toxigenic fungi

Microbiological spotlights

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Sub-lethal injury of *Listeria monocytogenes* in response to food related stress conditions

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Food processes within the food industry aim to eliminate microorganisms that may pose a potential threat for food safety, such as *L. monocytogenes* (Lm). However, a fraction of bacterial populations may be sublethaly injured. Apart from the evaluation of the processes' lethality, their design requires the quantification of bacterial sub-lethal injury to accurately assess the effectiveness of a treatment.

The viability and sub-lethal injury of Lm strains EGDe and ScottA (serotypes 4b, 1/2a) was investigated after exposure of 7log-CFU/ml cells to the following treatments: Starvation Stress, Starvation & osmotic stress (NaCl 7%), Lactic acid-LA pH3.0/8h; Heating 55°C/2h; Peracetic acid-PAA 10ppm/5days; quaternary ammonium compounds-QUAT 10ppm/5days, Benzalkonium chloride-BC 10ppm/4h, prepared in i)1/4 strength-Ringer; (R) and ii)Microcosm Water (ddH_2O-sterile; MW) incubated at 4°C and 20°C. Further, the effect of stress-adaptation (R-10ppm/4°C/24h) on cell-injury was evaluated under exposure to QUAT: i)M-10, ii)M-20, ii)R-20 (ppm).

TSAYE, was used as growth medium and determination of injury was performed with the maximum non-inhibitory concentration (MNIC) method. The MNIC of NaCl for Lm was 5%. Additionally, the recovery capacity on TSAYE+3% NaCl was evaluated.

The type of treatment affected both the cell viability and the extent of injury in Lm. Under exposure to LA-M/4°C, 69% of EGDe cells were injured compared to 98% of ScottA. In contrast, ScottA surviving population was 2logCFU/ml, higher than that of EGDe. Heating led to extensive cell injury compared to other treatments; while both strains were only decreased by 1log, cell-injury was ~99%. Exposure to BC resulted in 47% injury for EGDe in 2h and 66% injury for ScottA in 4h. On the other hand, PAA, did not affect EGDe, contrary to ScottA that was reduced by 1logCFU/ml after 5days in PAA-M/20°C, and survivors in TSAYE+NaCl 5% were 2logCFU/ml lower than TSAYE-survivors. The 80% of 4-week starved ScottA cells were injured despite their higher cell densities compared to EGDe. This observation was reversed for the two strains when 7% NaCl was added in the starvation medium. Adaptation to QUAT-R-10ppm/4°C/24h enhanced the resistance of cells upon exposure to QUAT-R-10ppm/4°C but also increased the injured subpopulations within the surviving cells.

Understanding factors, which influence the sub-lethal injury of Lm might contribute to better selection of treatments applied in food processing.

Keywords: cell injury, inactivation, Stress, disinfectants, Listeria monocytogenes

Microbiological spotlights

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Microbial safety of fresh produce in seven African countries

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Fresh produce consumption has increased in recent years because of its health-promoting properties. Similarly, the number of outbreaks caused by foodborne pathogens associated with fresh produce has increased worldwide. The aim of this study was to gain insight on food safety issues of fresh produce in seven African countries (Benin, Botswana, Burkina-Faso, Ghana, Nigeria, Sudan, and Uganda). In each country, food safety information regarding fresh produce was collected based on literature and expert opinion. Lettuce (*Lactuca sativa*) ranked first among fresh produce that could be involved in outbreaks by five out of the seven countries. The most incriminated pathogen in all the seven countries was pathogenic *Escherichia coli*, which is in agreement with a recent meta-analytical approach involving the same countries. Data on time and temperature profiles along the lettuce supply chains have not yet been documented in the selected African countries. Data on the prevalence and load of pathogens associated with fresh produce were mainly from grey literature and based only on conventional methods. In all the selected countries, there was no documentation or reporting on outbreaks caused by foodborne pathogens associated with fresh produce. The study highlights the necessity of implementing microbiological safety surveillance of fresh produce across African countries.

Keywords: Lettuce; Pathogenic bacteria; Food safety

Microbiological spotlights

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A study on the use of *Vernonia amygdalina* extract to control fungi associated with groundnut seeds

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A study was carried out on the use of *Vernonia amygdalina* del. extract to control fungi associated with groundnut (*Arachis hypogeae* L) seeds. *Aspergillus niger* van Tiegh, *A. flavus* link ex fries, *Cercospora arachidicola* Hori, *Phoma exigua* desm., *Macrophomina phaseolina* (Tassi) Goid, *Fusarium oxysporium* schl., *Cercosporella* sp and *Phyllosticta* sp were isolated from groundnut seeds obtained from retail markets in Rivers state. The water and ethanolic extracts of *Vernonia amygdalina* significantly (P=0.05) inhibited the spore germination and vegetative growth of *Phoma exigua* and *Macrophomina phaseolina*. Water extract was most active for *Phoma exigua* with an ED_{50} of less than 12.0%, followed by *Macrophomina phaseolina* 12.8%. the ethanolic extract showed fungitoxicity with ED_{50} of 13.2% (*M. phaseolina*) and ED_{50} of 15.9%(*P. exigua*). The results showed that *Vernonia amygdalina* leaf extract would be effective in controlling the leaf spot and charcoal rot of groundnut caused by *Phoma exigua* and *Macrophomina phaseolina* respectively.

Keywords: Bitter Leaf Extract, Fungal Disease, Groundnut.

Microbiological spotlights

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Diversity of food-borne pathogens and their antimicrobial resistance in food animals from the largest surveillance study, India

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Foodborne diseases are a major health concern worldwide. *Salmonella* is the leading cause of death followed by *Campylobacter.* Diarrhoeagenic *E. coli* (DEC)causes maximum hospitalizations while *Cronobacter sakazakii* is an emerging foodborne pathogen. Mostly these pathogens are found as commensals in food animals which act as their reservoirs.Their impact is even more grave due to antimicrobial resistance (AMR). Antibiotics are extensively used in livestock for therapeutic, prophylactic and growth promotion,of which many are critically important for humans. India has a high burden of foodborne illnesses and there is no national database or surveillance system. With population rise,animal meat has become one of the prime sources of food in urban India. The current study surveys the zoonotic reservoir of *Campylobacter*,Non-typhoidal *Salmonella* (NTS),DEC and *C. sakazakii* in food animals and their AMR in North India.Stool&meat samples from animal farms, slaughter houses & retail meat shops were processed for enteric pathogens. MacConkey agar, XLT4 agar&Rappaport vassiliadis broth,Campy-Cefex agar& Bolton broth, VRB broth were used for bacterial isolation. The isolates were confirmed by MALDI-TOF(Bruker,Germany). NTS serovars were confirmed by antigenic serotyping. *E. coli* pathotypes were identified by multiplex PCR. Antimicrobial susceptibility was tested by phenotypic and genotypic methods. A total of 839 meat and stool samples were collected from chicken, pig, goat/sheep from poultry farms, slaughter houses and retail meat shops covering a large geographic area in North India. A high burden of *Campylobacter*(25%), NTS(12%), EPEC(8%), ETEC(5.5%), C. *sakazakii*(7%) was found. Common Salmonella serotypes identified were Typhimirium, Enteritidis, Kentucky in poultry, Anatum & Agona in pigs and goats/sheep. Other less common serotypes found were Weltevreden, Saintpaul and Newport. High resistance to fluoroquinolones(28-50%), tetracyclines(55%) and third generation cephalosporins(11%) was seen by disk diffusion. Carbapenem resistance (17.2%) was seen in C. sakazakii. The proportion of resistance was higher in animal fecal samples than meat samples. Genotypically CTXM-15, CMY and NDM were the predominant AMR genes found mostly in poultry isolates followed by pig isolates. There is a high burden of foodborne bacterial pathogens in food animals in India and there is an utmost need of a strong surveillance network to monitor the spread of these pathogens and their role in AMR

Keywords: Campylobacter, Salmonella, Cronobacter sakazakii, food animals, Antimicrobial resistance

Microbiological spotlights

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Prevalence of *Bacillus cereus* and *Staphylococcus aureus* in "Gappal", a fermented food made with milk and millet dough from Burkina Faso

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"Gappal" is a food made with millet dough and milk typical of the Fulani ethnic group of Burkina Faso. It is produced artisanally, however its consumption in the cities of Burkina Faso is growing. It could develop a risk to the health of consumers due to the lack of heat treatment and the lack of control of the fermentation process. The aim of this study was to evaluate the prevalence and *Bacillus cereus* and *Staphylococcus aureus* in "Gappal" marketed in Burkina Faso. A total 106 samples of "Gappal" from six 6 cities in Burkina Faso were analyzed. The results indicate that 2.2% of the liquid "Gappal" samples and 9.4% of the dried "Gappal" samples were of unsatisfactory microbiological quality according to the criteria applicable to *Staphylococcus aureus* while all samples (100%) were of acceptable quality or satisfactory quality according to the rules applicable to *Bacillus cereus.* The load of *Bacillus cereus* and *Staphylococcus aureus* varied following producers ($p = 0.03$). The mean of *Bacillus cereus* was $5x10^6$ ufc/mL in the liquid gappal and $4.8x10^4$ ufc/mL in the dried "Gappal" while the mean of *Staphylococcus aureus* was $3.9x10^2$ ufc/mL in the liquid "Gappal" and $2.0x10^4$ ufc/mL in the dried "Gappal". Milk and cereal nature gives interesting nutritional properties to "Gappal", however, the presence of germs that can produce toxins in the food increases the risk to the health of consumers.

Keywords: Gappal, Bacillus cereus, Staphylococcus aureus, prevalence, Burkina Faso

Microbiological spotlights

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Assessment of fungal contamination in peanuts marketed in the municipality of Rio de Janeiro

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Peanut (*Arachis hypogaea*) is known for its many food options and high nutritive value with high percentage of digestive protein. In Brazil, peanut production grows exponentially, due to domestic market expansion and export prospects. However, as Brazil is a tropical country, the occurrence of fungal contamination and the production of mycotoxins, which are toxic secondary metabolites released by some filamentous fungi is frequent in agri-food products all over the world and can lead to the loss of the nutritional value of this legume. The objective of the present study was to assess fungal contamination in peanut kernels, sanitized with 2% sodium hypochlorite and sold in supermarkets of Rio de Janeiro city. Mycological analyzes and the determination of moisture and ash followed the methodologies proposed by Samson et al. (2000) and Lutz (2008), respectively. The results showed that all shells were contaminated with fungi of the Rhizopus sp. genus and most of the non-sanitized kernels presented growth, mainly, of the fungus Aspergillus niger, a great producer of Ochratoxin A and of highly mycotoxigenic Aspergillus flavus. After sanitizing the shell, it was possible to observe a significant reduction in the contamination of the grains, as only three samples, out of a total of 10, were positive for the fungus Aspergillus flavus. When ash and moisture were analyzed, all contaminated samples exceeded the values allowed by the Brazilian legislation, except samples 3 and 5, which were within the accepted values. This study proved that shells are important for kernel protection. The fungus isolated with the greatest frequency was *A. flavus*, an important producer of aflatoxin, which is extremely carcinogenic. Concerning the high humidity values found, high humidity rates increase the risk of fungal contamination, including microorganisms that produce mycotoxins. The present study demonstrated the importance of a more effective and rigorous monitoring to control fungi and ensure peanut food safety.

Keywords: Peanut, fungal contamination, mycotoxins.

Microbiological spotlights

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The awakening to enteric *Yersinia*

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Enteric Yersinia are elusive pathogenic microorganisms. While not emerging per se, regulators and public health professionals worldwide are reawakening to the extent of foodborne yersiniosis. This presentation will give an overview of the research currently undertaken to understand the epidemiology of this pathogen in New Zealand and the challenges to improve the recovery and detection methods in food and environment for enteric Yersinia. The incidence of yersiniosis has been decreasing in the United States and in Europe, but is still the 3rd most commonly reported zoonosis in Europe with some European countries having rates 5 time higher than the average European rate. In New Zealand, Yersiniosis is the fifth most frequently notified foodborne disease. While Y. enterocolitica remains the most commonly recognized cause of yersiniosis, Y. pseudotuberculosis has been associated with less prevalent but more important outbreaks, e.g. in Finland and New Zealand. Historically, pork is a food recognised to play a major part in the transmission of the disease as pigs are reservoirs of Yersinia enterocolitica 4/O:3. Although several animal species are hosts of other specific bioserotypes, the potential role of their products in the disease epidemiology is less understood. Moreover, the role of wildlife, environment and foods of non-animal origin needs to be better understood. Unfortunately, better attribution is hindered by the lack of robustness of existing detection analytical methods for food products, especially for Y. pseudotuberculosis, despite extensive efforts to develop better methods. Although they were classified as non pathogenic Y. enterocolitica, the recent observation of clinical cases linked to biotype 1A challenges the previous established dichotomy and classification scheme of Yersinia spp. and calls attention to the need for redefining the virulence and physiopathology of Yersinia. Advances in molecular methods and particularly the development of whole genome sequencing may give hope for improved detection methods, enabling the definition of a new identification and typing scheme. These developments will provide better tools to investigate Yersinia outbreaks and to attribute and manage the sources of infection, thereby reversing the current epidemiologic trend in some developed countries and particularly in New Zealand.

Keywords: Yersinia enteric yersiniosis enterocolitica pseudotuberculosis

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Origin of staphylococcal strains isolated from food in Europe

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Studying the population structure of foodborne pathogen, provides an understanding of how lineage clusters relate to ecology and is the first step of source attribution. In Europe, between 2005 to 2017, EURL CPS collected a large collection of Staphylococcal aureus strains responsible of foodborne outbreaks. The objectives of this study were to identify the population structure of this species in Europe and to infer the best population model for Staphylococcal strains.
A collection of 143 genomes, encompassing strains responsible for outbreaks, isolated from food, environment or humans, were sequenced with illumina sequencing technology. The core genome of these 143 genomes was defined using the variant calling method. To identify population structure, two methods were used using the R package “adegenet v 2.1.1” (Jombart T. 2008), the Discriminant Analysis of Principal Component (DAPC) and a clustering method based on maximum likelihood (Jombart et al. 2018). The DAPC is free of population genetic hypotheses whereas the clustering method infers populations at Hardy-Weinberg equilibrium. Once the genetic group number was determined, a population genetic study was performed using the Python library, Egglib (De mita et al. 2012). This allowed designing the divergence scenarii between the different population clusters to infer. Datasets of variants were simulated according these different scenarii. Finally, comparisons between these scenarii and observed dataset were performed with the Approximate Bayesian Computation (ABC) method (Beaumont et al 2002). Scenarii design, simulations and ABC analyses were realised using DiyABC v 2.1 (Cornuet et al).
The number of genetic groups is lower with the clustering method than the DAPC method (6 vs 18 genetic groups). The groups obtained by DAPC a method corresponds to a sub-structuration, indeed the 6 groups of clustering method encompass several DAPC groups. Furthermore, our results showed that population genetic structure of S. aureus is not of sampling dates or locations, as strains isolated in different countries and different dates belong to the same genetic group. These results highlight that several sources can be responsible for Staphylococcal foodborne poisoning outbreaks and that strains are widespread in Europe. Finally, it would be interesting to study evolutionary mechanisms (homologous recombination and transfer of virulence associated factors) in order to understand emergence of these strains in food.

Keywords: Staphylococcus aureus, SFPO, Europe, Approximate bayesian computation, population genomics

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STEC screening and STEC identifi cation using real-time PCR

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Every year, food-borne illnesses caused by Shiga toxin-producing *Escherichia coli* (STEC) claim many lives worldwide. STEC are most commonly transmitted through raw ground beef, raw or inadequately pasteurized milk, sprouts and vegetables. Most infections are caused by *E. coli* serotype O157 and a number of non-O157 *E. coli* serotypes (e.g., "Big Six"). In 2011, *E.coli* O104:H4, not a member of the "Big Six", caused a serious outbreak in Germany. ISO/TS 13136 requires screening for the virulence factors *stx1*, *stx2*, and the intimin *eae*, following identification of five serotypes (O26, O103, O111, O145 and O157). Besides O157, the US focuses on the "Big 6" (O26, O45, O103, O111, O121 and O145). Moreover, in the European Union there was an extension of regulation (EC) 2073/2005, which additionally requires testing for O104 serogroup. BIOTECON Diagnostics has developed two real-time PCR kits that enable the easy screening for STEC and the subsequent identification of the most important eight serotypes (O26, O45, O103, O104, O111, O121, O145 and O157) in less than 24 hours. The **food**proof® STEC Screening LyoKit detects *stx1*, *stx2*, *eae*, and the internal amplification control in one single PCR reaction. The assay is based on ISO/TS 13136, but it was further expanded to detect all known variants of the *stx*-genes. In particular, *stx2f* is missing from the ISO method, but can be easily detected with the screening assay. Following screening, the most important eight serotypes can be identified by melting curve analysis in just one additional PCR reaction using the **food**proof® STEC Identification LyoKit. During validation, all tested 81 STEC strains comprising at least 13 different O-serotypes STEC have been detected and correctly identified. The limit of detection of pure DNA is at least 20 genome equivalents for all eight target serotypes. For convenience, safety, and easy storage the reaction mixes in the **food**proof® LyoKits are pre-filled and lyophilized, so that the sample DNA can be added directly to the reaction tube. Thus, these kits allow an easy and convenient screening and identification of the "Big Six", O157, and O104 in less than 24 hours using real-time PCR technology.

Keywords: Shiga toxin-producing Escherichia coli,

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Risk associated with Shigatoxin-producing *Escherichia coli* (STEC) in Food: Overview of opinions of the French agency for food, environmental and occupational health & safety (ANSES)

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Shigatoxin-producing Escherichia coli (STEC) are enteropathogens causing human infections with a broad spectrum of clinical outcomes. Transmission of STEC to humans occurs through consumption of contaminated food or water and through direct contact from person to person or from infected animals (cattle especially). Although not all STEC are pathogenic for humans, some strains named enterohaemorrhagic E. coli (EHEC) are however responsible for severe illnesses such as haemorrhagic colitis and the Haemolytic Uraemic Syndrome (HUS). HUS is the leading cause of acute renal failure in young children.The French Agency for Food, Environmental and Occupational Health & Safety (Anses) has put the stress on risk assessments linked to this bacterium. The presentation makes an overview of risk assessments led by Anses dealing with pathogenic E. coli:
- Report on the general state of knowledge on pathogenic Escherichia coli (2003)
- Opinions related to the definition of the pathogenic STEC strains (2008, 2010, 2017)
- Quantitative risk assessment of EHEC in ground beef meat and evaluation of efficacy of sampling plans (2007, 2011, 2014, 2017)
- Risk assessment related to the consumption of raw sprouts, in light of developments in the health context following several cases of HUS observed in France in June 2011
- Dedicated datasheet on EHEC for food processing industry.
In the framework of these assessments, Anses reviewed the state of the scientific knowledge on the pathogenicity of STEC. Anses also assessed the impact of prevention and control measures applied throughout the food chain (hygiene measures, self-inspections, consumer cooking practices) on the reduction of the risk of HUS.

Keywords: Shigatoxin-producing Escherichia coli (STEC); Risk assessment

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A quantitative risk assessment model for highly pathogenic Shigatoxin-producing *E. coli* in ground beef in France

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In the framework of a collective expert appraisal, a quantitative risk assessment model was developed in order to estimate and compare the efficacy of three different sampling plans at reducing the risk of Haemolytic Uremic Syndrome (HUS) associated with the consumption of beef burgers in France.
The model developed simulates the different stages of beef burger manufacture from the slaughter of cattle through to the finished products. The exposure assessment considers the prevalence of the five highly pathogenic STEC serotypes in cattle feces, cross-contamination and decontamination during the slaughter process, the distribution of the STEC contamination levels in beef cuts and the variability of cooking methods of beefburgers.The dose-response relationship used is an exponential model in which the r parameter varies according to the age. The model estimates a “baseline risk” of HUS which is the probability of occurrence of HUS following consumption of a beefburger (from any mixture).The probability of detecting the presence of STEC in a ground beef batch with the three sampling plans and the related risk were assessed. The efficacy of these plans is expressed as a risk reduction in comparison to the “baseline risk”. Monte Carlo simulation was used to assess the effect on variability and uncertainty in the model parameters.
According to the model, the comparison of the sampling plans showed a risk reduction following application of the three plans tested. This risk reduction was greater in a high shedding period (92-98%) compared to a low shedding period (57-87%). Given the uncertainties in the model, the differences in the reductions observed between the three plans are not significant. As an example, in a high shedding period, a sampling plan n=1, c=0, m=absence in 25g, applied to all batches produced, would divide by 10 the “baseline risk” of HUS.
The results highlighted the interest to apply systematic microbiological criteria on ground beef batches in France. To achieve the levels of performance estimated in this opinion, the microbiological criteria must include the five major serotypes of highly pathogenic STEC and be applied to all ground beef batches.

Keywords: Shigatoxin-producing E. coli; Quantitative risk assessment; ground beef

Microbiological spotlights

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Vibrio parahaemolyticus biofilm formation and its control

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Vibrio parahaemolyticus is a major food-borne pathogen in aquatic products, which has been the first place of microbial food poisoning in China. *V. parahaemolyticus* is easily to adhere to the surface of most food and food contact materials, embedded within a matrix of extracellular polymeric substances (EPS) (including exopolysaccharides, proteins and extracellular DNA) and form a complex three-dimensional membrane structure, called biofilm. The cells in biofilm are more resistant to various stress factors (disinfectants, antibacterial agents, low temperature, pressure, etc.) than that of the planktonic form, so it causes serious burden on food processing. In naturally, the growth of *V. parahaemolyticus* is highly correlated with temperature and surface materials. Therefore, the objective of this study is to evaluate the effect of temperature and surface materials on *V. parahaemolyticus* biofilm formation, and the eradication of *V. parahaemolyticus* biofilm by acidic electrolyzed water (AEW). The results showed that *V. parahaemolyticus* biofilm formed better at high temperatures (25 and 37 °C) than low temperatures (4-15 °C). Meanwhile, 25 °C was the optimal temperature for *V. parahaemolyticus* biofilm formation. The surface materials for *V. parahaemolyticus* biofilm formation from high to low level are as follows glass > plastic > steel. In additionally, the effects of low temperature (4, 10 °C) cold shock on the pre-formed *V. parahaemolyticus* biofilm were analyzed. The results showed that biofilm biomass, extracellular polysaccharide and extracellular proteins increased significantly after cold shock, and reached the peak at 48h, while the biofilm formation rate was slow down. Plate counting results indicated that after 60 h cold shock, the number of viable bacteria in biofilm was significantly higher ($p < 0.05$) than that in control sample (without cold shock). Gene expression analysis showed that the temperature mainly influenced biofilm formation by changing the motility and quorum sensing of bacteria. The breakup and detachment of biofilm were occurred after acidic electrolyzed water (AEW) treatment, and Raman spectroscope analysis showed that AEW triggered EPS disruption, indicated by the changes in the carbohydrates C - O - C group, tyrosine and phenylalanine of proteins.

Keywords: V. parahaemolyticus, biofilm, temperature, surface materials, acidic electrolyzed water

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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. Within the Swedish *Campylobacter* broiler monitoring programme, all flocks are sampled and analysed for the presence of *Campylobacter*. Most cases of human campylobacteriosis and the highest prevalence of *Campylobacter* in broilers in Sweden are reported during the summer months, but additional peaks were observed during three consecutive winters, starting in the last quarter of 2014, 2015 and 2016, respectively. The temporary increases of domestic campylobacteriosis and of prevalence in broiler flocks were shown by whole genome sequencing (WGS) typing to be linked.

In 2017 the Public Health Agency in Sweden decided to start monitoring *Campylobacter* causing campylobacteriosis through WGS typing on isolates collected at the hospitals during one week in March and one week in August. At this time the National Veterinary Institute in Sweden also started to type all *Campylobacter* isolates collected in the *Campylobacter* broiler monitoring programme from the weeks preceding the collecting of the human isolates. The chicken isolates were sequenced with the Illumina technology and the cluster analysis was performed using cgMLST. The results from the first period (February/March 2017) showed that 44 out of 48 broiler isolates were identical and belonged to sequence type (ST) 918. The ST-918 isolates were from different producers but from the same slaughterhouse. The same ST was also dominant in Swedish patients and in retail chicken products from the same period. The results from the second period (July/August 2017) showed a bigger diversity of isolates. Nine different STs were identified from the 63 *Campylobacter jejuni* isolates from broilers. However, ST-918 was still the most apparent ST and represented 23% of the isolates. Isolates of ST-918 collected at the second time period were closely related to the ones of the same ST from the first period. Upon comparison with isolates from patients, more than half of the broiler isolates could be connected to human isolates in the second period.

The monitoring by typing of *Campylobacter* isolates has continued in 2018 and will hopefully be permanently established to understand, control and prevent further abnormalities in *Campylobacter* prevalence.

Keywords: Campylobacter, monitoring, WGS

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Molecular biology techniques in predictive microbiology: Past, present and future

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Predictive microbiology is an important area of food microbiology, and its essence lies in application of mathematical model to describe the behavior of microbiology under specific conditions. Predictive microbiology model could be used to predict the shelf life and contribute to the microbial risk assessment system for guaranteeing the food security and improving the public health. This study summarizes the history of predictive microbiology and the research hotspot of predictive microbiology. It emphatically introduces the application of molecular biology techniques in predictive microbiology including PCR-DGGE, Real-time PCR, DNA probe, Metagenomics etc. And then we expound the concept and construction method of molecular predictive model. The application of other molecular biology techniques in predictive microbiology and molecular predictive model are also prospected. This review could provide comprehensive data for promoting the progress of predictive microbiology.

Keywords: Molecular biology techniques, Predictive Microbiology, Application, Prospects

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Cell invasion and cell-to-cell spread of *Listeria monocytogenes* correlates with serotype-specific traits

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Listeriosis is a foodborne disease caused by the Gram-positive bacterium *Listeria monocytogenes*, a pathogen that modulates its intracellular survival via vacuolar escape and cytosolic replication. In the present study, we examined the ability of 58 *L. monocytogenes* strains recovered in Brazil (from food processing environment, beef, and clinical sources, 1978 to 2013) to invade, replicate and spread in a human intestinal epithelial cell line (Caco-2). The strains varied widely in their intracellular doubling times (between 40 to 100 min), and there was no clear relationship between serotype and isolate source. Premature stop codons were common in the *inlA* gene of serotype 1/2c strains from food processing environment and beef, and was correlated with decreased Caco-2 cell invasion when compared to other serotypes. Serotype 1/2a strains were generally impaired in their ability to spread between Caco-2 cells, with an average 30% smaller focus area than the 10403S reference strain. However, most serotype 1/2b strains from beef exhibited enhanced cell-to-cell spread, with an average 35% increase in focus area. Curiously, all strains from serotypes 1/2b, 1/2c, and 4b had a 105 bp deletion in *actA* whereas serotype 1/2a strains had the full *actA* sequence. Our findings are consistent with serotype being a better predictors of cell invasion potential and cell spread compared with source of isolate, although the most invasive strains were primarily isolated from beef. Additionally, we have identified isolates that could provide novel insight into the pathogenicity of *L. monocytogenes* that may not be revealed by studying common laboratory reference strains.
Acknowledgments: CAPES, CNPq, FAPEMIG

Keywords: Listeria monocytogenes, Caco-2, serotype, invasion, internalin A, cell-to-cell spread

Microbiological spotlights

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Antibiotic resistance of *Salmonella* spp. obtained from mesenteric lymph nodes of pigs slaughtered in Minas Gerais, Brazil

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Salmonella is an important foodborne pathogen transmitted by a variety of food, including pork. The pig is a common reservoir of this pathogen, and the presence in the lymph nodes can be associated with its excretion in the industries and the contamination of the final products. In addition, the indiscriminate use of antibiotics increases the resistance in *Salmonella* strains. Concerned with this problem, many industries and government applied several processes to controls the presence of *Salmonella* spp. in the pork production chain. This study aimed to verify the presence of *Salmonella* spp. in mesenteric lymph nodes of pigs slaughtered in Minas Gerais, Brazil, and their antibiotic resistance profiles. A total of 100 animals were tested; samples of mesenteric lymph-nodes were collected during slaughtering, weighted (25 g) and subjected to *Salmonella* spp. detection based on the Bacteriological Analytical Manual from Food and Drug Administration. Typical colonies were subjected to identification by PCR targeting *invA* and *ompC* genes. Resistance profiles were assessed by the disk diffusion method by 12 antibiotics (Amoxicillin 20µg, Ampicillin 10µg, Azithromycin 15µg, Cefaclor 30µg, Cefepime 30 µg, Ciprofloxacin 5 µg, Cotrimoxazole 25µg, Enrofloxacin 5µg, Florfenicol 30µg, Imipenem 10 µg, Neomycin 30µg, Norfloxacin 10µg). From 100 animals, 14% were positive to *Salmonella* spp. From these animals, 30 isolates were confirmed by biochemical characteristics and due to amplification of fragments of *invA* and *ompC*. The isolates presented antibiotic resistance to six antibiotics: Amoxicillin (15/30), Ampicillin (16/30), Azithromycin (2/30), Ciprofloxacin (2/30), Enrofloxacin (14/30), Florfenicol (13/30). Considering the occurrence of multiple resistance, 12 isolates presented simultaneous resistance to 4 antibiotics: Amoxicillin, Ampicillin, Enrofloxacin and Florfenicol. The present study showied a continuous circulation of *Salmonella* in the pork production chain, high prevalence of the antibiotic resistance, and the demand for monitoring tools to control this foodborne pathogen.
Acknowledgments: CAPES, CNPq and FAPEMIG.

Keywords: Salmonella; pig; pork; antibiotic resistance

Microbiological spotlights

P5.166

New staphylococcal enterotoxins type G, H and I involved in food born outbreaks: Method development and application on real food

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According to the European Food Safety Authority, Staphylococcal enterotoxins (SEs) represent the major cause of foodborne illnesses due to bacterial toxins and their notifications have been mandatory since 2005. Among the 24 SEs reported in literature, only five can be detected with commercially available immunoassays: SEA, SEB, SEC, SED, and SEE. Due to the lack of suitable antibodies, no validated method is available for the detection/quantification of enterotoxin types other than SEA-SEE, such as SEG, SEH and SEI, which are yet known to be a risk for consumers. Several food-borne outbreaks in Europe have shown a typical SE symptomatology but no enterotoxins types SEA to SEE could be detected. However, genes *seg*, *seh*, and *sei* were detected by PCR indicating, probably, the presence of toxins types SEG, SEH and SEI.

The objective of this work was to develop and optimise an enzyme-linked immunosorbent assay-based (ELISA) method using monoclonal murine antibodies anti SEG, SEH and SEI. The developed ELISA-based method was challenged for the detection of staphylococcal enterotoxins type SEG, SEH and SEI in food matrices according to EN ISO 16140-2. Therefore, standard curve, limit of detection, specificity and sensitivity were established.

More than 20 Samples issued from milk, milk products and ready to eat food categories were spiked by recombinant toxins at several levels of concentration, and prepared according to the ESM (extraction by dialysis concentration). Sensitivity and specificity were evaluated at > 90%.

For application, samples issued from three staphylococcal outbreaks occurred in France and Ireland were analysed using the validated in-house ELISA. Satisfactory agreement was established between SE genes identified in *S. aureus* isolates (seg, seh and sei) and SEG, SEH and SEI toxins detected in the samples.

Keywords: Staphylococcal enterotoxins, outbreaks, ELISA, validatio method

Microbiological spotlights

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Evaluation of antibiotic resistance in pediococci isolated from food and feed

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Pediococcus pentosaceus and *Pediococcus acidilactici* are mostly associated with food fermentations either as indigenous microflora or in starters. Both species have been found in natural and controlled fermentations of vegetables, sausages and dairy sources and they have also been used in silage fermentation and in several commercial probiotic feeds. Strain safety assessment criteria as the presence of acquired antibiotic resistance is recommended by European Food Safety Authority. The aim of this study was to evaluate the phenotypic and genotypic antibiotic resistance in 98 pediococci collected from different sources as silage, seeds and dairy products. Some of them were recently isolated whereas other pediococci, belonging to the UCSC collection, were isolated forty years ago. Pediococci used as meat starter culture were also considered in this work. All isolated were clustered by RAPD and identified as *P. pentosaceus* and *P. acidilactici* by specie-specific PCR. Antimicrobial susceptibility tests towards eight antimicrobial agents (ampicillin , chloramphenicol, clindamycin, erythromycin, gentamicin, tetracycline, kanamycin and streptomycin) was performed using the Minimum Inhibition Concentration (MIC) according to EFSA (2012). All the resistant strains to at least one antibiotic were screened for resistance genes by PCR. Sixty isolates showed different RAPD profile and were further identified as *P. pentosaceus* (45) and as *P. acidilactici* (15). Thirty-four *P. pentosaceus* and 2 *P. acidilactici* strains were isolated in this study, whereas 24 and 13 strains from the UCSC collection respectively, were detected. All strains were found susceptible to ampicillin , chloramphenicol, clindamycin, erythromycin, gentamicin, and streptomycin. All 60 pediococci strains showed resistance to tetracycline in the range of 16 or 64 µg/ml, but no gene *tet*K, *tet*L, *tet*M were detected. With regards to the kanamycin, the 40% of the *P. pentosaceus* and the 60% of the *P. acidilactici* strains were resistant mostly with 128 µg/ml MIC value. Among genes determining resistance to aminoglycoside antibiotics, we assayed *aacA-aph*D, *aph*A3, and *aad*D determinants, but only one strain harbored *aad*D gene. No difference was found between resistant strains isolated 40 years ago and those lately collected. From this study a moderate frequency of antibiotic resistance was observed so we were able to select several strains of each origin to use as starter culture.

Keywords: Pediococci, Antibiotic resistance, Food, Feed

Microbiological spotlights

P5.168

Detection and characterization of *Clostridium difficile* in cattle and sheep carcasses

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Clostridium difficile is an anaerobic, spore forming, rod shaped bacterium frequently isolated from butchery animals in recent years. The virulence of *C. difficile* is mainly related to the presence of Toxin A (enterotoxin) and Toxin B (cytotoxin) or both. Some strains have also binary toxin (ADP- ribosyltransferase) and some human pathogenic ribotypes such as 027 (R027) and 078 (R078) have been named 'hypervirulent' on account of their increased toxin production and enhanced sporulation attribute.
The aim of this study is to evaluate the presence of *C. difficile* in cattle and sheep carcasses and to characterize the ribotype of the detected isolates.
For this purpose, 555 isolates were enumerated and the bacterium was isolated in 83 out of 247 (33.6%) cattle and 78 out of 308 (25.3%) sheep carcass samples. 15/83 (18.1%) cattle and 6/78 (7.7%) sheep isolates were identified as ribotype 027, whereas the other hypervirulent isolate ribotype 078 could not be detected among the analysed samples.
In conclusion, the results demonstrate the presence of toxigenic *C. difficile* isolates in cattle and sheep carcasses on the slaughter line. This situation draws attention to animals, which are potential contamination source of *C. difficile* for human, and therefore, animal originated food varieties can be one of the possible transmission routes from animals to humans.

Keywords: Clostridium difficile, Ribotype, Butchery animal, C. difficile toxin

Microbiological spotlights

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Inactivation heterogeneity of *Vibrio parahaemolyticus* in simulated human gastric fluid

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Gastric fluid is a major factor for human to ward off infections of foodborn pathogens, such as *Vibrio parahaemolyticus* that is widespread in aquatic products and will cause acute gastroenteritis. In this study, a total of 60 *V. parahaemolyticus* strains with different sources (clinical and environmental) and genotypes (*tdh* and *trh* genes) were treated by simulated gastric fluid (SGF) at pH value of 2.0, 3.0, and 4.0, respectively. And the scanning electron microscope (SEM) was applied to study the bacterial morphological change. Obvious inactivation heterogeneity was observed in different *V. parahaemolyticu* under different pH of SGF with the survival rates varied from 0.000% to 0.069%. No strains can survive at SGF pH value 2.0, and the survival rates increased as the SGF pH value increased. Besides, comparing with environmental (30 isolates) and clinical (28 isolates) isolates, clinical isolates were significantly more tolerant towards SGF at all pH values ($p < 0.05$). And the strains carried both *tdh* and *trh* genes had greater survivability ($p < 0.05$) than strains that only carried *tdh* or *trh* gene. The pathogenic strains (*tdh*+ or *trh*+) had significantly greater survivability ($p < 0.01$) than nonpathogenic strains (*tdh*- and *trh*-). The SEM images revealed the rod-like *V. parahaemolyticus* turned into a crumpled-sphere after treated by SGF. This study provided the better understanding of the inactivation heterogeneity and survival fate of different *V. parahaemolyticus* under gastric fluid treatment, which was beneficial for further study on the pathogenesis of this bacterium.

Keywords: Vibrio parahaemolyticus, Inactivation heterogeneity, Simulated gastric fluid

Microbiological spotlights

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Evaluation of antimicrobial and probiotic potential of isolates from different food sources

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At the present time, the food industry actively search for alternative sources of probiotic microorganism to develop healthy and safer foods to satisfy consumer demands. Probiotics are defined as food supplements made up of living microorganisms that, when ingested in the right amounts, have a beneficial effect on the health of the consumer by improving their intestinal microbial balance. Among the beneficial activities that have been reported in the probiotics, the antagonism of pathogens and spoilage microorganisms has gained increased interest since the probiotic cultures could not only be used to improve the safety and maintain food quality, but also in other biotechnological and biomedical developments.

Probiotic isolated from nonconventional sources like traditional fermented foods or breast milk, have generated great attention since they are products destined to human consumption and have an active microbiota considered not harmful. They are ideal substrates for the search of different GRAS microorganisms with probiotic potential. Probiotic candidates need to exhibit the ability to survive under different types of stress such as antibiotic presence, low pH, lysozyme and be able to inhibit pathogens and spoilage microorganisms.

In this work, microorganisms were isolated on selective media, from different food sources, such as Bulgarian yogurt, (fermented milk), Tibicos (fermented whole cane sugar water), pozol (fermented maize dough), kombucha (fermented tea) and finally, from samples of breast milk. The isolates were screened by RAPD and for their capacity of pathogen growth inhibition and spoilage microorganisms such as Salmonella enterica var Typhimurium, Listeria, Staphylococcus aureus and Streptoccous pyogenes.

Selected isolates were tested for their probiotic potential against lysozyme, low pH and antibiotic presence and compared with Lactobacillus acidophilus, a recognized probiotic. Several isolates including bacilli and cocci Gram (+) and yeast presented improved antimicrobial and probiotic potential than L. acidophilus, being tibicos, pozol and Bulgarian yogurt the major sources where prospective probiotic microorganisms were isolated although kombucha and breast milk also has potential probiotics. Identification and inhibition of other food spoilage microorganisms are currently being made.

Keywords: fermented foods, probiotics, food spoilage

Microbiological spotlights

P5.171

Occurrence of filamentous fungi during cocoa post-harvest processing in Honduras and potential alfatoxin production

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Cocoa beans are the major raw material for chocolate production. The fermentation of cocoa beans is the first step in cocoa post-harvest processing and is characterized by a succession of yeasts, lactic acid bacteria (LAB), and acetic acid bacteria (AAB). Dependent of surrounding conditions e.g. humidity due to rainfall, filamentous fungi are often observed with predominance in the late phase of fermentation, during drying, and storage. Fungal presence in cocoa is generally regarded as undesirable and often related to the formation of off flavors, spoilage and mycotoxin accumulation. In this study, 516 filamentous fungi were isolated from cocoa beans during fermentation (n=29), drying (n=216), and storage (n=271) in Honduras. A selection of 206 isolates was identified using a multiphasic sequencing approach. The remaining 310 were indirectly identified by means of macroscopic characterization after growth on different fungal agars. In an overview, filamentous fungi of the genera *Aspergillus* (n= 218, corresponding to 42.2 %), *Penicillium* (n=85, 16.5 %), *Lichtheimia* (n=69, 13.4 %), and *Fusarium* (n=10, 1.9 %) were identified concomitant with other genera (n=26, 5%), yeasts (89, 17.2 %), and a share of 3.7 % unidentified isolates (n=19). 148 isolates of the sectio *Flavi* were further characterized regarding aflatoxin production potential. After growth on yeast extract sucrose agar with β-cyclodextrine and sodium deoxycholate (YCSD) and coconut cream agar (CCA), 41 (28%) *Aspergillus* strains showed aflatoxin production on both agars, 24 (16%) only on one agar, and 46 (31%) on no condition. The remaining 61 (41%) isolates showed no aflatoxin production on at least one agar, whereas the second agar did not reveal clear results. A PCR approach targeting *alfP*, *alfO*, and *alfD* confirmed the phenotypic production of aflatoxin in 5 strains of *A. flavus/A. oryzae* genotypically. This study revealed a high risk of aflatoxin producing fungal strains present during post-harvest processing of cocoa beans. A potential production of further mycotoxins is currently under investigation.

Keywords: filamentous fungi

Microbiological spotlights

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Growth potential of pathogens in reverse osmosis filtrated whey intended for water-reuse in cheese production

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Water consumption in cheese production is excessive and lead also to high costs for returning used water to wastewater plants. In cheese production huge amounts of whey is a side product, which is filtrated by reverse osmosis in order to extract valuable substances. The industry has shown interested in using the residual filtrate (RO water) for cleaning and cooling purposes, which will require at least one or two days of storage. Such storage has to be safe and will require a risk assessment. This project aimed to investigate the potential of RO water to support growth of the zoonotic pathogens *Salmonella, Listeria monocytogenes* and shigatoxin producing *E. coli* (STEC) and the opportunistic pathogen, *Klebsiella*. Further the aim was to investigate if it was possible to identify biochemical feature which could be used by the industry as indicator for growth potential of pathogens in RO water. RO water from four dairy companies was sampled three times over a period of 3 months. The RO water was frozen at the companies and brought to the laboratory. After thawing and sterile filtration samples were inoculated with a five strain cocktail for each zoonotic pathogen and a three strain cocktail for *Klebsiella* with a starting concentration of approximately 10^2 cfu/ml. Inoculated samples were incubated 10°C, 20°C and 30°C for seven days. Cell counts were established by plating on Tryptic Soy Agar. At 10°C the psychrotrophes *Klebsiella* and *Listeria monocytogenes* showed different growth capacity. After a lag phase of app 24 hours, *Klebsiella* grew and reached a cell density of 10^8 cfu/ml, while Listeria monocytogenes did not show any growth during the seven days. In comparison *Salmonella* and STEC showed a 2-3 log increase during seven days. At 20°C and 30°C all pathogens and *Klebsiella* exhibited substantial growth with lag phases of few hours and Nmax around 10^8 cfu/ml. Although the RO water looks like drinking water, it has a nutritional background for substantial growth support of pathogens. The factor inhibiting Listeria growth at 10 °C is unknown. As the zoonotic pathogens are urease negative they cannot utilise this as carbon source and their growth must be supported by other carbon sources. Combase was used to establish estimates for lag phase, growth rate (μmax) and maximum cell density Nmax. Biochemically, the samples were tested for pH, Chloride, Nitrate, Phosphate, Nitrogen, Urea and Chemical Oxygen Demand (COD).

Keywords: pathogens

Microbiological spotlights

P5.173

Safety assessment of fermented fishery products from Cambodia

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The status of food safety of fermented fishery products has not been extensively studied in Cambodia. Thus, forty-five fermented fishery products originating from Cambodia were analyzed for their microbial communities, water activity, pH value, salt content and the presence of biogenic amines. Water activities of 0.69 – 0.84, pH values of 4.84 – 6.96, and salt contents of 6.30 – 33.76% were found in all tested samples. The most significant biogenic amine is histamine which is produced by the breakdown of the amino acid histidine. About 22 % of the samples exceeded the EFSA regulated histamine level of 400 ppm. Pathogenic bacteria were not detected. Spoilage and hygienic indicator microorganisms were generally present in low numbers. These results showed that the investigated products are safe for consumers in term of microbiological parameters. However, some fermented fishery products contained high amounts of biogenic amines, especially histamine, which may present a health risk. The levels of histamine in fermented fishery products are influenced by microbial contaminations, inadequate handling and storage conditions as well as poor hygienic manufacturing practices. Therefore, processors and regulatory bodies are advised to reconsider their products regarding safe handling measures.

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