FOODMicro 2026

by ICFMH®

31st International ICFMH Conference

Ljubljana, Slovenia
September 7–10

BOOK OF ABSTRACTS

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International Committee for Food Microbiology and Hygiene (ICFMH)

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FOODMicro by ICFMH®

31st International ICFMH Conference 2026 Ljubljana, Slovenia September 7–10

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Prof. Dr. **Sonja Smole Možina**,
Chair of the Programme Committee

Asst. Prof. Dr. **Elizabeta Mičović**,
Chair of the Organizing Committee

International Committee on Food Microbiology and Hygiene (ICFMH)

Prof. Dr. **Peter Raspor**, Dr. h. c. mult.;
Professor Emeritus
Member of ICFMH and Honorary President

Prof. Dr. **Andreja Rajkovic**,
ICFMH President

WELCOME ADDRESSES

Welcome from president of International Committee on Food Microbiology and Hygiene

Dear colleagues and friends,

On behalf of the International Committee on Food Microbiology and Hygiene (ICFMH), it is my great pleasure to welcome you to the 31st International ICFMH Conference, FOODMicro 2026.

FOODMicro has a scientific tradition extending over seven decades. Its strength lies not only in this history, but also in its ability to change together with food microbiology and the community behind it. And that community is changing rapidly, in people and in science. Foodborne diseases, microbial hazards and food preservation remain central to our discipline, but antimicrobial resistance, changing food systems, environmental pressures, new technologies and global public health increasingly require us to work across traditional boundaries. At the same time, fundamental food microbiology knowledge remains as important as ever.

ICFMH is evolving as well. FOODMicro remains our flagship international meeting, but our activities now extend much further. FOODMicroLatino, the *International Journal of Food Microbiology*, scientific and educational initiatives, hands-on trainings, international partnerships and increasing participation from different parts of the world are helping us to connect a broader food microbiology community.

FOODMicro 2026 reflects this development. Established scientists, young researchers, students and professionals have come together in Ljubljana from across the world. For me, what matters most is not the numbers themselves, but what they represent: a truly international scientific community coming together through FOODMicro.

The theme of this meeting, **"Revisiting the Roots: Reviving Hygiene in Advancing Food Microbiology,"** is particularly appropriate. Moving forward does not mean leaving our foundations behind. On the contrary, our knowledge of microorganisms, hygiene and food safety gives us the basis to address new questions, technologies and risks. We have knowledge to fall back on, but also the courage to move forward.

But FOODMicro is ultimately about people. Your presence, your science, the discussions you have and the collaborations you start here are what give this meeting its value. What happens after Ljubljana can be just as important as what happens during these four days.

I would therefore especially like to welcome our students and early-career scientists. I hope that you will use FOODMicro not only to present your work, but also to meet people, discuss ideas and build scientific relationships that may stay with you for many years.

I would like to thank the Programme and Organising Committees, the International Scientific Committee, our partners and supporters, invited speakers, reviewers and everyone who has contributed to making FOODMicro 2026 possible.

FOODMicro has strong roots and a proud history, while the breadth and diversity of the community gathered in Ljubljana give us confidence in what comes next. **Our responsibility at ICFMH is to connect the two: to preserve what remains fundamental while creating space for our discipline, our community and generations of food microbiologists to move forward.**

Welcome to Ljubljana and welcome to FOODMicro 2026.

On behalf of the ICFMH Executive Board,

Prof. Dr. Andreja Rajkovic
President
International Committee on Food Microbiology and Hygiene (ICFMH)

Welcome to FOODMicro 2026

Food safety is one of the fundamental responsibilities of the state and an important part of the resilience of society. Food is a strategic commodity. Its safety, quality and availability are important not only for public health, but also for the resilience of our countries, economies and societies. Food safety must be ensured throughout the entire agri-food and nutrition chain – from primary production, feed and animals, through processing, laboratories and official controls, to retail, catering and consumers.

At the Ministry of Agriculture, we are committed to promoting competitive domestic production, greater food self-sufficiency, and shorter and more resilient food supply chains. This is an important part of food security and national resilience. At the same time, we want the food produced domestically and offered to consumers to be safe, high-quality and worthy of their trust.

The European food safety system is based on scientific knowledge, risk assessment, prevention, traceability, control and clear responsibility throughout the food chain. Its effectiveness also depends on the knowledge and skills of the people who implement this system every day. That is why we must invest in research, education, training and lifelong professional development throughout the agri-food chain.

In this context, food microbiology has a particularly important role to play. Foodborne diseases, antimicrobial resistance and other microbiological risks require continuous monitoring, a sound understanding of processes and timely action. The fundamental principles of hygiene remain essential, even as new technologies, analytical methods and production processes develop rapidly.

This is why the theme of this year's 31st International Conference of the International Committee on Food Microbiology and Hygiene – FOODMicro 2026, "Revisiting the Roots: Reviving Hygiene in Advancing Food Microbiology", is so relevant. Returning to the fundamentals does not mean turning away from progress. Rather, it reminds us that new technologies and methods must be built on principles that remain essential to ensuring safe food.

Slovenia has an important tradition of bringing together knowledge and expertise in food, nutrition and microbiology. Over the past decades, Ljubljana has been the starting point for a number of important international scientific connections. CEFood began its journey in Ljubljana in 2002, followed by the FEMS network in 2004 and EFFoST in 2008. Professor Peter Raspor played an important part in this tradition through his scientific and organisational work, making a significant contribution to the establishment of these international connections and to the development of microbiology, biotechnology and food safety.

FOODMicro 2026 continues this tradition and provides an opportunity to connect scientific knowledge with practice and to seek answers to the challenges facing farmers, food businesses, laboratories and public institutions. Weather-related crises, global supply chains, new production methods, changing dietary habits and technological development are creating new risks. At the same time, advanced analytical methods, better-quality data and new technologies enable us to identify and manage these risks with greater precision. Their true value lies in our ability to use them effectively in preventive measures and everyday practice.

FOODMicro 2026 therefore provides an important opportunity to advance food microbiology, exchange professional experience and expertise, and deepen our understanding of risks that know no national borders. I believe that the discussions and insights generated at this year's conference will contribute to further strengthening food safety and quality.

I wish all participants a successful conference, inspiring discussions and a pleasant stay in Ljubljana and Slovenia.

Janez Cigler Kralj
Minister of Agriculture of the Republic of Slovenia

Welcome from FOODMicro 2026 Conference Chairs and Presidents

Dear colleagues and friends,

It is our great pleasure to welcome you to the 31st International ICFMH Conference, FOODMicro 2026, in Ljubljana. Returning to Slovenia after more than two decades, the conference brings together scientists and professionals from 59 countries for four days of scientific exchange, discussion and collaboration.

The theme of FOODMicro 2026, **"Revisiting the Roots: Reviving Hygiene in Advancing Food Microbiology,"** connects the foundations of our discipline with the questions shaping it today. Understanding microorganisms and their behaviour, maintaining effective hygiene and preventing foodborne disease remain fundamental. At the same time, omics technologies, microbiome research, single cell approaches, predictive modelling, artificial intelligence and new biotechnological methods are expanding both our scientific possibilities and the complexity of the questions we address.

This combination is reflected in the programme. Foodborne pathogens, hygiene, food preservation, antimicrobial resistance, fermentation, microbiomes, predictive microbiology, risk assessment, water safety and sustainable food systems are addressed alongside emerging technologies and methodological developments. A strong One Health perspective further connects food microbiology with human, animal, plant and environmental health.

FOODMicro 2026 includes around 500 scientific contributions from 1,870 authors, selected from more than 600 submitted abstracts. Plenary and keynote lectures, oral and flash presentations, posters, round-table discussions, project presentations and specialised sessions provide many different opportunities to present results, question interpretations and exchange ideas.

The programme is further enriched through cooperation with international partners, including for the first time International Association for Food Protection (IAFP), the International Commission on Microbiological Specifications for Foods (ICMSF) and ILSI Europe, as well as contributions from European and international research initiatives. We hope that these different perspectives will stimulate discussions and collaborations that continue beyond the conference.

We sincerely thank all authors, speakers, reviewers, session chairs, partners, sponsors and exhibitors, the members of the Programme, Organising and International Scientific Committees, the team at Cankarjev dom and everyone working behind the scenes to make FOODMicro 2026 possible.

Most importantly, we hope that you will make the conference your own: discuss science, question ideas, meet old and new colleagues and enjoy being together in Ljubljana. We also hope you will find some time to discover the history, culture, hospitality and gastronomy of Slovenia.

We wish you an inspiring and memorable FOODMicro 2026.

Sincerely,

Prof. Dr. Sonja Smole Možina
Chair of the Programme Committee FOODMicro 2026

Asst. Prof. Dr. Elizabeta Mičović
Chair of the Organising Committee FOODMicro 2026

Prof. Emer. Peter Raspor
Honorary President, FOODMicro 2026

Prof. Dr. Andreja Rajkovic
President, International Committee on Food Microbiology and Hygiene (ICFMH)

Ljubljana, September 7, 2026

Food Microbiology at a Crossroads: From the Legacy of FOODMicro to a Vision for the Future

The 31st FOODMicro meeting brings us back to Slovenia, continuing a remarkable tradition that began in 1954. Over the decades, FOODMicro has travelled across Europe and beyond, bringing together food microbiologists from different countries, institutions and generations. More than thirty meetings have created not simply a series of scientific conferences, but an international community built on the exchange of knowledge, ideas, experience and friendship.

1. From a series of meetings to a global community

Under the umbrella of FOODMicro 2026 in Ljubljana, we have 500 presentations contributed by 1,870 authors from 59 countries across all continents. They will be presented as 52 oral presentations, 41 flash presentations, 12 keynotes, 3 plenaries and 366 posters, alongside an exhibition and related activities at the conference. The organising committee lists more than 500 participants delivering selected and invited scientific and professional contributions. The meeting is therefore both a continuation of and an opportunity for reflection on all past meetings, in terms of both subject matter and organisational framework. For me, this meeting also has a deeply personal meaning. Food microbiology has accompanied me throughout my professional life – from my early training and apprenticeship, through decades of research and education, to my professorship and my involvement in international professional organisations. Much of that journey has been devoted to learning, researching and teaching microbiology, but also to advocating for its appropriate place in both academic research and industrial practice. I have had the privilege of being involved with the International Committee on Food Microbiology and Hygiene (ICFMH) for more than two decades, including the organisation of congresses and symposia and direct involvement in two International ICFMH conferences in Slovenia. These experiences have reinforced my conviction that the strength of our discipline depends not only on scientific excellence, but also on the quality of the community we build around it. FOODMicro has become much more than a series of scientific meetings. It has provided a place where generations of food microbiologists have met, challenged established thinking, exchanged knowledge, established friendships and created collaborations. Just as important, it has provided a forum in which we can ask what our discipline should become. As we open the abstract book of FOODMicro 2026, it is therefore appropriate not only to consider where food microbiology stands today, but also to ask a broader question: **What must we do to ensure that food microbiology remains scientifically strong, socially relevant and prepared for the challenges of the future?**

2. Education: the foundation of the discipline

One issue that deserves considerably greater attention is education in food microbiology. Our discipline is changing rapidly. Molecular microbiology, genomics, microbiomes, bioinformatics, artificial intelligence, predictive modelling, systems biology and advanced analytical technologies are becoming increasingly important components of the food microbiologist's toolbox. At the same time, the classical foundations of our discipline – microbial physiology, hygiene, epidemiology, food safety, microbial ecology and risk assessment – remain indispensable. The challenge is therefore not simply to add new subjects to existing curricula. We need to reconsider how food microbiology itself should be positioned within the educational programmes of the future. Food microbiology should have a visible and productive place within programmes dealing with food science and technology, biotechnology, nutrition, public health, veterinary medicine, environmental sciences and related fields. Students should learn not only how micro-organisms behave, but also how microbiological knowledge connects with food production, food safety policy, sustainability, public health and society. The food microbiologist of tomorrow will increasingly work at the boundaries between disciplines. Microbiology will intersect with data science, engineering, ecology, epidemiology, environmental sciences and policy. Students and young scientists must therefore be given opportunities to move across these boundaries rather than remain confined within traditional disciplinary structures. This has been addressed effectively within dedicated symposia on food microbiology education. I hope, however, that this will stimulate the ICFMH Commission and the Working Party on Advanced

Education in Food Microbiology to reconsider the importance of education and make it a more visible component of future FOODMicro programmes. Education is not an accessory to scientific progress. It is the foundation on which the future of the discipline will be built.

3. From scientific needs to research priorities

The FOODMicro 2026 programme demonstrates the remarkable breadth and maturity of contemporary food microbiology. It encompasses fundamental microbial science, food safety, technological innovation, risk assessment, industrial implementation and regulatory perspectives, while also incorporating emerging areas such as artificial intelligence, systems biology, microbiomes and sustainability. This breadth is important because the major challenges facing food systems cannot be addressed by isolated disciplines or by single scientific approaches. We need fundamental research to understand microorganisms and their interactions. We need applied research to translate that knowledge into safer and more sustainable food production. We need risk assessment to understand the implications of microbial hazards. We need technological innovation to develop better detection, preservation and control strategies. And we need collaboration with industry and public authorities to ensure that scientific discoveries can be implemented in practice. Food microbiology is consequently moving from a collection of specialised research areas towards an increasingly integrated science. The task of the international community should be to identify emerging needs early, encourage research across disciplinary boundaries and create opportunities for scientists from different countries and sectors to work together.

4. From scientific knowledge to policy

Scientific knowledge achieves its full value when it informs decisions that protect public health, support responsible innovation and strengthen food systems. Food microbiologists therefore have an important role not only in laboratories and universities, but also in the development of standards, regulations, guidance and public-health strategies. Yet the relationship between science and policy is not always sufficiently structured. Scientists need to understand the realities and constraints within which policy decisions are made. At the same time, policy-makers need access to current scientific knowledge and to the practical expertise of those working directly with micro-organisms, food production and microbial risks. This requires sustained dialogue between scientists, regulators, industry and other stakeholders.

FOODMicro is particularly well positioned to facilitate such dialogue. Its international character, scientific breadth and long history provide a natural platform for bringing together communities that do not always have sufficient opportunities to interact. The aim should not be to blur the distinction between science and policy. Rather, we should strengthen the mechanisms through which robust scientific evidence can inform responsible policy.

5. Building focused communities of expertise

The increasing complexity of food microbiology also raises a question about our professional structures. Can existing organisations and conference formats provide sufficient continuity to address complex challenges that require sustained international effort? I believe there is an opportunity to establish dedicated task groups or expert working groups within the international food microbiology community. These groups could bring together specialists from different countries and sectors to address clearly defined priorities over a limited period of time. Their tasks could include developing scientific positions, identifying research priorities, preparing educational materials, supporting risk assessment, providing recommendations to policy-makers, or identifying areas where international cooperation is urgently needed. Such groups should not become additional bureaucratic structures. Their value should lie precisely in the opposite: they should be **productive communities of expertise**, established with a clear purpose, a defined task and a commitment to producing something useful for the wider community. In an increasingly complex scientific environment, focused cooperation may sometimes be more effective than creating ever larger organisational structures.

6. Beyond the conference: building platforms for the future

FOODMicro has always demonstrated the value of an international platform for scientific exchange. The next challenge is to ensure that collaboration does not end when a conference closes. We should consider mechanisms that maintain cooperation between successive FOODMicro meetings and provide continuity from one generation of participants to the next. Such platforms could connect researchers, educators, industry, regulatory authorities and students, facilitate international projects, support young scientists, identify emerging challenges and maintain institutional memory between conferences. The history of FOODMicro provides an excellent foundation for such developments. An interesting example from the past was the 2004 FOODMicro meeting, which introduced new approaches to the organisation of scientific exchange and included the special pre-conference EuroMicroDay2004 matchmaking event, designed to connect people, build knowledge and encourage cooperation. The principle behind such initiatives remains highly relevant: conferences should not only disseminate knowledge but also create opportunities for people to work together afterwards. Perhaps we should now take this idea further. One possibility would be to develop a **Declaration on Food Safety and Food Microbiology**, as discussed within the organising committee and drafted for initiation purposes. Such a document would stimulate structured discussion and strengthen food microbiology circles.

7. Food microbiology within a One Health framework

One of the strongest messages emerging from contemporary food microbiology is the growing importance of the One Health perspective. Human, animal, plant and environmental health are inseparably connected. Food safety depends not only on effective hygiene and process control, but also on sustainable agricultural practices, responsible antimicrobial use, resilient ecosystems, safe water and informed behaviour throughout the food chain. The increasing attention to microbiological risk assessment, microbiomes, water safety, antimicrobial resistance and global food security shows that the future of food microbiology lies in integrating these perspectives rather than treating them separately. This represents an important evolution in our thinking. More than two decades ago, the 19th FOODMicro Conference in 2004 highlighted many issues that remain central today: the continuing burden of foodborne diseases, the emergence of new pathogens, the importance of hygiene throughout the food chain, improved detection and preservation methods, and the protection of consumers. The scientific tools have changed enormously since then. The fundamental responsibility, however, remains the same. What has changed is the complexity of the context in which that responsibility must be fulfilled.

8. From scientific excellence to societal impact

Scientific excellence alone is not enough. Our discoveries achieve their greatest value when they are translated into practical solutions that improve public health, support responsible innovation and strengthen confidence in food systems. Collaboration between academia, industry, public authorities and international organisations is therefore more important than ever. FOODMicro has always served as a meeting place for these communities. Its mission is further strengthened through cooperation with organisations such as the International Life Sciences Institute Europe (ILSI Europe) and the International Association for Food Protection (IAFP). The strength of such networks lies not only in the number of participating organisations, but in their ability to connect different perspectives and transform scientific knowledge into action. This is particularly important as food systems are being reshaped by climate change, globalisation, technological development, demographic change and new patterns of production and consumption. Microbiology is at the heart of many of these transformations.

9. Organisational flexibility as a strength

One further challenge concerns not the science itself, but the structures through which our community meets. FOODMicro has been organised in different ways over the decades, with responsibilities distributed variously between ICFMH, host institutions and national societies. Rather than treating this as an inconsistency, we might see it as a resource: the meeting has been able to adapt to the conditions of each host and each period without losing its identity. Looking ahead, that adaptability matters more than it might appear. Strong food microbiology communities exist in many countries whose professional structures differ from the traditional model, and some operate outside the IUMS umbrella altogether. If the organisational form is allowed to follow the scientific opportunity rather than constrain it, FOODMicro can extend its reach while preserving the scientific and professional standards that have characterised it throughout its history. Organisational arrangements are not an end in themselves. Their value will ultimately depend on whether they enable the community to grow, diversify and remain relevant. Organisational innovation is not an end in itself. Its value will ultimately depend on whether it enables the community to grow, diversify and remain relevant.

10. Consolidation - Looking beyond Ljubljana

The scientific contributions presented at FOODMicro 2026 demonstrate the vitality of our discipline. They show remarkable progress in understanding microorganisms, controlling foodborne hazards, exploiting beneficial microorganisms, applying new technologies and addressing the increasingly complex interactions between food, people and the environment. Yet a strong scientific community is measured not only by the quality of its research, but also by its ability to **educate the next generation, influence responsible policy, create productive partnerships and anticipate challenges that have not yet fully emerged.** This is where the future of FOODMicro should lie. We should remain confident about the future of food microbiology, but we should also be proactive in shaping it. We need excellent science, but we also need excellent education. We need research, but we also need structures that translate research into practice. We need conferences, but we also need platforms that sustain cooperation between them. Most importantly, we need a community capable of thinking beyond its traditional boundaries. I hope that the discussions in Ljubljana will therefore extend beyond the individual presentations and posters. I hope they will lead to new educational initiatives, new research collaborations, stronger cooperation between science and policy, and perhaps new international structures capable of addressing specific challenges with the focus and continuity they require. The history of FOODMicro gives us a strong foundation. The international scientific community gathered in Ljubljana gives us the knowledge, experience and energy. What we need now is the ambition to translate these assets into a vision for the coming decades. FOODMicro should remain a place where we not only present what we have accomplished, but also ask what should come next. The future of food microbiology is not something that will simply happen to us; **it is something we can actively build together**.

Ljubljana is therefore not only a place to celebrate how far food microbiology has come. It can also be a place where we begin to shape where it will go next.

Prof. Dr. Peter Raspor, Professor Emeritus,

Honorary President, FOODMicro 2026

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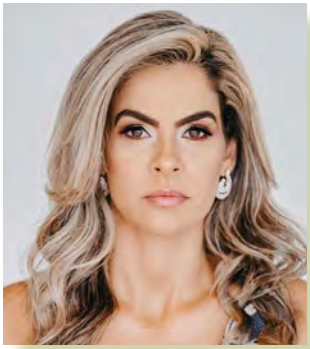
Plenary Speakers



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Andreja Rajkovic

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Jasna Kovač
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Fernanda Beatriz Mozzi
Cerela-Conicet, Argentina

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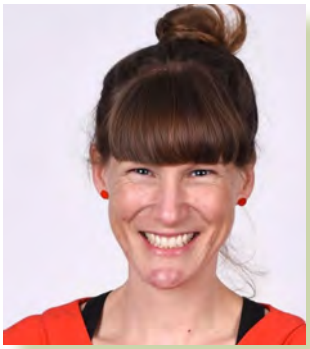

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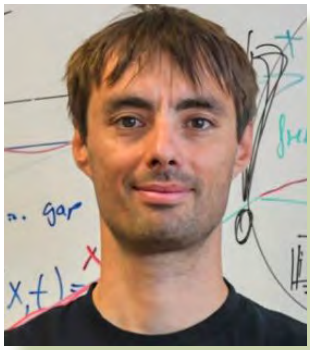

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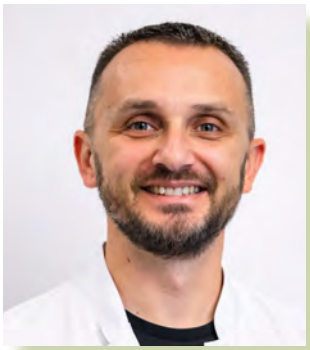

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ABSTRACTS

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ORAL PRESENTATIONS

Opening Plenary Lecture

PL-1: Safeguarding Food Safety in a Changing World: Understanding the Vital Role of Hygiene

Marciane Magnani[1]

[1]*Federal University of Paraíba, João Pessoa, Brazil*

Food systems are rapidly changing due to globalization, climate change, technological innovations, and evolving food consumption patterns. Ensuring food safety is also a key component of sustainable food systems and is directly linked to several United Nations Sustainable Development Goals related to health, food security, and sustainable production. These transformations create new microbiological risks and complicate efforts to ensure food safety worldwide. Changes in food production, distribution, and consumption have increased the complexity of food chains and the diversity of food products, creating new challenges for controlling foodborne pathogens and spoilage microorganisms. Despite major advances in food microbiology, including omics-based technologies, environmental monitoring, and quantitative microbial risk assessment, foodborne diseases remain a persistent global challenge. This paradox highlights the need to revisit one of the most fundamental principles of food safety: hygiene. This plenary lecture will discuss the central role of hygiene as the foundation of food safety systems and its importance across the entire food chain, from primary production to food processing environments and consumer practices. Effective hygiene practices are essential to prevent contamination, reduce microbial persistence in food environments, and minimize cross-contamination during food handling and processing. The lecture will explore how classical hygiene principles must be integrated with modern tools and risk-based approaches to better understand and control microbial risks. Modern food microbiology increasingly recognizes the role of microbial ecology and microbiomes in food environments, emphasizing that hygiene strategies must consider microbial communities and ecosystem dynamics, rather than focusing solely on individual pathogens. Special attention will be given to the role of human behavior, food processing environments as microbial reservoirs, and the impact of global changes on food safety risks. The lecture will also discuss how environmental monitoring programs, hygienic design, and food safety culture contribute to improving hygiene performance and reducing food safety risks. Food safety depends on combining fundamental hygiene practices with advanced scientific tools and risk-based management strategies. Revisiting hygiene is not a step backward but a necessary step forward to safeguard food safety in an increasingly complex world. Strengthening hygiene practices remains one of the most effective strategies to prevent foodborne diseases and protect public health.

Plenary Session

PL-2: Food Microbiome analysis: Chances and Challenges

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Due to technical limitations, food safety research was for years of limited understanding how pathogens interact with the commensal microbiome in specific environments. Together with the physico-chemical profile, this knowledge however is substantial to understand how pathogens transmit through a sequence of econiches on their way into the human body. Environment, transmission via food animals and food plants to the final products, and performance in the (human) host. Drawing such a holistic picture is sketched out by the word interconnectedness. Recent research has shown that substantial food microbiome diversity is present across different foods but only 3% of the food microbiome appears in the microbiome of the adult gut. There is an obvious difference in the part of the microbiome that stays in an econiche, and the part of the microbiome that travels (Carlino et al., 2024). With the microbiome floating around, contents such as virulence or antimicrobial resistance genes are carried away, and can be found in multiple environments (Quijada et al., 2025). To zoom into food processing in particular, there is a food business operation (FBO) specific microbiome that flows around between raw materials, food contact and nonfood contact surfaces, personal and the final product. Research has shown that this microbiome fluctuates dynamically through the processing chain as it reaches the final product state. In a meat microbiome example our initial hypothesis that vast part of microbiome is derived from fecal contamination of the slaughtered animals had to be revised as the largest part of the microbiome originate from the food environment (Zwirzitz et al., 2020).

Conclusively, microbial interconnectedness research spans from animal science to food science and nutrition science. It includes thorough planning of sampling and subsequent bioinformatic analysis of samples. A decisive pillar is the collection of sufficient metadata to understand the overall picture and translate the finding into meaningful risk management options for food business operations.

Carlino et al., 2024. Unexplored microbial diversity from 2,500 food metagenomes and links with the human microbiome. *Cell* 187, 20, 5775–5795.

Quijada et al., 2025. The food-associated resistome is shaped by processing and production environments. *Nature Microbiology* 10, 1854–1867.

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S1 Microbial Innovations in Food Fermentation Technologies

S1-KN1: Selenium-Enriched Fermented Beverages Using Lactic Acid Bacteria

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Selenium (Se) is an essential micronutrient for humans, and its intake is possible through food. Global Se intake depends on the amount present in the soil, which varies across geographic regions. Se is primarily known for its antioxidant, anti-inflammatory, and antiviral activity, and its deficiency is associated with various diseases related to oxidative stress, such as aging, cancer, male infertility, and thyroid dysfunction. The recommended daily intake (RDI) of Se is 25 to 34 µg for adults, depending on age and sex. Due to Se deficiency in many regions, it is necessary to supplement Se in the soil and in food. Some lactic acid bacteria (LAB), widely used in the production of fermented foods, can biotransform inorganic Se (selenate and selenite salts present in soil) into seleno-nanoparticles (SeNPs) and seleno-amino acids like selenocysteine (SeCys) and selenomethionine (SeMet), which are more readily absorbed and safer than inorganic Se. SeCys is present in the active site of many human selenoproteins involved in the immune system and oxidative defense. The biotransformation of inorganic Se into SeNPs and seleno-amino acids by LAB occurs to varying degrees, leaving residual toxic inorganic Se in the culture medium where it is added. An interesting strategy to prevent this problem and enrich foods with organic Se is to use selenized LAB, previously cultured with sodium selenite, as starter cultures. Following an initial evaluation with 100 LAB strains isolated from fruits, *Fructobacillus tropaeoli* CRL 2034 and *Lactiplantibacillus plantarum* CRL 2051 stood out for their ability to accumulate Se and produce SeNPs and SeCys in a culture medium containing sodium selenite. These strains, alone or in combination, were successfully used to formulate fermented beverages of fruit juice (mango-passion fruit) and a mixture of orange juice and milk. During microbial growth in the latter beverage, the strains accumulated 62.8–93.5 µg/L of total Se, with 32.7–47.8 µg/L composed of SeCys and 6.1–12.7 µg/L of SeMet. Beverages fermented with *L. paraplantarum* CRL 2051 alone and with the mixed culture showed the best sensory attributes, while those containing both strains exhibited high microbial resistance to cold storage, *in vitro* gastrointestinal tract conditions, and an acceptable sensory shelf life. Notably, a 250 mL serving of this beverage provides 64% of the Se RDI, of which 28% is SeCys, thus enhancing its potential nutritional benefits. Finally, the SeNPs produced by these strains were characterized, and their presence after gastrointestinal digestion was determined.

S1-RT1: A Tale from 30 French Vineyards: How Vine-to-Wine Microbial Shifts Shape Volatile Profiles in Berries and Wines

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Grape berry quality is highly influenced by the pedoclimatic conditions in vineyards and specific microbial communities colonizing the berry surface during ripening. Yet, microbial community shifts from vine to wine have not been fully explored, especially mycobiota. Fungi are well-known producers of volatile organic compounds (VOCs) that may impact wine quality. Some fungi as well as their interactions may contribute to wine defects such as fresh mushroom aroma (FMA).

To decipher the microbial succession from vine to wine, we monitored bacterial and fungal communities in 30 vineyards at five different stages from vine to wine during three successive years (2021–2023). Climatic conditions were recorded. The cultural and metabarcoding data generated were compared with sensorial analyses of still wines resulting from 30 grape must micro-vinifications to determine to what extent fungal composition and microbial interactions led to the FMA defect in wines.

We observed distinct shifts in microbial communities, mainly influenced by climatic data and berry ripening stage. In the early stages, yeasts dominated, followed by a progressive increase in mold diversity. Climatic conditions not only shaped mycobiota but also played a crucial role in FMA detection in wines. Wines exhibiting FMA off-odors were linked to vineyards with higher precipitation and lower temperatures during the 2021 and 2023 growing seasons. FMA was primarily linked to higher fungal counts and *Penicillium* species, especially *Penicillium crocicola*. Fifteen *Penicillium* species were identified.

On Meunier or Pinot noir grapes, *Botrytis cinerea* co-inoculated with *Penicillium bialowiezense* or *Penicillium citreonigrum* also led to detectable FMA off-odors and an increase in known FMA markers. Untargeted head space GC-MS identified known FMA-associated VOCs (8 carbon VOCs) but also unidentified VOCs, suggesting the FMA defect is most likely linked to combinations of multiple fungal derived compounds.

Overall, our results advance the understanding of how microbial communities evolve from vine to wine. We showed how specific individual and co-cultured grape-associated fungi thrived on berries and induced FMA off odors. This research provides the groundwork for the possible use of the identified repressive species to control fungal populations on berries.

S1-RT2: Deciphering Microbial Contributions to Fermentation and Flavour Development in Mediterranean Table Olives by MultiOmics

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Table olives are a traditional fermented food typical of Mediterranean countries. Fermentation is needed to degrade oleuropein, a bitter compound. Two main debittering approaches exist: in Spanish-style, it is performed synergistically by sodium hydroxide and fermentation, whereas Greek-style only relies on microbial activity. Microbial actors include lactic acid bacteria (LAB) and yeasts that not only remove bitterness but also develop the flavour of table olives. However, both the ecological factors influencing the microbiome composition and its metabolic potential are still overlooked.

In this study, we performed whole metagenome sequencing on a total of 271 samples from different Mediterranean countries (Italy, Spain, Greece, Cyprus and Egypt). In addition, volatilome was analysed through Solid Phase Micro-Extraction (SPME) and Gas Chromatography Mass Spectrometry (GC-MS) analysis. Metagenomes were screened for metabolic activities involved in flavour formation and in protection against exogenous factors and microbial contaminants, and the link between microbial species and concentration of volatile organic compounds was explored.

Lactiplantibacillus pentosus was the most abundant taxon in both Greek and Spanish-style olives, although showing a significantly higher abundance in the former. In addition, the length of fermentation strongly influenced the community assembly by increasing the diversity of LAB and selecting for bioprotective activities, thus reducing the presence of potentially spoilage microbes, which in turn reflected on the metabolic potential of the communities. In addition, the distribution of esterases and lipases involved in flavour development and of the carrying microorganisms discriminated between Greek- and Spanish-style olives. This was also reflected in flavour profiles: Greek-style olives exhibited a higher diversity of alcohols, ketones and esters than Spanish-style products.

Overall, these findings advance our understanding of the microbiome potential and its influence on volatilome in table olive production. These results may assist in the isolation of starter cultures with flavour-enhancing and bioprotective activities, thus supporting stakeholders in developing high-quality products.

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S1-RT3: Exploring Microbial Fermentation of Pea Flour to Enhance Its Sensory and Nutritional Qualities

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Pulses are a sustainable plant-based protein source for vegetarian diets; nevertheless, their broader use by consumers is hindered by limited sensory acceptability and the presence of anti-nutritional factors. Fermentation is a potentially effective strategy to address these challenges, improving both flavour and nutritional properties. This study investigated which diverse microorganisms, including yeasts, moulds, and aerobic bacteria, enhanced the organoleptic profile and reduced anti-nutritional compounds in *Pisum sativum* flour. Certain isolates, belonging to the *Bacillus* and *Rhizopus* genera, have already been identified as unconventional starters in European food applications. In our study, 169 strains, belonging to 55 species of bacteria, 22 species of yeasts and 36 species of moulds, were characterised for their growth and proteolytic, lipolytic, amylolytic and phytic-degrading activities. Among them, 50 strains were further selected, and a multidisciplinary metabolomics, volatilomics and orthonasal sensory analyses approach was used to evaluate their impact after pea-flour fermentations. These analyses enabled the classification of microbial groups according to their impact on flavour and anti-nutritional factor degradation.

Overall, moulds outperformed bacteria and yeasts in terms of enhancing sensory qualities. Several species generated fruity aromas as *Aspergillus oryzae*, *Rhizopus* sp., *Mucor* sp., and *Neurospora*, while brioche-like odors were detected, especially with *Mucor* sp.. Certain yeasts and bacteria also produced distinctive flavours. *Yarrowia lipolytica* and *Mammaliicoccus lentus* contributed to cheesy notes, while *Bacillus* spp. strains generated cocoa or hazelnut descriptors. Conversely, certain strains produced undesirable odors, often described as "rotten" or "cowshed." A significant correlation between sensory descriptors and odour-active molecules, including pyrazines and esters, emphasised the chemical basis of flavour development. All these selected strains were also identified as safe for use as no biogenic amines or mycotoxins were produced. Furthermore, 23 strains exhibited the capacity to degrade galacto-oligosaccharides, achieving up to 100% reduction. This finding suggests a possible enhancement of product digestibility.

Overall, the present study identified multiple microbial strains capable of thriving on pea-flour media and positively modulating both flavour and nutritional attributes. The selected strains produced a wide range of aromatic profiles, demonstrating to what extent fermentation generated desirable sensory properties. Fermentation of *P. sativum* flour can therefore be regarded as a valuable substrate to develop innovative, palatable, and nutritionally enhanced pulse-based foods or ingredients, thus contributing to more sustainable and acceptable plant-based diets.

S1-RT4: Fungal-Driven Fermentation of Plant-Based Blue Cheese Analogues: Integrating Metabolomic and Metagenomic Insights into Substrate Influence and Safety

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The increasing demand for sustainable foods has driven the development of plant-based cheese analogues. However, reproducing mold-ripened varieties remains challenging, as their sensory attributes depend on microbial activity during ripening. Blue cheese analogues (BCAs), in particular, require controlled fungal metabolism to achieve characteristic flavor and texture while ensuring safety.

In this study, *Penicillium roqueforti* (Roquefort population), previously isolated from PDO Roquefort cheese, together with selected lactic acid bacteria, was used to ferment cashew- and tofu-based substrates to produce BCAs. A combined targeted and untargeted metabolomics approach was used to characterize the metabolite profile of *P. roqueforti* of the BCAs and assess the potential presence of mycotoxins originating from raw materials. The targeted analysis covered approximately 40 metabolites, including *P. roqueforti* secondary metabolites (e.g., roquefortine C, mycophenolic acid, isofumigaclavine A, andrastin A, PR toxin), selected mycotoxins associated with raw material contamination (aflatoxin B1, ochratoxin A, patulin, citrinin, sterigmatocystin, citreoviridin), fatty acids, and terpenoids. The untargeted approach enabled the discovery of unknown, substrate-associated features. This was complemented by shotgun metagenomics to provide additional insights.

Metabolomic profiling confirmed active fungal metabolism across all samples, with the detection of characteristic *P. roqueforti* secondary metabolites, including roquefortine C, mycophenolic acid, isofumigaclavine A, and andrastin A, alongside fatty acid-related metabolites. The tetrapeptide cyclo(Phe–Val–Val–Phe) was consistently detected, indicating active peptide-related metabolism. PR toxin and its biosynthetic pathway intermediates (ErmA and ErmB) were not detected, indicating that this biosynthetic pathway was not activated under the applied fermentation conditions. In addition, no other regulated or emerging mycotoxins potentially associated with raw material contamination were detected. Untargeted metabolomics revealed substrate-dependent differences, with tofu-based analogues showing higher relative abundances of fungal secondary metabolites.

Shotgun metagenomic analysis demonstrated distinct microbial community structures between substrates. Cashew-based samples were dominated by *Lacticaseibacillus paracasei* (~75% relative abundance), indicating a primarily bacterial-driven fermentation. In contrast, tofu-based samples exhibited a more diverse microbial ecosystem, with increased abundance of *P. roqueforti*, alongside *Leuconostoc mesenteroides* and *Enterococcus hirae*. The highest *P. roqueforti* abundance was associated with enhanced fungal metabolite production.

Overall, substrate composition critically shapes microbial dynamics and metabolite production in plant BCAs. The absence of regulated mycotoxins and PR toxin from *P. roqueforti* supports the safety of controlled fermentation. Integrating metabolomics and metagenomics offers a strong framework for optimizing fungal-ripened plant-based foods.

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S1-RT5: Water Kefir Ecosystem Multiomics Brings New Understanding of Microbial Activities During Fermentation

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Water kefir (WK) is a beverage produced by fermenting a sucrose solution with dried fruits, using water kefir grains (WKGs) as inoculum. WKGs are natural dextran granules that host fermentative microorganisms, which not only ferment the available substrate but also build the WKGs themselves. WKGs always contain lactic acid bacteria (LAB) and yeasts, and often contain also bifidobacteria, acetic acid bacteria, and *Zymomonas*. The WK microbial composition and the impact of technological parameters in WK production have been well-researched. However, much remains to be uncovered about the functioning of WK(G) microorganisms during fermentation and the interactions between them.

In this study, we aim to bring new knowledge on the functioning of WK and WKGs by combining classical microbiology, genomics, metagenomics, metatranscriptomics, and metabolite target analyses – all applied to a single WK ecosystem. LAB strains belonging to *Lacticaseibacillus paracasei*, four *Liquorilactobacillus* species, and *Schleiferilactobacillus harbinensis* have been isolated from the WK ecosystem under study, along with strains of yeast species *Brettanomyces bruxellensis*, *[Candida] boidinii*, *Hanseniaspora valbyensis*, and *Zygotorulaspora florentina*, as well as strains of two *Acetobacter* species and *Bifidobacterium psychraerophilum*. Moreover, long-read metagenomics revealed the presence of species not yet isolated from this WK, including *Lentilactobacillus hilgardii*, *Oenococcus sicerae*, and a *Zymomonas mobilis* relative. Enabled by isolate genome sequences and metagenome-assembled genomes, metatranscriptomics revealed *Liquorilactobacillus nagelii* as the most transcriptionally active bacterium in the experimental fermentation, followed by *O. sicerae*. Hence, a deeper analysis of transcriptional activities focused on these two bacteria and uncovered traits of likely importance during WK fermentation. For instance, *L. nagelii*, although homofermentative, expressed genes typical of heterofermentative metabolism. It also expressed genes needed to utilise gluconic acid and mannitol produced by acetic acid bacteria and heterofermentative LAB (e.g., *O. sicerae*), respectively. Both species expressed ATP synthase subunit genes to a high degree, but only *O. sicerae* expressed the arginine deiminase pathway, revealing different approaches to acid resistance mechanisms and amino acid management. Moreover, genes possibly involved in rudimentary respiration were expressed by both species, and *L. nagelii* expressed the genes involved in menaquinone biosynthesis. Thus, multiomics revealed genes involved in microbial metabolism (e.g., amino acid and cofactor biosynthetic capabilities) and stress resistance, as well as potential metabolite-based microbial interactions between WK microorganisms. The analyses delivered novel insights into the WK ecosystem function and metabolic versatility of WK microorganisms, uncovering multiple directions for future studies.

S1-FT1: From Waste to Taste: Developing a Novel Water Kefir

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The concept of novel food fermentation has emerged as a response to converging factors, including the increasing demand for diverse and functional fermented foods, efforts to find novel solutions to global food shortages, and the shift towards more sustainable and plant-based diets. Water kefir (WK) is a traditional fermented beverage. This study used genome-scale modelling (GSM) to design minimal synthetic microbial communities (SynComs) for novel WK fermentations. These SynComs were subsequently validated through multi-omics analyses following fermentation with two food waste-based substrates from Ireland. The study aims to assess the performance of SynComs in terms of fermentation efficiency and to evaluate the safety and health-promoting potential of the resulting beverages.

Six minimal SynComs were developed using GSM and used for the novel WK fermentation with two sustainable substrates from the Irish food waste stream. Shotgun metagenome sequencing was performed to identify the community composition after fermentation. Safety assessments, including mycotoxin screening and cytotoxicity evaluations, were also performed. Gut microbiome modulatory effects were evaluated using an *ex vivo* model of the distal colon. A comprehensive metabolomic analysis was performed to quantify non-volatile and volatile compounds.

All SynComs successfully fermented the novel WK, lowering pH to ~3.5. Ethanol content remained within the non-alcoholic range (< 0.3% ABV), and the sucrose content was 5 g/L, resulting in non-alcoholic, low-sugar beverages. Shotgun metagenomics confirmed SynCom composition. No mycotoxins and cytotoxicity were detected. Metabolomic analysis revealed the presence of favourable health-promoting compounds and volatiles, resulting in promising novel beverages.

This study demonstrates the successful application of GSM-guided SynComs to transform food waste-based substrates into novel, healthy, and sustainable fermented foods.

S1-FT2: Mechanistic Insights into the Role of Yeast Stress Responses in Driving Optimal Oven Rise Performance During Breadmaking

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Yeast is a key ingredient in breadmaking, yet the understanding of its performance during the critical oven rise phase is lacking. Especially in whole-wheat systems, optimal oven rise remains a challenge. Optimising yeast performance, leveraging the natural diversity of yeast, offers a sustainable, clean-label alternative to industrially used bread improvers. Traditionally, yeast strains are screened in liquid media; however, our previous research highlights the necessity of in-dough studies. Yeast viability during baking strongly correlates with the extent of oven rise, a correlation notably absent in liquid media. This raised a fundamental question: which characteristics enable baker's yeast to better withstand the stresses of the baking phase?

To address this gap, we first optimised the extraction of the yeast transcriptome directly from dough, analysed it and compared it with that obtained from a simple liquid screening medium. Building on these findings, we compared the stress responses in a dough matrix of three yeast strains that differ in the extent of oven rise they impart.

Our analysis revealed that the dough environment induces a complex, multi-stress response characterised by the upregulation of heat-shock proteins, osmotic stress protectants, and cell wall remodelling. This response is unique to the dough matrix; for instance, yeast in dough exhibited a 44-fold increase in glycerol and a 6-fold increase in trehalose during fermentation compared to liquid culture. Indicating yeast during dough fermentation is prepared for the harsh baking phase that follows.

We demonstrated that the three strains exhibit similar stress responses during breadmaking, reflected by comparable trends in stress metabolites. In contrast, amino acid metabolism shows differential expression among strains. The best oven-rise-performing strain demonstrated a unique ability to maintain redox homeostasis. This was evidenced by the significant upregulation of glutathione synthesis and precursor pathways, alongside the downregulation of glutathione degradation. This suggests that redox homeostasis, mediated by glutathione, may be a critical determinant of yeast survival during the thermal transition of baking. This research demonstrates that the dough matrix is an active stressor that impacts yeast physiology in ways simple liquid screenings cannot capture. By analysing yeast behaviour in dough, we identified glutathione-mediated redox stability as an interesting baker's yeast trait for optimal oven rise performance. We provide a new mechanistic framework for selecting robust yeast strains, ultimately supporting the development of clean-label, additive-free breadmaking processes.

S1-FT3: Ancient Brews in New Bags: Recreating the Historical Beverage Fuqqāʿ

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Within medieval Arabic food culture, fuqqāʿ ("bubbly beer") was a popular fermented beverage. It consisted of more than 20 variants, according to historical records. Despite this variability, a number of shared characteristics can be identified, including a relatively brief fermentation time (1–2 d), a low alcohol content (0–2%), and natural carbonation. The most used ingredients included grains and/or bread as carbohydrate sources, supplemented with dried fruits, spices, sugar, honey, and herbs. The fermentation process is speculated to have been carried out in small, sphero-conical clay vessels.

In this study, fuqqāʿ production was first simulated in glass vessels with a water lock, and subsequently in historically reconstructed clay vessels. Three fuqqāʿ recipes were followed, using a flat bread for soaking in water (and thus carbohydrate delivery) complemented with either 1) pomegranate seeds and Arabic gum, 2) raisins, or 3) dates. Based on the microbial and metabolic results obtained from the glass vessel experiments, the first recipe was selected for further experiments in clay vessels. Within each clay vessel, spontaneous fermentations were repeated five times to study backslopping effects (based on microbial colonization of the pores within the clay).

Across setups, fermentations converged towards mostly similar microbial and metabolic profiles (sugars, sugar alcohol, organic acids, volatile organic compounds, etc.), with some differences. In glass vessels, the early stages were dominated by lactic acid bacteria (LAB), particularly *Leuconostoc citreum* and *Lactococcus lactis*, which drove rapid acidification within 24 h, while yeasts such as *Saccharomyces cerevisiae* emerged later, contributing to low ethanol levels (0–1%). In clay vessels, undesirable fungi (e.g., *Fusarium* sp.) emerged in the early stage but disappeared upon backslopping, paralleling the establishment of a LAB-dominated microbiota with the later onset of *S. cerevisiae*.

Overall, fuqqāʿ fermentation was driven by specific LAB species and *S. cerevisiae*, regardless of recipes, raw materials, vessels, and batch variations. Nonetheless, reusing clay vessels proved important, as it enhanced microbial stability via backslopping, thereby contributing to product consistency and biosafety.

S1-FT4: Targeted Isolation and Characterization of Propionic Acid Bacteria for Plant-Based Food Fermentations

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Demand for plant-based alternatives to dairy and meat products is increasing, yet many plant-derived matrices pose multiple limitations. A major nutritional limitation is the lack of vitamin B_{12}, a micronutrient predominantly found in foods of animal origin. Propionic acid bacteria (PAB), particularly *Propionibacterium freudenreichii*, are established food-grade organisms and are known to synthesize bioavailable vitamin B_{12} forms, making them attractive adjacent starter cultures for plant-based applications. The aim of this work was to isolate PAB from raw milk and assess their suitability for future use in plant-based fermentations.

PAB were isolated from raw milk samples using selective and non-selective media. Colonies were pre-selected based on typical PAB colony morphology. Isolates were further screened using a PAB-targeted PCR with specific primers. For taxonomic identification, 16S rRNA gene analysis was performed. Phenotypic differentiation employed RAPID ID 32A, with emphasis on α/β-glucosidase and α/β-galactosidase activities as indicators of potential interactions with plant-derived glycosides. Complete genomes of isolates identified as *P. freudenreichii* were obtained using Nanopore sequencing. Phylogenetic relationships among isolates were inferred by core-genome analysis using Roary. Scoary was applied to identify genetic features associated with enzymatic activities. Genomes were also compared for key genes involved in vitamin B_{12} biosynthesis.

From over 20 raw milk samples, multiple *P. freudenreichii* strains and *Acidipropionibacterium* spp. were isolated, with *P. freudenreichii* representing the most abundant taxon. Metabolic profiling revealed strain-dependent enzyme activity patterns, indicating functional diversity potentially relevant for plant-based matrices. While some *P. freudenreichii* isolates exhibited α-galactosidase activity, none fermented raffinose, in contrast to the *Acidipropionibacterium* isolates, making them perhaps less relevant for legume-associated substrates. Despite high similarity, *P. freudenreichii* isolates formed distinct genomic clusters based on core-genome comparisons. Scoary analysis showed distinct genotype–phenotype links underlying enzyme activity differences. All *P. freudenreichii* genomes shared the core vitamin B_{12} biosynthesis genes. Ongoing work will evaluate vitamin B_{12} production under food-like fermentation conditions to identify starter candidates.

S1-FT5: Creation of a Catalogue Containing 1,593 Metagenomes Unearths the Microbiome Role in the Terroir of Cheese

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Cheese microbiomes shape unique signatures reflecting diverse manufacturing, geographical origins, and potential health benefits. Yet, their diversity and functional links to terroir and bioactive potential remain underexplored.

High-throughput shotgun metagenomic sequencing raw reads from curatedFoodMetagenomicData (*n* = 1044), publicly available dataset (*n* = 233), and newly sequenced Protected Designation of Origin and Protected Geographical Indication cheeses (*n* = 316) were merged and analysed. Furthermore, a metadata collection and standardisation (*n* = 18) allowed the harmonisation of diverse information about cheeses.

Thus, a comprehensive metadata-curated catalogue of cheese microbiomes by collecting a total of 1,593 shotgun metagenomic samples was developed. Our findings revealed different values of α- and β-diversity depending on the metadata considered, as well as different distribution patterns of microbes and key functional genes associated with flavour, quality, and health benefits. Explainable machine learning models further elucidated the cheese microbiomes classification among metadata, also suggesting potential biomarkers. Additionally, phylogenetic analyses were employed to investigate strain-level microbial diversity, whilst functional phylogenomic approaches linked microbial taxa to functional cheese patterns. In summary, our analysis yielded 4,170 high- and medium-quality metagenome-assembled genomes, over 28% of which represented unknown species, alongside strain-level patterns linked to cheesemaking practices.

This curated cheese microbiome catalogue advances our understanding of cheese microbial ecology, offering novel insights into microbial contributions to cheese terroir and safety, and supporting future applications for precision fermentation and health-oriented food innovation.

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S1-FT6: Optimizing Recombinant Bovine Protein Production in *Komagatella phaffii* Using Modular Cloning: A Safety and Yield Assessment of Precision-Fermented β-Casein

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Precision fermentation enables the sustainable production of animal-free milk proteins, offering a scalable alternative to conventional dairy. Among these, β-casein plays a pivotal role due to its amphiphilic structure and its capacity to form micelles, which are essential for the texture, stability, and nutritional functionality of dairy products. However, expressing bovine caseins in yeast remains challenging due to their intrinsically disordered structure and proline-rich sequences. These features often trigger endoplasmic reticulum (ER) stress and significant secretion constraints. To address these limitations, we developed a modular cloning (MoClo) strategy using the OpenPichia toolkit to engineer *Komagatella phaffii* for the production of bovine beta-casein (β-CSN), with beta-lactoglobulin (BLG) as a comparative control.

Utilizing a Golden Gate assembly-based approach, we systematically constructed expression cassettes to evaluate how genetic architecture influences protein yields. All constructs were designed and verified in silico to optimize codon usage and select ideal regulatory elements. We compared methanol-inducible versus constitutive promoters, alongside different signal peptides, to facilitate correct folding and efficient extracellular export of the target phosphoproteins.

Scale-up was performed in 10-L bioreactors using a fed-batch strategy, with protein recovery managed via tangential flow filtration (TFF). Parallel to yield optimization, a safety evaluation was conducted following international Risk Assessment standards, including the detection of residual recombinant DNA, the absence of viable genetically modified yeast cells, and the screening of endogenous metabolites. Through an integrated omics pipeline, we verified amino acid sequences and characterized post-translational modifications, specifically phosphorylation patterns, to ensure equivalence with bovine counterparts.

This study provides a standardized molecular framework to overcome yeast secretory constraints and a robust methodology for the safety characterization of next-generation sustainable dairy ingredients.

S2 Microbiomes in Sustainable Food Systems, Mitigation of Food Spoilage, Losses and Wastes

S2-KN1: Mapping the Invisible: How Microbiomes Influence Contamination and Control Strategies

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Microbiomes in food processing environments are increasingly recognized to influence the efficacy of sanitation efforts and thereby microbial food safety and quality. Environmental microbiota is shaped by raw ingredients, season, human activity, food processing, and sanitation protocols, among others. These factors impact the composition and temporal stability of microbial communities and, by extension, the persistence and behavior of foodborne pathogens such as *Listeria monocytogenes*. Longitudinal surveillance across food processing facilities is revealing that environmental microbiota is highly facility-specific, with communities clustering by location more strongly than by sampling time or sanitation treatment applied. Commonly identified core genera, particularly *Pseudomonas*, *Acinetobacter*, *Flavobacterium*, and *Stenotrophomonas*, recur across facility types and geographic contexts, suggesting broad environmental adaptation. Despite this shared composition at the genus level, finer-scale taxonomic variation and seasonal dynamics mean that cross-sectional snapshots can misrepresent the resident microbiota, demonstrating the need for longitudinal, spatially resolved sampling strategies. Beyond community characterization, microbiome data carry predictive value. Differential abundance analysis and machine learning approaches have identified microbial taxa in tree fruit processing facilities, including *Pseudomonas*, *Stenotrophomonas*, *Microbacterium*, whose relative abundance is elevated in *L. monocytogenes*-positive samples. In meat processing environments, *Pedomicrobium* species showed positive associations with *Listeria* spp. recovery from drain samples, the predominant harborage niche for *L. monocytogene*s across facility types. Mechanistic studies have revealed why these co-occurrence patterns matter for food safety. Multi-species biofilms formed by *Pseudomonadaceae*, *Xanthomonadaceae*, *Microbacteriaceae*, and *Flavobacteriaceae* substantially increased the attachment and growth of *L. monocytogenes*, and some have substantially extended its tolerance to benzalkonium chloride and peroxyacetic acid at concentrations routinely applied in food processing facilities. In some cases, extending the minimum duration for killing beyond the recommended sanitizer contact time. In dairy environments, the presence of *Pseudomonas* similarly reduced the antilisterial efficacy of lactic acid bacteria proposed as potential biological control agents, illustrating that biocontrol performance is context dependent. Taken together, this body of work demonstrates that food safety and quality cannot be assessed in microbiological isolation. The resident microbiome of a facility shapes pathogen survival, sanitizer efficacy, and the performance of alternative control strategies. Mapping this invisible landscape is therefore foundational to designing effective, facility-informed sanitation and surveillance programs.

S2-RT1: Framework for the Integration of Multispectral Imaging Technologies and AI-Based Methods for Assessing the Microbiological Quality of Beef Slices (*M. longissimus dorsi*)

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Ensuring the microbiological quality of food products remains a critical challenge in food systems, particularly for highly perishable items like meat. Traditional microbiological analyses, though accurate, are labor-intensive, time-consuming, and unsuitable for real-time monitoring in processing and storage environments. The need for continuous evaluation of the microbiological status of food products, together with limitations of currently available methods, has created a strong demand for rapid, non-destructive, and automated techniques capable of providing reliable indicators of microbial proliferation.

The study focused on exploring the potential of integrating multispectral imaging with deep learning as a novel framework for assessing the microbiological quality of beef slices (*M. longissimus dorsi*). Reflectance data covering 18 wavelengths (405–970 nm) acquired with a VidiometerLab system were employed to capture structural, and colour-related changes within beef matrix stored for up to 20 days at different temperatures (0, 5, 10, and 15°C) in air and under modified atmosphere (40% CO_2 – 30% O_2 – 30% N_2) conditions. Simultaneously, together with multispectral imaging microbiological analyses were performed to determine total viable counts (TVC).

The proposed framework integrates multiple steps covering data acquisition, preprocessing, feature extraction and interpretation. To efficiently perform this complex task, convolutional neural networks (CNNs) were employed using Python the Keras/TensorFlow library. The 2D-CNN structure was optimized to balance sensitivity to microbial changes with robust generalization to new data. The developed model architecture consisted of a single-channel input of raster images illustrating waveforms of reflectance changes across wavelengths, followed by three convolutional layers with ReLU activation functions (with 6, 9, and 12 filters, respectively; kernel size 5×5), each paired with by 2×2 max-pooling layers for feature extraction. The generated signals were further processed by fully connected dense layers, including one hidden layer with six neurons, and output layer with single neuron for predicting bacterial populations in beef samples. The applied deep learning algorithm effectively extracted features from image-based inputs, enabling the detection of subtle patterns associated with microbial activity that may remain imperceptible to conventional analytical techniques. The developed CNN model demonstrated high predictive performance for TVC in beef slices, both in air and under modified atmosphere conditions, as reflected by strong agreement between predicted and observed values (RMSE = 0.54 log CFU/g, R^2 = 0.93). The integration of multispectral imaging and CNNs can contribute to the development of next-generation food quality and safety management strategies, enabling proactive, data-driven interventions that enhance product quality and consumer protection.

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S2-RT2: Microbial Spoilers at Home: Investigating the Link Between Domestic Hygiene and Fresh Fruit and Vegetable Spoilage and Losses at the Household Level

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Fresh fruit and vegetable (FFV) spoilage represents a main part of consumer's food losses. While most losses stem from consumer behaviour, the link between hygiene of storage compartments and spoilage at home remains unclear. In this context, this work aimed at identifying microbial spoilers of FFV and studying the microbiota of storage surfaces at home as well as evaluating the biocidal effect of different products.

FFV loss was measured in 49 volunteer households using connected bins, over two periods (summer/autumn) while nearly 400 swabs were taken from domestic storage compartments (refrigerator FFV bin, basket, etc.) and analysed using culture-dependent and -independent approaches. Besides, over 350 spoiled FFV were collected and the microorganisms associated with them were identified. The biocidal effect of "chemical" disinfectants (sodium hypochlorite, commercial disinfectant) and "green" alternatives (lemon juice, alcohol vinegar, household alcohol) was evaluated against nine spoilage bacteria and fungi and three foodborne bacterial pathogens.

FFV storage compartments were reservoirs of fungi and bacteria including FFV spoilage species with important variations among households. Low but significant positive correlations were also found between microbial variables and FFV losses. Among tested green alternatives, lemon juice showed limited disinfection efficacy.

For the first time, the link between domestic hygiene and FFV spoilage is being investigated at the household level.

S2-RT3: Biotic and Abiotic Determinants of *Secundilactobacillus collinoides* Spoilage During Cider Fermentation

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Cider is a fermented apple beverage with rich tradition, particularly in France, where it is elaborated using spontaneous fermentation. While cider fermentation can develop complex and desirable flavors, it is also susceptible to microbial spoilage. One such defect is an alteration due to the metabolism of glycerol known as "piqûre acroléique", which severely impacts its taste and quality. This issue is mainly caused by *Secundilactobacillus collinoides*. The bacterium metabolizes glycerol, a natural cider component produced during alcoholic fermentation, into 3-hydroxypropionaldehyde (3-HPA), which is then dehydrated into acrolein. Several factors increase the risk of acrolein formation, including high glycerol content, low acidity, and high temperatures. This problem is particularly concerning for French distillation ciders, where the use of sulfites (SO_2) is not a viable solution due to regulations and product specifications.

To better understand this defect, this study analyses genetic determinants of subpopulations of *S. collinoides*, key microbial interactions and environmental conditions that modulate 3-HPA formation by *S. collinoide*s during cider fermentation. On one hand, a combination of genomic analysis and infrared spectroscopy were used to characterize genetic variations of *S. collinoides*. It was possible to distinguish two subpopulations of *S. collinoides* exhibiting different operon organisations. On the other hand, the interactions between *S. collinoides*, *Oenococcus oeni* and *Brettanomyces anomalus* were investigated, as these tripartite relationships remain largely undocumented despite being potentially determinant in modulating 3-HPA and acrolein production, whether through competition for substrates, alteration of physicochemical conditions, or direct metabolic cross-talk between these co-occurring microorganisms. Metabolic activities were used to characterize bacterial populations and their activity throughout fermentation. qPCR targeting the *pduC* gene, which encodes the key subunit of glycerol dehydratase, the enzyme directly responsible for 3-HPA production, enable sensitive and specific detection of relevant subpopulations. This method allows detection down to concentrations as low as 10^3 CFU/mL, providing a reliable quantitative monitoring tool even at early stages of contamination. Metabolic activity were tracked through HPLC measurements of key parameters including sugars, ethanol, and malic acid, which are central to both alcoholic and malolactic fermentation dynamics. pH was also monitored throughout the process, as it is known to influence bacterial growth and enzymatic activity. Preliminary results suggest that microbial co-cultures significantly modulate 3 HPA accumulation. Understanding this mechanism could provide new strategies to control bacterial activities and prevent the defect, without adding chemical preservatives.

S2-KN2: Finding Needles in a Haystack: Enumerating Bacterial Food Spoilers in Raw Materials

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Microbial food spoilage represents a critical yet often underestimated challenge at the intersection of food quality, safety, security, sustainability, and public health. As a major driver of global food loss – accounting for a substantial proportion of the approximately one-third of food produced but not consumed – spoilage contributes significantly to environmental burdens, including greenhouse gas emissions. Tackling food spoilage is therefore not only a matter of quality control, but a key step toward improving food security and advancing global sustainability goals.

This presentation will explore recent advances in understanding and managing bacterial food spoilage, focusing particularly on the concept of detecting the 'needles in the haystack' – low-abundance, highly specialized bacteria that can nevertheless cause severe product defects. Despite their impact, these organisms often escape routine detection, as conventional methods lack the sensitivity and selectivity required to reliably identify them at low concentrations, limiting effective risk assessment and spoilage mitigation. To overcome these challenges, novel methodological approaches have been developed, tailored to specific spoilage phenomena relevant to dairy and bakery products. These include late blowing in cheese caused by butyric acid-producing clostridia, gas defects in raw milk cheese associated with propionic acid bacteria, and rope spoilage in bread linked to *Bacillaceae*. Together, these examples illustrate both the diversity of spoilage bacteria and the need for targeted analytical solutions.

At the same time, food spoilage is inherently complex. It does not arise from a single cause, but from the interplay of microbial communities, microbial variability, and environmental conditions. Understanding contamination pathways and ecological niches along the food chain – from primary production to the final product – is therefore essential. In addition, genetic characterization increasingly reveals substantial phenotypic and genotypic heterogeneity within species, highlighting that spoilage potential is often strain-dependent.

Strengthening our ability to detect, understand, and control microbial spoilage will ultimately contribute to reducing food loss and supporting a more sustainable and resilient food system.

S2-RT4: Effect of Regulated Irrigation of Field-Grown Baby Leafy Greens Crops on Native Microbial Assemblages and Foodborne Pathogen Susceptibility at Time of Harvest and During Post-Harvest Storage.

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Regulated irrigation can help optimize water use in vegetable agriculture but effects on crop microbial quality, that impact shelf-life and gut microbiome health, remain understudied. We investigated how two watering regimes, adopted a week prior to leafy greens harvest, affected post-harvest microbial assemblages in the leaves at harvest and for up to nine days post-harvest. *Brassica napus* L. var. *pabularia* (kale) 'Red Russian' and *Lactuca sativa* L. (lettuce) 'Red Sails' were planted in a high tunnel and drip-irrigated, with soil volumetric water content (VWC) monitored continuously. At harvest day, VWC had attained 30/32% in the continuous irrigation beds, and 11%/16% in the restricted irrigation beds of kale and lettuce, respectively. Four-week-old plants were harvested and processed immediately, or stored at 4°C for 3, 6 or 9 days prior to processing for microbiome, native microbial assessment and enteropathogen susceptibility. Amplicon sequencing targeted the 16S rRNA gene for microbiome analysis. Total aerobic bacteria (AC), total lactic acid bacteria (LAB), yeast and mould were quantified on Petrifilm® Plates and *Pseudomonas* spp. on Pseudomonas Agar F. For enteropathogen susceptibility, ~4.5 log CFU/mL of *Escherichia coli* O157:H7, *Salmonella enterica* Enteritidis and *Listeria monocytogenes* were infiltrated into kale while lettuce was spot inoculated, followed by storage for 1, 3, 6 and 9 days at 4°C. Leaves were homogenized for pathogen enumeration by plate counting or modified MPN. At harvest, kale LAB and yeast counts ($p < 0.05$), and lettuce yeast, mould and *Salmonella* counts ($p < 0.05$) were lower from restricted irrigation than continuous irrigation beds. Over the entire 9 days, irrigation was a significant factor for AC, LAB, yeast and mould in kale ($p < 0.05$), and *Salmonella* in lettuce ($p \leq 0.05$). Additionally, storage time had a positive effect on AC, *Pseudomonas*, yeast and *Listeria*, and a reducing effect on *E. coli* O157:H7 in kale ($p < 0.01$). In lettuce, storage time was a factor for all enteropathogens ($p < 0.01$), and all epiphytic native microbe groups ($p < 0.01$), except LAB. PERMANOVA indicated that irrigation treatment was a factor in shaping resident microbial assemblages in kale ($F = 5.0$, $R^2 = 0.07$, $p < 0.01$), although storage time had a stronger effect ($F = 9.4$, $R^2 = 0.41$, $p < 0.001$) and accounted for a greater proportion of the variation. Similarly, in lettuce both storage time ($F = 5.6$, $R^2 = 0.30$, $p < 0.01$) and irrigation ($F = 2.0$, $R^2 = 0.04$, $p < 0.05$) were significant. Taken together, findings indicate that deficit irrigation of leafy greens affected post-harvest microbial quality by restricting resident microbes and Listeria in storage, relative to controls and diminished *Salmonella* persistence during storage.

S2-RT5: From Waste to Function: Exploring Antimicrobial Activity of Orange By-Product Derived Extracts and the Effect of Fermentation Process

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The citrus processing industry generates large amounts of solid by-products, whose management represents an environmental and economic challenge. In line with circular economy principles, recent research has explored innovative strategies to valorize orange by-products through the recovery and enhancement of antimicrobial compounds. The present work synthesizes experimental evidence on the antimicrobial potential of orange-derived matrices obtained after essential oil extraction or juice production, with a particular focus on the role of lactic acid fermentation and formulation approaches in improving bioactivity and applicability in food systems.

Solid residues remaining after cold-press extraction of orange essential oil were subjected to ethanolic extraction showing to retain antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, despite the removal of the essential oil fraction.

In parallel, fermentation of orange pomace prior to essential oil extraction markedly modified the volatile profile of the oils, promoting the bioconversion of limonene into α-terpineol, a compound strongly associated with enhanced antimicrobial efficacy. Essential oils obtained through guided fermentation showed increased activity against a broad range of food-related microorganisms, including bacterial pathogens and spoilage microorganisms. However, when applied directly to complex food matrices such as oat-based beverages, the antimicrobial performance of essential oils was strongly reduced due to matrix effects.

To overcome these limitations, nanoemulsion were evaluated and proved effective in improving the antimicrobial efficacy of essential oils, particularly those richest in limonene, potentially enhancing dispersibility and bioavailability in aqueous food matrices. Overall, the combined application of controlled fermentation and nanoemulsion technologies emerges as a promising strategy to tailor the antimicrobial functionality of citrus by-products, enabling their use as natural preservatives and contributing to sustainable food processing and waste valorization.

S2-FT1: Impact of Spoilage-Associated Microbial Communities on the Evolution of Headspace Volatile Organic Compound Profiles in Refrigerated Paella

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This study investigates the differential effects on the evolution of volatile organic compounds (VOCs) exerted by different spoilage-associated microbial groups inoculated on refrigerated paella. Therefore, freshly cooked paella portions (90 ± 0.5 g) were placed in hermetically sealed jars, sterilised, and individually inoculated (below 4 log CFU/g) with psychrotrophs, *Pseudomonas* spp., *Enterobacterales*, lactic acid bacteria (LAB), and yeasts, followed by storage at 6°C, while uninoculated samples stored under similar conditions served as controls. The VOCs were periodically trapped and analysed using two-dimensional gas chromatography with thermal desorption, coupled with flame ionisation and mass spectrometry detectors, along with microbial and pH analyses. Among all samples, several aldehydes and hydrocarbons were consistently detected, including hexanal, pentanal, 2-methylbutanal, 3-methylbutanal, pentane, heptane, and 2-octene, which were also found in the control samples. These compounds are therefore mainly associated with lipid oxidation processes in the food matrix induced by cooking. In contrast, VOCs such as 1-hexanol, 1-pentanol, 2-methylbutanol, and 3-methylbutanol were detected in all the inoculated samples, indicating their formation through general microbial metabolic activity.

In addition, distinct VOC profiles were observed for different microbial groups. For instance, psychrotrophs produced a wide range of unique metabolites, such as 1,1-diethoxyethane, 2,3-butanedione, acetone, acetoin, 3-pentanone, n-propyl acetate, 2-methyl-3-pentanone, isopropyl acetate, and allyl methyl sulfide. *Pseudomonas* spp. was mainly associated with the formation of isopropyl lactate and carbon disulfide. *Enterobacterales*, LAB, and yeasts exhibited similar alcohol-metabolising activity, which was also observed in other inoculated samples. However, a sharp decrease in pH changes was observed in samples inoculated with LAB and yeasts.

Overall, the above results revealed a significant overlap in VOC profiles among spoilage-associated microbial communities in refrigerated paella. Specifically, psychrotrophs comprise of diverse spoilage-associated microorganisms that exhibited the broadest VOC profiles. From an application perspective, these findings support the development of VOC-based smart sensing systems to detect common spoilage-related VOC patterns, thereby enabling non-destructive shelf-life monitoring of refrigerated foods.

S2-FT2: Genetic and Phenotypic Diversity of Nitrate-Reducing Mesophiles and Thermophiles Isolated from a Dairy Powder Site

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Raw milk can get contaminated via soil, bedding, milk equipment, or transport from the farm to the processing plant. At the dairy production site, mesophilic and thermophilic bacteria survive heat treatments and can form biofilm communities in factory equipment. Their survival can provide a source of nitrite contamination for dairy powders. It has been reported that some of these mesophilic and thermophilic microorganisms can convert nitrate to nitrite, thereby affecting nitrite levels in final products. Thus, it is important to control these microorganisms throughout the production process to prevent spoilage and ensure product quality.

This study explores the nitrate conversion capabilities and biofilm formation of different mesophilic and thermophilic species isolated from a dairy powder site. The genomes are sequenced and mined to identify key genomic differences among the isolated bacterial species and isolates to explain phenotypic behaviour.

In this work, 155 strains were isolated from different locations (n = 4) along the spray-drying process, during multiple sampling campaigns (n = 3). Samples were obtained from the milk evaporator, the concentrate after the pre-heater and the final powder samples. The strains were identified at the species level using MALDI-TOF MS. The biofilm-forming capacity of relevant strains was evaluated by crystal violet staining, and nitrate conversion was assessed using the Griess reagent test. The genomes of 58 representative strains, selected based on phenotypic diversity, were sequenced.

Bacillus licheniformis was the most frequently isolated microorganism in the evaporator, while in the concentrate, *Geobacillus stearothermophilus* was the most prevalent. In powder samples, both *G. stearothermophilus* and *Anoxybacillus flavithermus* were dominant. Among the overall sequenced strains, 44.8% showed complete nitrate reduction, 46.6% showed strong reduction, and 8.6% showed poor conversion to nitrite. Furthermore, differences in biofilm-forming capabilities are apparent among species and strains, with many *B. licheniformis* strains being good biofilm producers. Five of these *B. licheniformis* strains isolated from all locations showed high nitrate conversion and biofilm-forming capabilities. Both biofilm and nitrate conversion capabilities seem to be strain-dependent and independent of the isolation source.

Genome mining and phenotypic characterisation are key to identifying nitrate-reducing bacteria and preventing spoilage at the dairy powder factory, enabling control of the dairy production line and powder quality.

S2-FT3: Processing Shapes the Spore-Forming Bacterial Contamination Dynamic from Seeds to Plant-Based Ingredients

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Spore-forming bacteria in plant-derived ingredients may affect the stability of minimally processed foods. However, contamination dynamics from seeds to ingredients remain underexplored, particularly in pulses and oilseeds. This study investigates spore loads and diversity across lentil and sunflower seed-to-ingredient chains.

Twenty-two samples from two batches were collected across lentil (dehulling, milling, then either extrusion or turbo-separation) and sunflower (cold-press, milling, ethanol-washing, drying) processing chains. For each sample, total viable counts (TVC) and bacterial spores were enumerated at pH 7 and 5. Specific culture conditions were used to quantify total psychrotrophic (TPS, 10°C), mesophilic (TMS, 30°C), and thermophilic (TTS, 55°C) spores, as well as presumptive *Bacillus cereus* (BCES; MYP medium, 30°C) and sulfite-reducing *Clostridium* (SRCS; ISA medium, 37°C). Highly heat-resistant thermophilic spores (HRTS, 55°C) were also enumerated after a 100°C/30 min heat-treatment. From each spore count plate, 5–10 morphologically distinct colonies were identified by MALDI-TOF MS to assess diversity.

Bacterial loads varied across seeds, batches, and processing stages (from < 1.0 to 4.1 $\log_{10}$ CFU/g). TMS levels approached TVC, indicating a high spore proportion. Across all samples, TMS showed the highest counts (1.0–3.8 $\log_{10}$ CFU/g), followed by TTS, TPS, and BCES (< 2.5 $\log_{10}$ CFU/g), while SRCS and HRTS remained mostly below 1.0 $\log_{10}$ CFU/g. TVC, TMS, and HRTS levels were similar under acidic and neutral recovery pHs. In lentil processing, dehulling reduced microbial loads by 0.8 log, whereas turbo-separation partitioned spores, with higher levels in the protein-rich fraction (1.8 $\log_{10}$ CFU/g) than in the starch-rich one (1.0 $\log_{10}$ CFU/g). Extrusion effects could not be assessed due to low contamination in dehulled lentils. In sunflower processing, despite an increasing trend in TMS (from 1.98–2.95 $\log_{10}$ CFU/g to 2.4–3.8 $\log_{10}$ CFU/g), no single step significantly impacted overall contamination levels and profiles. Across all samples, most isolates belonged to *Bacillus* and *Paenibacillus*. In lentil-derived samples, the main species were *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus pumilus*, and *Heyndrickxia coagulans*, while sunflower samples were dominated by *B. licheniformis*, *B. pumilus*, *B. subtilis* group, and *B. cereus* group. Across both processing chains, *H. coagulans* was the main species recovered under acidic conditions.

This study demonstrates that lentil and oilseed-derived ingredients carry a significant endogenous spore load that can be modulated by processing steps such as turbo-separation and dehulling. However, overall microbial profiles remained largely unchanged, indicating spore persistence through the processing chains. These spores may challenge the formulation of minimally processed plant-based foods and acidic products, due to the spoilage potential of species such as *H. coagulans*.

S2-FT4: Managing Spoilage Potential of Acetic Acid Bacteria in the Beverage Industry: Facing Complexity Through an Integrated Approach

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Europe has a long tradition of production and consumption of fermented beverages, with associated economic, social and environmental value. The sector is increasingly challenged by multiple factors, including microbial spoilage phenomena, which are major contributors to significant quality and economic losses and negatively affect production sustainability. A comprehensive understanding of the microorganisms and mechanisms underlying microbial spoilage, together with control strategies, is still limited. Within this context, acetic acid bacteria (AAB) represent a major spoilage-associated group, whose diversity, ecological dynamics and spoilage potential remain poorly characterized. This PhD work aims to address these gaps by investigating AAB across different fermented beverages.

First, their diversity will be explored by performing new isolation screenings and using complementary culturomic, metagenomic and genomic approaches, along with the development of improved tools for studying unconventional AAB. The impact of AAB on beverage quality will then be examined, with particular attention to identifying key biotic and abiotic factors that influence their spoilage potential. Finally, the project will focus on developing rapid detection methods and innovative, integrated strategies to control AAB in fermented beverages.

To date, a collection of fermented beverage-associated AAB has been established, with strains isolated from spoiled wines produced in different European countries. Whole-genome sequencing of selected strains is ongoing, supporting the exploration of inter- and intraspecific diversity and enabling future investigations into their behavior and spoilage potential. In parallel, culturomic and metagenomic approaches were directly compared to assess their respective potential for AAB detection, highlighting their complementarity rather than a direct replacement. In addition, we are exploring the design of a loop-mediated isothermal amplification (LAMP) assay for the rapid detection of spoilage-associated AAB, aiming to provide a fast, practical tool for monitoring fermented beverage systems.

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S2-FT5: Granadaene Production, Biofilm Formation, and Co-Hemolysis in *Acidipropionibacteria*: Implications for Raw Milk Cheese Quality

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Acidipropionibacterium belongs to the group of dairy propionic acid bacteria (dPAB), characterized by their ability to produce propionic acid. These bacteria are anaerobic to aerotolerant and are considered contaminants in several long-ripened raw milk cheeses, where they enter the milk via biofilms in milking systems. Their presence may lead to sensory defects and spotting in the cheese matrix, partly due to the production of the red pigment granadaene. Granadaene is a hemolytic cytotoxin with antioxidant properties and is therefore undesirable in food production. Despite their relevance, the knowledge of dPAB is currently limited, partly due to taxonomic changes.

In this study, granadaene production was evaluated in a strain set of 47 isolates under anaerobic, microaerophilic, and aerobic conditions on yeast extract lactate agar at 30°C. Colony color was quantified using an IRIS electronic visual analyzer based on the CIELAB color space. Based on pigment production, nine representative strains were selected for further characterization. Biofilm formation was assessed at 20, 30, and 37°C under anaerobic and microaerophilic conditions using a crystal violet assay. Additionally, co-hemolytic activity with *Staphylococcus aureus* was investigated.

Notably, despite being described as anaerobic to aerotolerant, 75% of strains exhibited growth under aerobic conditions following prolonged incubation (10–14 days). Colony coloration varied with atmospheric conditions, with darker and more intensely red pigmentation observed under microaerophilic and aerobic conditions. Biofilm formation was strain-dependent but generally increased at higher temperatures, while atmospheric conditions had a lesser effect. All pigmented isolates demonstrated enhanced hemolysis in proximity to *S. aureus*, indicating co-hemolytic activity. While this phenomenon is known for skin-associated propionic acid bacteria, it has not previously been described for dairy-associated species.

Given the occurrence of these bacteria in raw milk cheeses and their relevance to food production, these findings highlight potential safety and quality concerns and underscore the need for further investigation of this bacterial group.

S3 Novel and Sustainable Bioprocesses in Circular Economy

S3-KN1: Sustainable Microbial Production of Foods Using Carbon Dioxide as Feedstock

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The breach of six planetary boundaries underscores the urgent need for sustainable food production systems. Modern biotechnology relies on a handful of well-studied microbial species cultivated on sugar-derived feedstocks. Although highly successful, their dependence on agricultural resources and limited tolerance to unconventional conditions constrain their role in future sustainable production. Growing attention is therefore directed towards non-model organisms with native metabolic capabilities uniquely suited to next-generation bioprocesses. Among these, HOBs are interesting, converting atmospheric CO_2 and green hydrogen (H_2) into biomass through gas fermentation, a process already deployed for food-grade single-cell protein production, potentially enabling supply chains independent of agriculture, with minimal land and water inputs and potential for carbon-neutral production. To move beyond single-cell protein, HOBs must be engineered into versatile cell factories capable of precision fermentation. Recent advances in synthetic biology, metabolic engineering, computational modelling, and bioreactor design have provided a valuable blueprint for scalable gas-based fermentation platforms. Using secreted recombinant milk protein as a case study, in this talk I will highlight the key challenges and towards engineering HOBs into cell factories.

S3-RT1: Sustainable Lipid Production: A Combined Approach for Enhancing Production Efficiency in Oleaginous Yeast Fermentations

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Edible oils are essential ingredients in the food industry. The growing global population, the rise of the alternative protein market which lacks a complementary fat production industry, and the use of oil for biofuel production are all driving an impending oil supply crisis. Oleaginous yeast, a group of lipid-accumulating yeast, have emerged as promising candidates for alternative oil production due to their capacity to produce lipids from a wide range of substrates. This study focuses on *Lipomyces starkeyi*, a prominent oleaginous yeast species, for lipid production through a circular economy-based approach. In the first phase, we designed fermentation processes utilizing various food production wastes, byproducts, and lignocellulosic biomasses as sustainable fermentation feedstocks. We collected and characterized multiple types of feedstocks with diverse compositions from various sectors of the food industry and formulated distinct fermentation media out of them. The propagation and lipid accumulation capacities of *L. starkeyi* were evaluated across these media. Several media demonstrated superior support in yeast propagation and lipid accumulation, and notably, some induced spontaneous lipid release from cells. A simple centrifugation step effectively separated the lipid phase, potentially eliminating the need for downstream lipid extraction processes. In the second phase, we conducted, for the first time, genetic breeding program of *L. starkeyi* with the goal of developing new strains genetically optimized for the formulated fermentation media. Our focus was directed toward lignocellulosic-based media representing cost effective and sustainable raw material. The breeding process leveraged the natural genetic diversity among *L. starkeyi* founder strains combined with innovative genetic breeding technologies developed by our team, enabling the creation of improved, non-genetically modified strains with enhanced performance. *L. starkeyi* breeding populations were constructed and subjected to multiple generations to enhance growth capabilities in the lignocellulosic media. Post-breeding populations demonstrated significant growth improvements of 30%, validating the effectiveness of our breeding program. Single clones with superior growth abilities were isolated, showing further improvement in the lipid content after 48 h of fermentation compared to the founder strains. Fatty acid analysis of these strains revealed varying triglyceride compositions, with some resembling palm oil. Overall, the integration of media selection, formulation, and genetic breeding approaches demonstrated an effective strategy for designing new sustainable lipid production processes. This integrated approach improved yield and process efficiency while enabling precise modulation of triglyceride composition tailored to meet target specifications. These advancements pave the way for scalable, sustainable, and cost-effective production of yeast-derived lipids.

S3-RT2: Building Beneficial Microbial Partnerships to Advance Microalgal Food Production

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Microalgae hold strong potential as future food ingredients due to their high nutritional value and sustainable production. However, largescale microalgal production is often constrained by low productivity. Cultivating microalgae with upcycled industrial side streams offers a valuable opportunity for resource-efficient production, though these substrates often introduce additional nutritional and physiological challenges to microalgal growth. Addressing how to overcome such limitations is essential for enabling sustainable and market-competitive microalgal food systems.

Here, we explored a food microbiology based approach to enhance microalgal performance during mixotrophic cultivation on alternative carbon sources. We investigated whether introducing food grade, Qualified Presumption of Safety (QPS) bacterial communities could support the model microalga *Chlamydomonas reinhardtii* under conditions where growth is otherwise restricted. Using glycerol as an example of an upcycled carbon substrate, we examined how different bacterial community compositions influence microalgal robustness, productivity, and culture stability.

Our findings show that co cultivation with specific food grade bacterial combinations can markedly improve microalgal growth under these conditions, assessed by total cell count and chlorophyl content. The ability of *C. reinhardtii* to grow mixotrophically in a glycerol-containing media was only observed in combinations with two bacterial genera, whereas single genus communities fail to generate comparable results. This indicates that community-level interactions, rather than individual strains, play a decisive role in shaping microalgal performance. The bacteria and microalgae were able to coexist without detrimental overgrowth, and the microalgae maintained a stable macromolecular composition (carbohydrates, lipids, and proteins), suggesting that the increased productivity was achieved without imposing physiological stress.

To better understand the mechanisms driving these inter-kingdom interactions, we performed metabolomic and community-level analyses. The results highlight an interplay within the mixed bacterial consortium that shape the microalgal environment in ways not reproduced by individual genera. While the specific mechanisms require further exploration, the results collectively demonstrate that food grade multispecies communities can be strategically assembled to enhance microalgal robustness under resource limited cultivation.

This work establishes QPS listed bacterial consortia as promising microbiological tools for improving the stability, efficiency, and food safety of microalgal production systems. By leveraging ecological principles from food microbiology, our approach opens opportunities for designing microbial communities that support next generation food production using upcycled substrates. These findings highlight the potential of community-based strategies to drive more resilient, scalable, and sustainable microalgal cultivation without relying on genetic modification or non-food-grade organisms.

S3-RT3: From Rumen Fermentation to Milk and Cheese: Integrated *in vitro* and *in vivo* Evaluation of Grape Stalks as a Dairy Cow Feed Supplement

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Grape stalks are abundant winery by-products rich in structural carbohydrates and phenolic compounds, and their use in ruminant nutrition may support more circular and sustainable livestock systems. The aim of this study was to evaluate the effects of grape stalk inclusion in dairy cow diets by integrating results from an *in vitro* rumen simulation trial (RUSITEC) and an *in vivo* feeding trial, with particular attention to rumen fermentation, microbiota, milk characteristics, and cheese traits.

In the RUSITEC trial, diets containing grape stalks from three cultivars (Lagrein, Cabernet Sauvignon, and Merlot) at 8% of diet dry matter or grape seed meal were compared with a control diet under steady-state conditions. Overall fermentative efficiency was maintained across treatments. pH, redox potential, total gas production, methane proportion, short-chain fatty acid profile, acetate/propionate ratio, protozoal counts, and most nutrient disappearance parameters were not significantly affected, indicating that the inclusion levels tested did not impair rumen function. By contrast, ammonia-N concentration was reduced in grape stalk treatments, with the strongest decrease observed for Cabernet Sauvignon, suggesting a specific effect on ruminal nitrogen metabolism. Rumen microbiota analysis, performed by 16S rRNA gene sequencing on the Illumina MiSeq platform, showed significant treatment associated shifts superimposed on a stable core community, with *Prevotella* emerging as the main taxon associated with dietary modulation.

In the *in vivo* trial, dairy cows received grape stalk supplementation at 2% of diet dry matter. Milk fat, protein, lactose, and casein were not significantly altered, supporting good dietary tolerance. Likewise, no major diet related modulation was observed in the milk microbiota, indicating that supplementation did not markedly alter the overall microbial profile of milk. Effects were, however, detected in cheese, where total polyphenol content was highest in the Cabernet Sauvignon group (224 ± 34 mg/kg), together with cultivar dependent differences in metabolomic and volatile profiles.

Overall, the combined *in vitro* and *in vivo* results indicate that grape stalks can be included in dairy cow diets without disrupting rumen fermentative efficiency, milk composition, or the overall milk microbial profile. Their main effects were a modulation of ruminal nitrogen metabolism, a selective shift in the rumen microbiota involving *Prevotella*, and subtle but measurable changes in cheese biochemical traits, supporting the valorisation of grape stalks as a local feed resource for sustainable dairy production.

S3-RT4: Olive Pomace as a Sustainable Feed Supplement: Effects on Rumen Microbiota and Methane Production, in a RUSITEC Model

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The agri-food system in Trentino includes relevant olive oil production, generating by-products such as olive pomace (OP) that can be reused within a circular economy framework. Among the possible applications, OP could be used as a feed supplement for dairy cows. Recent studies have shown that daily supplementation with OP can enhance milk nutritional quality without compromising milk yield. Due to its content of phenolic compounds, residual oils, and tannins, OP may modulate rumen microbial communities and fermentation pathways. This study aimed to evaluate the effects of OP supplementation on rumen fermentation, microbiota and methane (CH_4) production using the in-vitro Rumen Simulation Technique (RUSITEC). Two RUSITEC systems were used in three experimental runs to compare a control diet (CTRL) with a diet supplemented with 4% OP on a dry matter basis. Samples were collected over 5 days to assess fermentation parameters and rumen microbial composition. No significant differences were observed between CTRL and OP in terms of pH or protozoa counts, although redox potential increased under OP supplementation but still within the rumen physiological range. Mean gas composition ranged from 14.0 to 15.8% for CH_4, 76 to 78% for CO_2, and 0.07 to 0.09% for H_2. A tendency toward reduced ammonia production was observed with OP compared to CTRL. Caproate concentration decreased with OP supplementation, suggesting a shift in microbial activity. Microbiota analysis showed stable alpha diversity but changes in community composition, indicating a partial modulation of taxa potentially involved in alternative fermentation pathways, and weaker associations between *Methanobacteriota* and CH_4. Overall, OP supplementation showed the potential to modulate rumen fermentation without disruption of core rumen parameters, supporting further investigation of sustainable ruminant feeding systems.

S3-RT5: From Lab Bench to Field: *Bacillus subtilis* PS-216 as a Probiotic Candidate for *Campylobacter jejuni* Control in Poultry

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Poultry is the primary reservoir of the foodborne pathogen *Campylobacter jejuni*, the leading cause of bacterial gastroenteritis in the EU and US. The absence of EFSA-approved products specifically targeting *Campylobacter* in broilers represents a critical gap in food safety management, underscoring the urgent need for effective, green, and sustainable interventions.

Within our research group, *Bacillus subtilis* PS-216 has been systematically evaluated as a probiotic candidate for *C. jejuni* control over the past five years. Initial screening of 20 *B. subtilis* strains identified PS-216 as the top performer, demonstrating strong anti-*Campylobacter* activity in planktonic co-culture with reductions of up to 4 $\log_{10}$ CFU/mL. Mechanistic studies identified the antimicrobial compounds bacillaene and bacilysin as primary drivers of this effect, with activity shown to be temperature and oxygen dependent. The maximum efficacy was determined at 42°C under microaerobic conditions, mirroring the chicken gut environment. PS-216 was tested against a collection of 35 natural *C. jejuni* isolates (human, chicken, slaughter environments, water). While 33 isolates proved sensitive, two exhibited a resistance, suggesting natural variability in *C. jejuni* susceptibility to probiotic-mediated inhibition.

A controlled *in vivo* challenge study (21 days) in broiler chickens showed that continuous treatment with PS-216 spores in drinking water significantly reduced *C. jejuni* cecal load, although short-term treatment (7 days) failed. A longer-term study (42 days) demonstrated additional host benefits, including improved growth performance, feed conversion ratio, and immune parameters. A favorable shift in the cecal microbiota toward butyrate-producing bacteria (*Selenomonadales*, *Clostridium* sensu stricto) and reduced stress-associated *Anaeroplasma*, coupled with increased cecal butyrate concentrations, was also observed. Improvements in meat quality parameters, including reduced electrical conductivity and improved breast meat color distribution, were additionally recorded.

Together, these results establish *B. subtilis* PS-216 as one of the most thoroughly characterized probiotic candidates for sustainable *Campylobacter* control in poultry, while also highlighting strain-level variability as a key factor to address when translating probiotic efficacy to commercial broiler production.

S3-KN2: Valorisation of Grape Pomace for Sustainable Production of Bacterial Nanocellulose by Acetic Acid Bacteria

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Bacterial cellulose (BC) is a versatile natural biomaterial with unique physicochemical properties and considerable potential for industrial applications. However, its broader utilisation remains limited by high production costs, particularly those associated with the culture medium. We successfully valorised grape pomace (GP), an abundant food-processing by-product, as a low-cost substrate for BC production by acetic acid bacteria. The GP hydrolysate was optimised without enzymatic treatment, and screening of different hydrolysates and bacterial strains identified the recently described species *Komagataeibacter melomenusus* AV436^T as the most efficient BC producer, yielding up to 1.24 g/L of dry BC membrane. Furthermore, BC production in GP hydrolysate was optimised by combining bacterial adaptation to the substrate with optimisation of medium supplementation. *K. melomenusus* AV436^T was adapted to the GP-hydrolysate medium to enhance its growth and cellulose-producing capacity. The adapted strain showed a shorter lag phase and approximately 40% higher BC production, mainly due to a 15.6% increase in membrane diameter compared with the non-adapted strain. We also evaluated different alcohols and organic acids at various concentrations to identify supplementation strategies that could enhance BC production or modify its structural properties. The combination of 0.5% ethanol and 1% acetic acid proved most effective for increasing BC production, whereas supplementation with 2% glycerol combined with 0.02% butyric acid or 0.025% lactic acid resulted in noticeably thicker cellulose fibres. Adaptation did not affect the fundamental crystalline structure of the synthesised BC. Genome analysis of the adapted strain revealed a mutation in the *bcs1* operon, providing a potential genetic explanation for the observed changes in cellulose production. Overall, this study shows that adapting *K. melomenusus* AV436^T to GP hydrolysate, combined with targeted medium supplementation, can improve BC production and influence selected fibre properties. The findings represent an important step towards developing more efficient and sustainable BC production processes based on food-waste-derived substrates.

S3-FT1: *Bacillus cereus* sensu lato is a Challenge in Solid-State Fermented Brewers' Spent Grain

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Brewers' spent grain (BSG) is the main by-product from the beer industry and has gained attention within circular bioeconomy which promotes the use of BSG in food applications. Fungal solid-state fermentation (SSF) with *Rhizopus* sp. as starter cultures for production of tempeh-like foods is emerging for its numerous advantages. In this study, we investigated potential microbiological safety issues regarding the use of BSG for SSF with *Rhizopus oryzae*. Our results have shown that spore forming bacteria are the dominant microorganisms in BSG, particularly members from the *Bacillus subtilis* group. The presence of coliforms in two BSG samples (13%, *n* = 15) indicated unsatisfactory handling of some BSG batches from the brewery in Denmark. The presence of molds in two other BSG batches (13%, *n* = 15), draws attention to potential issues with mycotoxins in BSG. *Bacillus cereus* sensu lato (s. l.) was identified as the most significant microbiological hazard naturally present in BSG, with 100% occurrence, 1.8–3.7 $\log_{10}$ CFU/g in frozen BSG and 3.2–4.2 $\log_{10}$ CFU/g in fresh BSG. Characterization of 149 *B. cereus* s. l. isolates showed diversity of *B. cereus* s. l. in BSG from the same brewery in Denmark, but *panC* group II was found to be the dominant group. The results of SSF with *R. oryzae* performed both in a microclimate-controlled bioreactor and under lab-simulated conditions showed that *B. cereus* s. l. could grow to over the critical level of 5 $\log_{10}$ CFU/g. Even though *R. oryzae* fermentation showed inhibitory effect against *B. cereus* s. l., which was attributed to the production of organic acids and pH drop in the growth phase of *R. oryzae*, growth of *B. cereus* s. l. was still observed. Therefore, it is important to investigate more control measures to prevent the growth and toxin production of *B. cereus* s. l. during SSF of BSG, which are intended for food applications. Furthermore, our study reveals that the maturation and aging phase of tempeh-like products should not be longer than 3 days, due to the increased risk of *B. cereus* s. l. growth and spoilage. It also stresses that the further storage of fermented BSG and other BSG based products should be kept at cold temperatures (< 5°C), to prevent the growth and toxin production of *B. cereus* s. l. in the products during the shelf-life.

S3-FT2: Valorisation of White Wine Lees: Green Strategies Uncover Key Molecules Stimulating Lactic Acid Bacteria Growth

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Wine production generates substantial by-products, among which wine lees are the second most abundant in volume. These solids, deposited at the bottom of fermentation tanks after alcoholic fermentation (AF), contain organic acids, carbohydrates, proteins, phenolic compounds, and yeast biomass. Given their rich biochemical composition, these by-products represent a promising resource that can be valorized to enhance subsequent stages of the winemaking process.

Malolactic fermentation (MLF), which typically follows AF, converts malic acid into lactic acid, thereby reducing wine acidity and improving sensory quality and stability. It is carried out by lactic acid bacteria (LAB), mainly *Oenococcus oeni*. However, due to the harsh physicochemical conditions of wine (low pH, high ethanol content) and the complex nutritional requirements of LAB, their growth and metabolic activity are often limited. Although commercial starter cultures and yeast-derived nutrients are used to facilitate MLF, their effectiveness remains inconsistent.

In this study, we evaluated the potential of recycling white wine lees as a nutrient source to promote LAB growth and malolactic fermentation. Initial results showed that the addition of 0.5 g/L of white wine lees significantly stimulated *O. oeni* growth and enhanced MLF performance during red wine fermentation. These beneficial effects were confirmed under various inoculation conditions, regardless of the bacterial strain, physiological phase (exponential, stationary, late stationary, and death), or inoculum concentration (10^3, 10^4, or 10^5 cells/mL). The addition of lees accelerated LAB growth by more than threefold, depending on the strain and inoculation conditions. Moreover, the stimulatory effect of lees exceeded that of commercial nutrient products.

To further identify the bioactive components of wine lees responsible for promoting LAB growth, we developed a solvent-free, environmentally friendly separation approach based on sequential tangential flow filtration using membranes with different molecular weight cut-offs. This method represents an original approach, employing a green and sustainable strategy to recover oenologically relevant compounds from winemaking by-products, thereby reinforcing circular economy principles within the wine industry.

S3-FT3: Substrate-Driven Metabolic Adaptation and Gastrointestinal Resilience of *Lacticaseibacillus paracasei* AF43 in Dairy and Plant Protein Matrices

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Lactic acid fermentation is a promising strategy to valorize food industry by-products and alternative proteins, supporting sustainable food systems and the ongoing protein transition. Understanding how lactic acid bacteria adapt to different protein substrates is essential for tailoring both the functional properties and the probiotic potential of fermented foods. This study explores the metabolic adaptation, functional outputs, and gastrointestinal resilience of *Lacticaseibacillus paracasei* AF43 grown on three protein-based media: whey protein with lactose (WM), pea protein with glucose (PM), and their hybrid mixture (WPM). An integrated approach combining label-free LC-MS/MS proteomics, targeted metabolite analysis, and *in vitro* gastrointestinal digestion was applied.

Proteomic analysis revealed pronounced substrate-dependent metabolic shifts. The strain displayed predominantly homolactic fermentation in WM and switched to a phosphoketolase-driven heterofermentative metabolism in PM, with an intermediate profile in WPM. These strategies influenced growth, proteolytic activity, and organic acid production. WM supported slower growth, mild proteolysis, and accumulation of low-molecular-weight peptides, whereas PM promoted faster growth and increased citrate and acetate formation. The hybrid medium showed intermediate behavior. These metabolic differences translated into distinct bioactivities. Whey fermentates exhibited combined antioxidant, ACE-inhibitory, and antimicrobial effects; pea fermentates showed the highest antioxidant capacity, mainly associated with citrate production; and hybrid fermentates displayed enhanced ACE-inhibitory activity. Pre-cultivation conditions also significantly impacted gastrointestinal survival. Cells grown in WM maintained higher viability during simulated digestion, while those grown in PM and WPM showed marked reductions, particularly under gastric conditions. Proteomic data indicated that both substrate and digestion phase modulated ribosomal functions, carbon metabolism, and stress-response pathways. Gastric stress induced a general downregulation of biosynthetic processes, reflecting a shift toward energy conservation. Notably, WM-grown cells already exhibited a pre-adapted, stress-oriented proteomic profile before digestion, characterized by reduced ribosomal investment and sustained activation of stress-response pathways. This physiological state translated into improved proton homeostasis and recovery capacity during digestion, supported by a flexible regulation of F_1F_o-ATPase.

Overall, *L. paracasei* AF43 demonstrates high metabolic and physiological plasticity, which can be modulated by substrate composition to tune both functional properties and gastrointestinal robustness. Linking proteomic signatures to bioactivity and stress resilience provides a basis for the rational design of sustainable protein-based fermented foods with targeted health benefits.

S3-FT4: Improved Enzymatic Conversion of Starch During Brewing Using Hydrodynamic Cavitation as a Process Intensification Strategy

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This study investigates hydrodynamic cavitation (HC) as a process intensification strategy to enhance enzymatic starch conversion during brewing operations, more specifically the mashing step.

Mashing is the first step in beer brewing where sprouted barley (malt) is mixed with water and heated to different temperature. Malt naturally contains both starch (long chains of glucose) and enzymes that can break the starch into smaller sugars. The goal of mashing is to heat the mixture to optimal enzymatic temperatures to break down the starch into smaller fermentable sugars that the yeast can use in the fermentation process later. Efficient conversion of starch into fermentable sugars is a key step in food fermentation processes, directly impacting yield and resource utilization. However, incomplete starch accessibility during conventional mashing can limit conversion efficiency.

HC was applied during the mashing step, and its effect on sugar yield was evaluated against a conventional mashing process without cavitation. Process conditions, including temperature profile and enzyme activity, were kept comparable to isolate the effect of HC. Sugar release was monitored throughout mashing to assess conversion efficiency.

Application of HC resulted in a consistent increase in fermentable sugar yield of up to 10% compared to the control. The enhanced sugar release is attributed to cavitation-induced disruption of starch granules, increasing substrate accessibility for amylolytic enzymes. The mechanical and physicochemical effects generated during bubble collapse, including localized shear forces and micro-mixing, are proposed to enhance enzyme–substrate interactions and mass transfer.

Importantly, the improved conversion was achieved without increasing enzyme dosage or extending processing time. These findings highlight HC as a scalable and energy-efficient approach to intensify starch conversion processes.

By increasing fermentable sugar yield, HC has the potential to reduce raw material consumption and improve overall process sustainability. This work demonstrates the applicability of hydrodynamic cavitation as a tool for process intensification in starch-based food systems, with direct relevance for fermentation-driven industries.

S3-FT5: Definition of Ecosystem Services in Fermented Food Ecosystems as Perspectives for Raw Milk Cheese Production in a Changing World

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Ecosystem services can provide information on food quality, production yields and sustainability of food ecosystem. These services must be assessed using bioindicators. As part of the ANR cAnOPee project, the Normandy PDO cheese sector was chosen as a case study to create and evaluate bioindicators and indices focusing on the production of raw milk and associated cheeses. Current bioindicators are primarily linked to food spoilage or safety. However, especially in the case of fermented products, where microbial communities confer specific organoleptic characteristics while protecting the food against undesirable microorganisms, functional indicators can also be assessed. Indeed, dairy herds environments are a source of microbial diversity at the community level, currently threatened by untargeted and stringent methods and global change. These communities are of technological interest for cheese processing and may offer properties of interest for human and bovine health that are worth exploring. An ecosystem-based approach was first conducted, which identified six services associated with the microbial communities in raw milk: reservoir of microbial diversity, provision of functional microbial diversity, preparation of the matrix for processing, creation of organoleptic characteristics, resistance to colonization, and biological activities related to human health. Raw milks were collected once in 30 selected farms over a three month-period. Milk from two successive milking was collected from the bulk-tank. Selective media were used to enumerate eight microbial groups. 50 mL of milk was centrifugated for DNA extraction before metabarcoding using the V3–V4 region of the 16S rRNA gene and the ITS1 region (Illumina MiSeq platform). Right after collection, milk pH and oxidoreduction potential were monitored for 36 h at 30°C. Volatilome was analyzed by SPME GC-MS (Schimadzu) using 2 mL-milk samples.

The analyses from this sampling campaign validated the methodologies and provided sufficient data to select relevant bioindicators. For example, the variability observed among the 30 farm samples regarding the microbial diversity of bacterial and fungal communities suggests that associated indices, especially Chao and Pielou, are relevant for describing the ecosystem service of reservoir of microbial diversity. A total of 10 bioindicators were validated and represented into a pie chart for each of the farm studied. Each farm has a specific profile of services acknowledging distinctive practices among them leading to diverse microbial communities and variability of services. Deeper understanding of the links between practices, bioindicators, and ecosystem services will define pathways toward more efficient and resilient food systems, while continuing to protect ecosystems and their biodiversity.

S4 Foodborne Pathogens and Their Resistance

S4-KN1: Small Toxins but Significant Impact: Cereulide Challenges Across the Food Chain

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Cereulide, a small, highly heat- and acid-stable emetic toxin produced by *Bacillus cereus*, is gaining increasing prominence, particularly after the global recall of cereulide-contaminated infant formula in 2025/2026. Despite its classical association with reheated rice dishes, cereulide contamination spans a broad range of food matrices, including dairy, cereal-based products and composite ready-to-eat foods, and remains heavily underdiagnosed in routine surveillance. This keynote will trace the "life cycle" of cereulide along the food chain – from primary production and processing environments to catering and domestic settings – and highlight critical points where toxin formation is favoured and where current control concepts fail. Analytical and molecular tools for discriminating emetic *B. cereus* strains and accurately quantifying cereulide in food will be presented, together with examples of their application in outbreak investigations and product evaluation. Finally, I will summarise recent advances in understanding cereulide biosynthesis and regulation, including the contribution of structurally related isocereulides, and discuss why these findings call for a shift from qualitative presence/absence testing towards quantitative, effect-based risk assessment to mitigate this still-underestimated hazard.

S4-KN2: Targeting Noroviruses in Food – Paving New Analytical Approaches in Food Virology

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Human noroviruses remain the foremost cause of foodborne viral disease worldwide, yet their detection, characterization, and risk interpretation continue to challenge conventional food microbiology. Unlike bacterial pathogens, noroviruses cannot be routinely cultivated in a conventional laboratory setup, occur at low and heterogeneous levels in complex food matrices, and are highly resilient to environmental and processing stresses. Current analytical frameworks, largely centered on reverse transcription quantitative PCR (RT-qPCR), have enabled global surveillance but remain fundamentally limited, particularly due to their inability to discriminate between infectious and non-infectious viral particles and their susceptibility to matrix-associated inhibition. These constraints underscore the need to rethink analytical paradigms and transition toward more informative and risk-relevant approaches in food virology.

Targeting noroviruses in food systems now requires integration of next-generation analytical technologies that extend beyond conventional nucleic acid detection. Digital PCR (dPCR), including Nanoplate, microfluidic or droplet-based platforms, offers absolute quantification without reliance on calibration curves and demonstrates improved precision and reduced bias compared to RT-qPCR, particularly in the presence of sequence variability and inhibitors. Complementing this, advances in next-generation sequencing (NGS) enable whole-genome characterization directly from contaminated food matrices, facilitating outbreak tracing, source attribution, and viral evolution studies with unprecedented resolution.

Emerging analytical frontiers further include CRISPR-based detection systems, capable of highly sensitive and rapid identification of viral genomes, and novel infectivity-linked approaches employing human intestinal enteroids and zebrafish surrogate models to bridge the critical gap between genome detection and public health risk. In parallel, emerging biosensor technologies like microfluidic platforms, aptamer-based assays, and lab-on-chip devices, are advancing toward real-time, on-site detection, reducing analytical turnaround time and enabling proactive monitoring across the food supply chain.

Importantly, future analytical strategies must adopt a systems-based perspective, integrating improved sample preparation, viral concentration, and inhibitor removal with multi-modal detection platforms. Coupling molecular quantification with infectivity assessment and genomic characterization will allow a more accurate linkage between analytical results and actual consumer risk. Such an approach also supports the development of quantitative microbial risk assessment (QMRA) models and evidence-based regulatory frameworks.

Paving new analytical approaches in food virology therefore demands a shift from detection-centric methodologies toward integrated, high-resolution, and risk-oriented analytical ecosystems. By harnessing innovations in molecular biology, genomics, and biosensing, the field can move closer to accurately targeting noroviruses in food, ultimately enhancing food safety management, outbreak prevention, and global public health protection.

S4-RT1: From Outbreaks to Eradication: Identifying Contamination Sources and Removing a Persistent *Listeria monocytogenes* ST121 from a Salmon Processing Facility

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This presentation describes an investigation of a 'Seek and Destroy' process conducted at a cold-smoked salmon producer linked to two listeriosis outbreaks caused by *Listeria monocytogenes* ST121. The study also includes an extensive evaluation of the effect of a total of 24 cleaning and disinfection agents as well as UV-C and heat treatments to eradicate *L. monocytogenes* from identified harborage sites.

In the 11 week Seek and Destroy process, 329 samples were analysed (140 food-contact sites, 41 non-food-contact, and 148 salmon/product samples). Studies showed that the skinning machine was the primary contamination source of fillets. Whole genome sequencing showed that the ST121 outbreak strain persisted in two woven conveyor belts and an air-filled roller of the skinning machine, despite extensive cleaning and disinfection and hydrogen-peroxide whole-room treatment. Replacing the skinning machine, combined with enhanced sanitation (including disassembly/boiling of brine-injector needles), resolved the *L. monocytogenes* contamination.

Different control strategies were further evaluated using coupons cut from one of the contaminated discarded woven conveyor belts. *L. monocytogenes* survived 42 different combinations of 24 cleaning and disinfection agents tested at recommended user concentrations for 10 min exposure time. Increasing cleaning agent concentrations (10×) did not eliminate *L. monocytogenes*, nor did UV-C treatment (6 J/cm²). Complete inactivation was achieved only by immersing belt coupons in a water bath at 60°C for 1 h. Heat treatment also successfully eradicated *L. monocytogenes* from the air-filled roller.

This study highlights the critical importance of hygienic design of food-processing equipment. It also demonstrates the strong potential of heat treatment and the significant limitations of conventional cleaning and disinfection methods as well as UV-C for eliminating *L. monocytogenes* from difficult-to-access harborage sites.

S4-RT2: Strain-Dependent Variability and Different Extrinsic Factors Govern Surface Adhesion and Gastrointestinal Resilience in *Cronobacter sakazakii*

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Cronobacter sakazakii is an opportunistic foodborne pathogen associated with severe, often fatal infections in neonates and young infants. However, its infective potential beyond this high-risk group remains insufficiently understood. In this study, we systematically investigated strain-dependent traits linked to contamination and infection, focusing on surface adhesion and gastrointestinal resilience.

A collection of 28 *C. sakazakii* strains from food and clinical origins was screened for their adhesion capacity using 96-well plate assays under varying temperatures, incubation times, and growth media. From this set, eight representative isolates were selected for in-depth analysis of (*i*) adhesion to nasogastric feeding tubes and (*ii*) survival under simulated gastrointestinal conditions relevant to both infants and adults.

Adhesion to nasogastric tubes was assessed at 37°C in reconstituted powdered infant formula (PIF), closely mimicking clinical feeding practices. Gastrointestinal resilience was evaluated using *in vitro* digestion models, combining culture-based enumeration with viability-based qPCR to capture both culturability and viability.

Our results reveal pronounced strain-dependent variability. Temperature (37°C) and growth medium (PIF) were the primary drivers of adhesion on polystyrene, while incubation time and strain origin had no significant effect. Notably, all selected strains demonstrated the capacity to adhere to nasogastric feeding tubes in PIF, underscoring their biofilm-forming potential in clinical feeding environments.

Furthermore, *C. sakazakii* exhibited substantial resilience under simulated infant gastrointestinal conditions. Under adult gastric conditions, cells maintained membrane integrity despite loss of culturability, suggesting a transition into a VBNC-like state, with subsequent recovery of culturability during the intestinal phase.

Collectively, these findings highlight critical, strain-specific survival strategies of *C. sakazakii* that may facilitate persistence and infection beyond infancy. This work underscores the need to reconsider current risk assessments and strengthen surveillance of *Cronobacter* infections in broader vulnerable populations.

S4-RT3: Study of Antimicrobial Resistance in Pathogenic Microorganisms Isolated from Food Industries and Its Potential Influence on Biocide Products Effectiveness

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Antimicrobial resistance (AMR) in foodborne pathogens represents a growing concern for public health and the food industry, particularly regarding the potential cross-resistance between antibiotics and commonly used biocides. This study aimed to investigate the prevalence of antibiotic resistance in pathogenic microorganisms isolated from diverse food industry environments and to evaluate whether such resistance is associated with reduced susceptibility to industrial biocides.

Samples were collected from multiple food processing sectors, encompassing a wide range of environmental conditions and hygienic practices. Target microorganisms were isolated and the presence of antibiotic resistance genes was analysed by genomic analysis. In parallel, phenotypic resistance to selected antibiotics was assessed using the disk diffusion method, allowing the determination of inhibition halo diameters and classification of resistance profiles.

To further explore the potential link between antibiotic resistance and tolerance to disinfectants, strains exhibiting the highest levels of antibiotic resistance were selected for biocide susceptibility testing. A range of disinfectants commonly used in the food industry was applied at concentrations representative of routine industrial use. The effectiveness of these biocides was evaluated by measuring microbial survival following exposure following the European standards.

The results demonstrated the presence of some antibiotic resistance genes among isolates from different food industry sectors, confirming the relevance of these environments as reservoirs of AMR. Phenotypic analyses revealed varying degrees of resistance to the antibiotics tested, with some strains showing multidrug-resistant profiles. However, despite this observed antibiotic resistance, no direct correlation was found between the presence of resistance genes or phenotypic resistance and decreased susceptibility to the tested biocides at commonly used concentrations.

It is important to emphasize that all selected biocides maintained their efficacy at standard in-use concentrations, even against strains with elevated antibiotic resistance. These findings suggest that, under proper application conditions, commonly used disinfectants in the food industry remain effective tools for controlling resistant foodborne pathogens.

In conclusion, while antibiotic resistance is prevalent among food-associated microorganisms, it does not necessarily confer cross-resistance to biocides at operational concentrations. This highlights the continued importance of correct sanitation practices in mitigating microbial risks and supports the effective role of biocides in ensuring food safety.

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S4-RT4: Decoding *Listeria monocytogenes* Stress Adaptation in Fruit Puree via Transcriptomic Biomarkers

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Listeria monocytogenes is a major foodborne pathogen responsible for listeriosis, currently ranked fourth among reported zoonoses in the European Union, with incidence rising over 2020–2024. Its persistence is largely driven by its ability to adapt to diverse environmental stresses. Prior exposure conditions, such as pH and temperature, can strongly influence its subsequent survival, growth, or stress resistance – a phenomenon known as "cross-protection" or "stress hardening."

Building on our previous work showing that the survival of *L. monocytogenes* strain 10403S in fruit puree depends on prior growth conditions (pH 7.2 vs 5.5; temperature 10, 25, 37°C), the current study aimed to identify transcriptomic biomarkers predictive of survival. Cells cultivated under these pH–temperature combinations were subjected to RNA sequencing before inoculation and storage in fruit puree at 10, 25, and 37°C, allowing gene expression to be linked with subsequent survival.

Differential expression analysis identified four co-expressed gene clusters representing metabolic pathways (gene modules) strongly influenced by prior growth conditions. A cold-induced module was enriched in branched-chain amino acid biosynthesis genes (*ilv–leu* operon). Another module, repressed under acidic and low-temperature conditions, involved arginine and ornithine biosynthesis pathways. A third module highlighted iron homeostasis functions, including siderophore-mediated transport, metal ion uptake, and purine biosynthesis, reflecting extensive metabolic reprogramming from environmental exposure. The fourth module associated with genes specifically repressed at 10°C and 25°C, and therefore induced at 37°C, was enriched in glycine catabolism via the glycine cleavage system, iron response pathways, and Tat-dependent protein transport.

Sparse partial least squares (sPLS) regression was applied to associate transcriptomic data with subsequent inactivation kinetics during storage at 10, 25 and 37°C, fitted with a log-linear model (D-values). Although the model explained ~9% of variance ($r = 0.31$), it enabled selection of 60 biologically relevant candidate genes, including ABC transporters, phosphotransferase systems, DNA repair genes, nucleotide biosynthesis, and central metabolism genes – potential biomarkers of stress adaptation.

These findings provide a foundation for future validation studies and advance predictive microbiology frameworks. They improve understanding of *L. monocytogenes* survival in ready-to-eat acidic foods and contribute to more robust risk assessment strategies.

S4-RT5: Aeromonas spp. as reservoirs and vectors of antimicrobial resistance within a One Health framework

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Aeromonas spp. are ecologically widespread bacteria found in aquatic environments, animals, humans, and a variety of foodstuffs. Several species are recognized as opportunistic human pathogens, and soft tissue infections may occur after exposure to contaminated water, while ingestion of contaminated food or drinking water represents the main route of Aeromonas-associated gastrointestinal disease. In light of the emerging global threat concerning development and spread of antimicrobial resistance (AMR), Aeromonas spp. have received increasing attention as potential reservoirs and vectors of AMR within a One Health context.

Bacteria inhabiting aquatic environments influenced by human and agricultural activities can acquire resistance genes through horizontal gene transfer from clinically relevant bacteria. High bacterial densities, combined with selective pressures from chemical residues, can facilitate the transfer of antimicrobial resistance genes (ARGs) between ecologically diverse bacteria. Ultimately, such bacteria may enter the food chain via several transmission routes, for example through filter-feeding organisms such as bivalve mollusks.

In this study, more than 170 Aeromonas isolates were collected from various aquatic environments in Norway, Sweden and Belgium, as well as from blue mussels in Norway, to assess the role of Aeromonas spp. as reservoirs and potential vectors of ARGs that can be spread across One Health compartments and ultimately enter food products. All isolates were identified to genus or species level using 16S rRNA or gyrB gene sequencing, and their susceptibility to a panel of 16 antibiotics representing major antibiotic classes was evaluated using conventional disc diffusion. A subset of isolates was whole-genome sequenced (WGS) to characterize their resistome and mobile genetic elements, and all strains displaying phenotypical resistance to cefotaxime (a third-gen. cephalosporin) were tested for their ability to transfer resistance determinants via conjugation.

Overall, the level of phenotypical resistance was low and not alarming compared with other enteric Gram-negative bacteria. Resistance to clinically important antibiotics such as cefotaxime and ciprofloxacin (a fluoroquinolone) was observed in only 1.1 and 1.8% of the isolates, respectively. Notably, one A. allosaccharophila isolate exhibited resistance to 12 agents, and WGS analysis confirmed that resistance genes were located on a plasmid of potentially foreign origin. Conjugative transfer of ARGs using this strain as a donor could not be demonstrated experimentally, but further optimization is required to fully assess this potential. Nevertheless, this study highlights the role of Aeromonas spp. as hosts of a broad range of clinically relevant ARGs that may be spread across One Health compartments, including different food chains.

S4-FT1: Plant-Based Meat and Dairy Alternatives as a Novel Food Safety Challenge: Microbiological Surveillance and Genomic Typing of Bacterial Isolates

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Plant-based food products represent a rapidly expanding market niche, however their microbiological safety profile remains insufficiently characterized, particularly regarding emerging pathogens. This study assessed the microbiological quality of 145 plant-based food samples, including vegetable burgers, alternative deli products, vegan cheeses, tofu, seitan, tempeh, plant-based beverages, and ready-to-eat (RTE) or ready-to-cook (RTC) preparations, purchased from supermarkets in Lombardy (Italy) between 2024 and 2025, within the framework of a national research project.

All samples were analysed using official microbiological methods for the detection of *Listeria monocytogenes*, *Salmonella* spp., STEC, coagulase-positive staphylococci, *Bacillus cereus*, *Enterobacteriaceae*, and *Escherichia coli*.

Salmonella spp., STEC and coagulase-positive staphylococci were not detected. *Enterobacteriaceae* and *E. coli* counts were under the limit of detection (< 10 CFU/g) in all samples, reflecting adequate manufacturing hygiene practices. *B. cereus* was detected in 9/145 samples (6.20%) at low concentrations (~5.0×10^1 CFU/g), consistent with its ubiquitous environmental presence and the plant origin of the matrices. *L. monocytogenes* was isolated from 9/145 samples (6.20%) belonging to RTE and RTC categories, including vegetable mozzarella, vegetable stracciatella, pea-based burgers, vegetable nuggets and vegetable cheese. No co-contamination was observed within any single sample.

WGS characterization of the nine *L. monocytogenes* isolates identified five sequence types (ST2, ST7, ST8, ST9, ST26), all STs previously associated with clinical cases of listeriosis or food-related outbreaks in Europe and North America. AMR profiling revealed the presence of *fosX* and *tet(M)* genes in ST26 isolates, with *tet(M)* representing a resistance determinant of particular relevance for surveillance purposes. Similarly, WGS analysis was performed for *B. cereus*, with 7/9 isolates characterized, belonging to different STs and including both *B. cereus* sensu stricto and *B. mosaicus*. Virulence (*cytK*-2, *sph*) and AMR genes (*bla*, *bla2*, *fosB*/*fosBx1*) were detected; notably, one isolate carried the *ces* gene cluster, indicating potential cereulide production.

Overall, most analysed samples showed satisfactory microbiological quality, with no major safety concerns for most pathogen categories. The detection of *L. monocytogenes* in RTE products, for which no further thermal treatment is applied prior to consumption, represents a critical issue. These products fall within the scope of Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs, which establishes safety criteria for *L. monocytogenes* in ready-to-eat foods. The present findings nonetheless underscore the importance of maintaining rigorous hygienic controls throughout the entire production and distribution chain, as well as careful management of shelf-life and cold chain conditions, to prevent pathogen growth and ensure consumer safety.

S4-FT2: Impact of Acquired Resistance to Sodium Sulfite and Sodium Nitrite on Biofilm Formation and Cross-Contamination of Meat by *Salmonella* Typhimurium

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Salmonella Typhimurium persistence in the food industry is associated with its adaptation to chemical stresses. Understanding the correlation between this stress adaptation, the biofilm-forming capacity and complexity, and cross-contamination risk is essential for food safety.

This study characterized the biofilm-forming ability, 3D architecture and surface-to-food transfer rate of *S.* Typhimurium resistant variants evolved under sodium sulfite and sodium nitrite stress, integrating phenotypic data with whole genome sequencing (WGS).

Ten *S.* Typhimurium variants resistant to sodium sulfite (S-RVs) and ten to sodium nitrite (N-RVs), obtained via constant-dose evolution, were screened alongside the parental strain (PS) for biofilm-forming ability using a colorimetric micro-method assay (Stepanovic et al., 2007). Biofilm production indices (BPIs) were quantified on polystyrene and stainless steel coupons. Biofilm structure and cells organization were visualized using confocal laser scanning microscopy (CLSM) with SYTO9/propidium iodide staining. The biofilm transfer rate (BTR) was assessed using a representative subset of strains (PS and one RV per preservative) by exposing beef samples to 24-h biofilms for 30 s (three sequential contacts) (based on Wang et al., 2022). WGS (Illumina) was performed on all 20 strains to identify the genetic basis of the observed phenotypes.

Evolutionary adaptation significantly altered biofilm-formation dynamics, with surface type playing a critical role. BPIs were higher in the RVs than the PS, particularly on stainless steel (PS: 0.144 ± 0.018; S-RVs: 0.232 ± 0.037; N-RVs: 0.423 ± 0.074). Moreover, significant differences ($p < 0.05$) were observed between the PS (0.276) and several RVs on polystyrene. Biofilm architecture on both surfaces was characterized by CLSM, highlighting differences in the proportion of viable and non-viable adherent cells. The BTR assay revealed high cross-contamination risk on stainless steel: S-RV exhibited the highest initial transfer (3.94 ± 0.30 $\log_{10}$ CFU/cm^2) and all strains sustained elevated contamination (> 2.5 $\log_{10}$ CFU/cm^2), even following three successive exposures of beef samples. Conversely, transfer from polystyrene was minimal after the initial exposure, suggesting that stainless steel biofilms exhibit enhanced resilience. WGS characterization of PS and RVs identified genetic mutations (e.g. metabolism, membrane structure and motility) driving these enhanced phenotypes.

Resistance to sulfite and nitrite enhances the ecological fitness of *S.* Typhimurium by increasing its biofilm-forming abilities and transferability on stainless steel. These biofilms' ability to contaminate numerous food contact events poses a considerable challenge to food safety. Future work will integrate these data into a comprehensive risk assessment framework to quantify the impact of resistant variants on consumer health.

S4-FT3: Antimicrobial and Quorum Quenching Activity of Bioactive Compounds Against *Pseudomonas aeruginosa* Used as a Proxy for Food Microbiology Applications

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Bacteria communicate through a system known as quorum sensing (QS), which allows a coordinated behavioral response to changes in cell density. This communication modulates the transcription of genes and functions such as biofilm formation and dispersion, motility, toxin production, among others. The potential presented by plant secondary metabolites at inhibiting QS is of special interest due to the dynamic interactions between plants and microbes. However, the specific mechanisms involved in quorum quenching (QQ) processes are still not well understood. The present study aims to evaluate the inhibitory effects of different classes of bioactive compounds, with antimicrobial and QQ potential, on *Pseudomonas aeruginosa* PAO1 as a model for bacteria of relevance to the food industry and human health. Initially, bioactive compounds with recognized antimicrobial potential and the ability to interfere with QS in food-relevant bacteria were selected based on a systematic literature review. The antimicrobial activity of the selected compounds was assessed by determining the minimum inhibitory concentration (MIC) and monitoring microbial growth in liquid culture, using the *P. aeruginosa* PAO1. The inhibitory effect on QS and virulence factors was investigated by evaluating the expression of virulence factors including rhamnolipids, elastase, protease, pyocyanin, motility, and biofilm formation on three cultivation times (24, 48 and 72 h) in *P. aeruginosa* PAO1. The literature screening led to the selection of 27 compounds with antimicrobial activity and potential QS inhibition against different microorganisms for *in vitro* studies, including the classes of flavonoids, phenolic acids, terpenes, and curcuminoids. Although the compounds exhibited MIC values higher than the maximum concentrations tested, concentrations that did not interfere with PAO1 growth kinetics were selected to evaluate QS regulated phenotypes. Following an initial screening, the most promising compounds were selected for assays involving the expression of virulence factors. Vanillic acid, curcumin, epicatechin, eugenol, farnesol, kaempferol, linalool, and quercetin inhibited at least two of the four evaluated phenotypes in PAO1 and were selected for subsequent analyses. Regarding motility interference, these compounds also demonstrated QQ activity, particularly vanillic acid, farnesol, and linalool, with approximately 50% inhibition. Biofilm formation, assessed at three time points, indicated that the compounds continued to inhibit biofilm development, especially vanillic acid, curcumin, and quercetin, achieving up to 60% inhibition even after 72 h of incubation. The results suggest that these compounds hold significant potential for the development of QS inhibition strategies, with possible applications in the food and pharmaceutical industries.

S4-FT4: Persistence and Targeted Control of Foodborne Pathogens in Two Soilless Farming Systems

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Limited data exists on the persistence of foodborne pathogens in soilless farming systems and targeted interventions to control them. The objective of this study was to characterize the persistence of *Salmonella*, *Escherichia coli*, and *Listeria monocytogenes* in soilless systems and to evaluate bacteriophage and bacteriocin-based products as early-stage interventions during seedling development.

Salmonella (Javiana, Thompson), *E. coli* (STEC O179:H8, EPEC O13:H6), and *L. monocytogenes* (two strains) were inoculated (~6 $\log_{10}$ CFU/mL) into reverse osmosis water and applied to rockwool containing butterhead lettuce seeds. Pathogen persistence was monitored during a 3-week nursery stage and a 4-week growth stage in deep water culture (DWC) and Kratky (KR) systems. During plant growth, samples were collected from nutrient solution near roots and system corners, as well as from rockwool, leaves, nutrient solution, and roots at harvest.

E. coli and *Listeria* concentrations declined during both stages ($p < 0.001$), whereas *Salmonella* increased during the nursery stage ($p = 0.029$; 0.01 log units/day) and remained stable during plant growth. After transplanting the seedlings into the systems, *E. coli* and *Salmonella* concentrations were higher near roots than at system corners ($p = 0.047$ and $p = 7.14 \times 10^{-9}$, respectively). No significant difference in pathogen persistence was observed between nutrient solutions from the DWC and KR systems. At harvest, all three pathogens were detected on roots, leaves, nutrient solution, and in rockwool, with *Salmonella* persisting at the highest levels.

To evaluate interventions, separate seedling groups in rockwool were inoculated (~4 $\log_{10}$ CFU/mL) with either a three-pathogen cocktail (*Salmonella*, *E. coli*, and *Listeria*) or *Salmonella* alone and treated once at the start of germination with bacteriocins or bacteriophages, respectively. Reverse osmosis water served as the control. Both treatments significantly reduced pathogen concentrations ($p < 0.001$), with bacteriocins achieving the greatest reduction for *Listeria* (1.66 log CFU), followed by *Salmonella* (0.80 log CFU) and *E. coli* (0.62 log CFU), and bacteriophages reducing *Salmonella* by 0.44 log CFU.

These findings demonstrate that soilless farming systems can support the persistence of foodborne pathogens, with *Salmonella* exhibiting the greatest capacity to persist through production. Root-associated microenvironments emerged as consistent hotspots for persistence, independent of the system design, highlighting a critical control point in soilless farming. Importantly, targeted early-stage interventions significantly reduced pathogen loads, with bacteriocins showing strong, pathogen-specific efficacy. Moving forward, development of targeted intervention strategies is critical for reducing food safety risk.

S4-FT5: Reassessing Autoinducer-2 Signalling in *Campylobacter jejuni*: Evidence for a Metabolic Role of LuxS Rather Than Canonical Quorum Sensing

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Campylobacter jejuni, a leading foodborne pathogen, produces autoinducer-2 (AI-2) via the enzyme LuxS. Although AI-2 is widely regarded as a signalling molecule involved in intraspecies and interspecies communication, whether its production in *C. jejuni* represents a canonical quorum-sensing (QS) system remains unresolved. To date, no AI-2 receptor has been identified in *C. jejuni* and previous experimental evidence showed no response to exogenous AI-2 during exponential growth *in vitro*, arguing against a demonstrated receptor-mediated QS mechanism. At the same time, LuxS is a key enzyme of the activated methyl cycle, raising the possibility that many phenotypes related to LuxS reflect metabolic rather than signalling effects.

To reassess the relationship between LuxS and QS in *C. jejuni*, we combined whole-cell biosensor assays with direct analytical and molecular measurements. AI-2-related activity was monitored using the highly sensitive *Vibrio harveyi* MM30 AI-2 biosensor and compared with direct AI-2 quantification by HPLC-FLD, while *luxS* expression and LuxS protein abundance were used to assess effects on *C. jejuni*. This framework was further challenged using lavender-derived preparations and the monoterpenes linalool and linalyl acetate as potential QS quenchers. Using this multi-method approach, reduced *V. harveyi* bioluminescence after exposure to spent growth medium (SGM) from lavender-treated *C. jejuni* cultures initially suggested suppression of AI-2 signalling. However, direct HPLC-FLD measurements did not support decreased AI-2 abundance, and neither *luxS* transcript levels nor LuxS protein abundance changed accordingly. These results were further supported by spiking the active compounds into untreated *C. jejuni* SGM, which resulted in the same reduction in *V. harveyi* bioluminescence, indicating that the active compounds interfered with AI-2 detection by the biosensor rather than inhibiting AI-2 production in *C. jejuni*, a conclusion further supported by in silico analysis. Together with our previous findings that *luxS* deletion strongly affects central metabolism and survival, our data support a predominantly metabolic interpretation of the role of *luxS* in the AI-2 system in *C. jejuni*.

Overall, we propose that AI-2 biosensor assays should be treated as sensitive screening tools, not as standalone evidence for QS in signal-producing microorganisms. For *C. jejuni*, conclusions on AI-2 signalling require multi-method validation and should be interpreted in the context of LuxS-dependent metabolism. This perspective may help avoid overstatement of anti-QS effects in food microbiology studies involving *Campylobacter* spp.

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Plenary Session

PL-3: Microbiome Interconnectedness Throughout Environments – Impact on Human Health and Well-Being

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Microorganisms form an immense and highly dynamic network that connects soils, plants, food systems, and the human microbiome. These invisible connections underpin fundamental ecosystem processes that sustain plant productivity, shape food quality, and influence human health and well-being. Increasing evidence suggests that microbiomes should not be viewed as isolated communities, but rather as interconnected systems spanning environmental compartments from soil to crops to food and ultimately to the human body.

Healthy soils harbor extraordinarily diverse microbial communities that drive nutrient cycling, regulate plant growth, and suppress pathogens. These soil microbiomes act as central hubs shaping plant-associated microbial communities, supporting resilient plant growth and influencing the biochemical composition of plant-derived foods. In turn, healthy plants reinforce beneficial microbial processes in the rhizosphere through root exudates and organic inputs, creating positive feedback loops that stabilize ecosystem functioning and enhance crop productivity and nutritional quality.

Microbial interactions continue to shape food systems beyond the field. Plant-associated microbiomes influence nutrient uptake and plant metabolism, affecting the nutritional and functional properties of crops. During harvest, storage, and food processing, microbial communities further transform agricultural products, driving fermentation processes, stabilizing food matrices, and contributing to flavor development and food safety. These microbial transitions along the food chain ultimately determine the microbial exposures associated with food consumption and may influence the composition and function of the human microbiome.

Healthy soils also contribute to animal and human health through less direct but equally important pathways. Soil- and plant-associated microbiomes can influence animal health via feed quality, microbial exposures, and biologically active compounds entering the food chain. Diverse soil microbial communities additionally shape the surrounding air microbiome, enriching the microbial diversity of the environments in which humans and animals live and work. Exposure to diverse environmental microbiota has been linked to beneficial effects on immune development and microbial balance in the human body. At the same time, biologically active and diverse soils can increase ecosystem resistance against biological invasions and may reduce the risk of pathogen emergence and zoonotic disease transmission.

Recognizing microbiomes as interconnected networks across environmental compartments opens new opportunities to harness microbial processes for productive agriculture, high-quality food, and healthier living environments. Understanding and managing these microbial connections — from soil ecosystems to the food on our plates and the air we breathe — represents an exciting frontier in microbiology with profound implications for resilient ecosystems and human well-being.

S5 Food and Gut Microbiomes for Human Health Benefits

S5-KN1: The Gut as a Bioreactor: Translating Food Microbiology into Microbiome Function and Human Health

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Understanding the mechanisms by which foods and microorganisms interact with the human gut remains one of the major challenges in food microbiology. The human gut is characterized by a complex interplay between digestive, physiological, and immunological processes and nutritional and microbiological factors. Mechanistic research within the *in vivo* human gut is extremely challenging, while animal models fail to adequately reproduce human gut physiology. Here, we will discuss the development of advanced *in vitro* and *ex vivo* technological platforms that progressively recapitulate the longitudinal and cross-sectional environmental complexity of the human gastrointestinal tract. Several case studies will be highlighted to illustrate how *in vitro* models of the human gut facilitate mechanistic research in the domain of food microbiology. More in particular, we will demonstrate the modulatory potential of probiotic strains and postbiotic products towards the viability and functionality of foodborne pathogen and enteropathogen, as well as their impact on the gut microbiome and mucosal health parameters. Finally, we will highlight recent technological advances aimed at mimicking the diverse micro-environments of the human small intestine and its resident microbiota.

S5-RT1: Differential Impact of Undigestible Plant Proteins and Fibers on the Gut Microbiota Environment During *in vitro* Gut Fermentation

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The transition toward more sustainable diets involves a higher intake of plant-based protein sources. However, plant proteins generally have lower nutritional quality than animal-derived proteins, including reduced bioavailability due to lower digestibility. Thus, a larger fraction of plant proteins reaches the colon, with potential to alter the gut environment in ways that are poorly studied. In this study, we have evaluated the intestinal impact of plant protein ingredients (isolates, concentrates) derived from cereals (oat, wheat) and pulses (faba bean, pea), and used cereal fibers (arabinoxylan, beta-glucan) or raffinose family oligosaccharide (pea) as references. The digestibility varied between the protein ingredients and was related to the amount of fiber present, e.g. the protein concentrates had more fiber and showed lower digestibility than the protein isolates. The undigestible fractions were subjected to *in vitro* fermentation with human feces as inoculum and their fates were followed for 24 h. The ingredients had differential impact on the gut microbiota where the fibers caused more similar microbiota than the plant protein ingredients. Largest variation was between the fibers and the protein isolates, with enrichment of proteolytic bacteria such as *Clostridium* and *Sutterella* with isolates. Protein fermentation was evidenced with increase of iso-valerate, and larger metabolic profiling with NMR. Highest amounts of short chain fatty acids (SCFAs) were produced with the protein concentrates including highest amount of beneficial butyrate. Cross-feeding between beneficial Bifidobacterium and butyrate-producing bacteria is suggested as mechanism. To conclude, our study indicates that a mixture of plant proteins and fibers, as present in the protein concentrates, is most beneficial for the gut environment. These findings are currently being verified in a mouse trial.

S5-RT2: Modulation of the Human Gut Microbiome by Cricket-Based Fortified Foods: A Randomized Controlled Trial

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The increasing demand for sustainable dietary solutions has accelerated interest in novel protein sources, including insect-derived ingredients. In this context, the present study aimed to evaluate the impact of cricket-based fortified foods on the human gut microbiome. A randomized, double-blind, placebo-controlled intervention was conducted in 58 overweight/obese adults affected by Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD). Participants were assigned to receive either a cricket-fortified snack (80 g/day, providing approximately 9 g of cricket proteins) or a control product for two weeks. Clinical, dietary, and fecal samples for shotgun sequencing and metabolomic profiling were analyzed. Cricket-fortified food did not alter alpha diversity, while beta diversity analysis revealed a shift in microbial community composition in the cricket arm. Taxonomic analysis identified significant modulation of specific bacterial taxa, including an increase in *Bacteroides cellulosilyticus*, *Bacteroides eggerthii*, and *Blautia* sp., alongside a reduction in *Clostridium*. Within-group comparison in the cricket-supplemented arm showed significant enrichment of genes involved in amino acid metabolism and nitrogen cycling. In particular, pathways related to amino acid catabolism (including aromatic, branched-chain, serine, threonine, and proline metabolism), peptide utilization, arginine and polyamine metabolism, and ammonia assimilation were enriched at T1. These functional and taxonomic shifts are consistent with the biochemical composition of the cricket-based ingredient, which is rich in free amino acids such as proline, glycine, histidine, phenylalanine, and glutamate, as well as nitrogen-containing compounds including ornithine. In line with previous evidence showing that specific amino acids can selectively modulate gut microbial taxa, such as histidine-associated increases in *Bacteroides* and glycine-associated enrichment of *Blautia*, the observed changes support a substrate-driven microbial response to insect-derived proteins. In addition, metabolomic analysis revealed an increase in propanoic acid in the cricket-supplemented group, supporting the functional relevance of the observed microbial and genetic adaptations. This pattern is consistent with evidence that amino acid-rich substrates promote microbial amino acid catabolism and nitrogen metabolism, resulting in the production of short-chain fatty acids, including propionate, through substrate-specific fermentation pathways. Importantly, microbiome modulation occurred without alterations in host metabolic markers, as liver enzymes and inflammatory indicators remained stable throughout the trial. Overall, these findings demonstrate that insect-derived proteins can act as modulators of the human gut microbiome, promoting coordinated shifts in microbial composition and in amino acid utilization and nitrogen metabolism while maintaining host metabolic stability. This study supports the role of novel protein sources as sustainable and functional dietary components capable of shaping gut microbial ecosystems.

S5-RT3: Microplastics Meet the Microbiome: Insights from Adult and infant *in vitro* Gut Models

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Microplastics (MPs) are recognized as a global threat due to their prevalence in environments and food chain. The gastro-intestinal tract (GIT) is the front door of MPs, but to date, the fate and potential effects of MPs in the human digestive environment remain largely unknown. This study aimed to investigate the effects of chronic ingestion of virgin MPs of polyethylene (PE), the most manufactured plastic polymer worldwide, on the gut microbiota using *in vitro* systems reproducing the colonic environment of adults (Mucosal Artificial COLon, M-ARCOL) and infants (Toddler Mucosal Artificial COLon, Tm-ARCOL).

These *in vitro* models were set-up to reproduce the main physicochemical (pH, transit time, anaerobiosis), nutritional (ileal effluent composition) and microbial (mucus- or lumen-associated microbiota) parameters of the human colon. Bioreactors were inoculated with fecal samples (n = 4 adults, n = 4 infants) and PE MPs (21 mg) were daily injected for 14 days. The metabolic activity (gaz, short chain fatty acids) and composition (16S metabarcoding) of the gut microbiota were determined prior and after exposure to PE MPs. The indirect impact of gut microbe metabolites after exposure to PE MPs on the intestinal barrier was evaluated using a co-culture (Caco-2 and mucus-secreting HT29-MTX cells).

We reported that PE MP impact on gut microbiota was donor-dependent and resulted in an increase abundance of potential pathobionts, like *Enterobacteriaceae*, regardless of age conditions. Exposure to PE MPs was associated with a significant decrease in butyrate production in infants, while skatole levels were significantly increased in adults. Conversely, no significant impact of PE MPs on the intestinal barrier, as mediated by changes in gut microbial metabolites, was evidenced.

To conclude, this study provided significant insights into PE MPs interactions with human gut microbiota and intestinal barrier of two age groups, under relevant human colonic conditions. These data further enhance understanding of MPs interactions with physicochemical and microbial parameters in the human GIT, supporting better human health risk assessment. Next steps will be dedicated to study the impact of MPs on at-risk populations such as pathological situations associated with gut microbial dysbiosis (e.g obesity).

S5-KN2: Our Missing Microbiome: How Traditionally Fermented Foods Support Health

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Fermented foods are dietary staples that were developed by our ancestors and consumed for their nutritive, preservative, and culturally significant properties. The process of fermentation results in microbial growth and transformation of food substrates in ways that remove, modify, and add to the composition of the final product. The fermented food microbiome is therefore an extension of the gut microbiome, providing additional exposures to microorganisms and their associated cell constituents and metabolites. This talk will examine current evidence on how fermented foods affect human health and will present a framework for preventing and treating disease through the food-to-gut microbiome continuum. The presentation will consider fermented plant, meat, and dairy foods through the lenses of microbial community assembly and microbe-food matrix interactions, with a focus on their effects on gut health.

S5-RT4: Impact of Formulation and Food Matrix on Probiotic Functionality: A Multi-Analytical Study on *Lactobacillus acidophilus* LA-5®

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The increasing use of probiotics in functional foods and dietary supplements highlights the need to better understand how formulation and food matrices influence probiotic performance under human gastrointestinal conditions. Conventional culture-based methods may underestimate probiotic potential, particularly in complex and multi-strain formulations.

In this study, four commercial formulations containing *Lactobacillus acidophilus* LA-5® were evaluated using a multi-analytical approach combining culture-dependent and culture-independent methods, including plate counting, flow cytometry (FC), whole-genome sequencing (WGS), digital droplet PCR (ddPCR), functional assays, *in vitro* gastrointestinal digestion, and metabolomic profiling.

Flow cytometry revealed that viable cells accounted for 59–88% of total fluorescent units, exceeding culturable counts and indicating the presence of viable but non-culturable populations. Functional assays demonstrated formulation- and dose-dependent antimicrobial activity against selected enteropathogens, with up to 40–50% growth inhibition at higher probiotic cell densities.

Simulated gastrointestinal digestion highlighted marked differences in probiotic survival depending on formulation and carrier matrix. Fresh cells and fermented matrices showed improved resistance compared to lyophilized formulations, which were strongly affected by gastric conditions. 1H-NMR metabolomic profiling further revealed matrix-dependent metabolic responses, with distinct signatures observed in apple juice and yogurt systems.

These findings demonstrate that probiotic functionality is strongly influenced by formulation and food matrix, highlighting their key role in determining probiotic performance and potential health relevance.

S5-RT5: Describing the "Healthy" State of Food-Related Microbiomes Through Metrics and Functional Stability

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Microbiota associated with soil, plants, animals, and humans are central to food microbiology and ecosystem/host health, influencing food safety, quality, and sustainability. Advances in metagenomics and high-throughput sequencing have generated extensive microbial datasets across these domains. Despite this progress, there remains limited consensus on how to interpret such data, particularly in defining what constitutes a “healthy” microbiome.

Existing studies have introduced various metrics, including diversity, resilience, and robustness of a microbiome. While informative, these indicators are often context-dependent and do not fully capture microbiome functionality. In particular, the concept of functional stability, namely, the ability of a microbiome to maintain key functions under perturbations, remains insufficiently defined. Furthermore, although functions such as pathogen suppression, nutrient cycling, antimicrobial resistance, and metabolic balance are recognized as important, a standardized framework linking these functions to different microbiomes and their “healthy state” is still lacking.

Finally, most studies have historically focused on Bacteria, yet modern research suggests that a comprehensive understanding requires consideration of all relevant microbial taxa, including fungi, viruses, and archaea. These additional members may influence community dynamics, functional stability, and responses to environmental changes in food-related systems.

In this work, we aim to describe a “healthy” microbiome by leveraging multi-omics data, including metagenomics and metatranscriptomics across soil, plants, livestock, human gut, and food-related environments, including diverse food matrices, while including data from all relevant microbial taxa (Bacteria, Fungi, Viruses, and Archaea). A central objective is to define quantifiable key microbiome attributes such as compositional and functional stability and then develop metrics to measure these attributes, which could later be combined to inform a composite index of a ‘healthy’ microbiome. Established measures such as diversity, resilience, and robustness will also be incorporated. Functional profiling and network analysis will then be used to assess stability, develop preliminary metrics for cross-domain comparisons, and identify shared traits that may underpin ecosystem or host health.

By integrating compositional and functional data across microbiota, this work seeks to contribute to a more coherent understanding of what constitutes a “healthy” microbiome in food-related contexts. Such an approach may help identify key universal microbial functions that support food safety, quality, and sustainability, providing a foundation for future research aimed at translating microbiome insights into practical applications.

S5-FT1: Redefining Polysaccharide Choice: Linking Technology, Gut Health, and Sustainability from Chitosan to Resistant Starch

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Food polysaccharides are increasingly being reconsidered not only as technological ingredients, but also as microbiota-targeted components (Xue et al., 2025). The objective of this work was to explore whether sustainable strategies based on chitosan valorisation and resistant starch tailoring could improve the gut microbiome-modulating potential of food ingredients beyond that of classical prebiotics.

In a first pilot study, we evaluated commercial chitosan (ChiC), seafood waste-derived chitosan (ChiW) and hydroxypropyl methylcellulose (HPMC), using fructooligosaccharides (FOS) as a positive prebiotic control. CHIC and CHIW were associated with enrichment of beneficial taxa (ruminococci, lactobacilli, and bifidobacteria) and increased production of key short chain fatty acids (SCFAs), notably butyrate and propionate (Addazii et al., 2026). Chitosan-derived oligosaccharides (COS) effects depend on physicochemical features (e.g., degree of deacetylation and molecular weight) and have been linked to selective stimulation of beneficial taxa, SCFAs modulation, and barrier-supporting functions (Edo et al., 2025).

In a second pilot study, type 4 resistant starch (RS4) was modified by plasma-activated water (PAW), annealing (ANN) and their combination (PAW+ANN), with untreated RS4 used as control. PAW (acidic pH and reactive species) and ANN (increased gelatinisation temperatures and narrowed gelatinisation range) were applied to induce structural changes potentially affecting starch accessibility and fermentability (Gebremical et al., 2024). Our results indicate that treated samples, especially PAW+ANN, can positively modulate the intestinal microbiome in terms of taxa shift and production of fatty acids. These observations are consistent with reports that RS fermentation favours acetate/butyrate production and shifts taxa such as *Ruminococcus* and *Lachnospiraceae* (Klostermann et al., 2024).

Overall, both pilot studies support a redefinition of polysaccharide selection, incorporating strategies to enhance their sustainability and promote a more favorable gut microbiome profile. Chitosan-based, particularly those derived from seafood waste, were associated with enrichment of beneficial taxa and increased SCFA production, while PAW+ANN emerged as the most promising approach for enhancing the microbiome response of RS4. Taken together, these findings suggest that sustainable sourcing and structural tailoring of food polysaccharides may represent a promising route for the development of next-generation prebiotic ingredients.

S5-FT2: Development of a Novel Psychobiotic Fermented Product for Gut-Brain Axis Modulation

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Modulating the gut microbiome through diet could positively influence mental health, offering new perspectives for dietary approaches in the treatment and prevention of psychiatric disorders. Many lactic acid bacteria (LAB) found in fermented food are major microbial producers of neuroactive compounds. In order to exploit the gut-brain axis and to evaluate the possible role in gut microbiome modulation, a plant-based yogurt-like (pYL) product was developed and tested in a mSHIME® model of depressed patient. Firstly, we analysed 1,185 fermented food metagenomes, revealing that genes related to the biosynthesis of neuroactive molecules were enriched particularly in ripened cheeses and mainly associated with LAB genomes. Genomic screening of 74 LAB strains isolated from fermented foods confirmed species-specific genomic patterns for γ-aminobutyric acid (GABA), acetylcholine, serotonin, and catecholamine pathways, particularly prevalent in *Levilactobacillus brevis*, *Lactiplantibacillus plantarum*, and *Limosilactobacillus fermentum*. Three strains (*L. fermentum* TO24, *L. brevis* TO10, and *Lentilactobacillus diolivorans* B92) were selected as the most promising candidates for mental health modulation and their neuroactive activity was confirmed by *in vitro* functional screening. The strains were used to ferment a plant-based matrix (mixing rice : pea : chickpea in a ratio 2:1:1) to develop a pYL product and tested in a M-SHIME® model of depressed patient. Treatment significantly increased the relative abundance of *L. brevis* and *L. fermentum* in both the lumen and mucosa samples, with *L. brevis* persisting after the washout phase. The pYL treatment significantly inhibited potentially pro-inflammatory taxa (*Acidaminococcus intestini* and *Megasphaera massiliensis*) in both compartments. Notably, the neuroactive potential is not limited to the introduced strains. A significant functional redundancy emerged, as commensal Metagenome-Assembled Genomes (MAGs) were associated with key functional hubs involved in GABA biosynthesis. These results suggest that the pYL treatment can modulate and partially restore the dysbiotic gut microbiome in depressed patients, representing a key mechanism for improving the gut-brain axis. This study successfully provides a comprehensive probiogenomics pipeline for identifying and validating novel psychobiotic strains.

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S5-FT3: Screening of 103 Probiotic and Proteolytic Bacterial Strains for Lupin-Based Food Applications Targeting Allergy Modulation

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The challenge of feeding 10 billion people by 2050 necessitates the incorporation of more plant-based proteins in our diets. In this context, legumes such as lupin are of particular interest, thanks to their high nutritional value and agrosystemic benefits (nitrogen fixation). However, lupin is known to induce IgE-mediated allergic reactions in a minor part of the population and requires obligatory labelling on prepackaged foods. More specifically, lupin is known to cause allergic reactions due to cross-reactivity with peanut. The allergenic potential of lupin proteins can be reduced via food processing methods, such as fermentation, which can act on both the structure of allergenic proteins (allergens) and the host immune response. Fermentation by specific bacterial strains has also a high potential to enhance consumer acceptance of plant-based protein products, as it improves the taste and digestibility.

In this study, carried out as part of the PULSAR project (grant number ANR-23-PLEG-0003), we aimed to identify lactic acid bacteria, propionic acid bacteria, and/or bifidobacteria with proteolytic activity toward lupin proteins and immunomodulatory properties. We screened a total of 103 strains and assessed their ability to hydrolyse lupin proteins, galacto-oligosaccharides, as well as to induce anti-inflammatory cytokine production in human peripheral blood mononuclear cells (PBMCs). Next, we clustered strains by phenotype and designed bacterial consortia using combinatorial optimization and decision matrix strategies. Constructed consortia were further characterized through analyses evaluating the growth of the bacteria and their immunomodulatory potential, lupin proteolysis, and changes in immunoreactivity. In the near future, we will further explore the utilisation of the identified consortia for the formulation of rationally designed lupin protein-based functional fermented foods.

S5-FT4: Probiotic-Fermented Jambolan (*Syzygium cumini*) Pulp Modulates Microbial Communities and Metabolite Profiles in Elderly Gut Microbiota

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Age-related alterations in gut microbiota are associated with impaired metabolic functionality, potentially compromising nutrient utilization and intestinal health. Food-based strategies, particularly probiotic-fermented matrices, have been proposed as approaches to modulate the gut microbiome and microbial metabolism in elderly populations. This study investigated the effects of jambolan (*Syzygium cumini*) pulp fermented with *Lacticaseibacillus casei* 01 on microbial composition and metabolic activity in elderly microbiota. Pooled fecal samples from volunteers aged ≥ 65 years (n = 5) were subjected to *in vitro* colonic fermentation for 48 h in the presence or absence of probiotic-fermented jambolan pulp. The 16S rRNA gene (V3–V4 region) was analyzed by amplicon sequencing. Raw reads were quality-checked (FASTQC), trimmed (Cutadapt), and processed using DADA2 for ASV inference and chimera removal. Taxonomic assignment was performed using the SILVA 138 database, and alpha diversity indices were calculated. Key metabolites, including organic acids, sugars, short-chain fatty acids (SCFAs), and phenolic compounds, were determined and quantified by liquid chromatography. Experiments were performed in triplicate, and statistical significance was considered at $p < 0.05$. The fermented pulp induced shifts in microbiota composition and metabolic profiles. The relative abundance of *Firmicutes*, *Bacteroidota*, and *Actinobacteriota* decreased ($p < 0.05$), whereas *Proteobacteria*, *Fusobacteriota*, and *Desulfobacteriota* increased ($p < 0.05$). *Enterococcaceae*, *Enterobacteriaceae*, and *Eubacteriaceae* increased ($p < 0.05$), while *Clostridiaceae*, *Fusobacteriaceae*, and *Prevotellaceae* decreased ($p < 0.05$) compared to the colonic fermentation control. Alpha diversity analysis indicated increased microbial richness, particularly based on Chao1 values, in the fermented jambolan treatment compared to the control. Metabolic shifts included reductions in malic and succinic acids and changes in SCFA profiles, with a significant increase in propionic acid and a decrease in butyric acid. The fermented pulp also increased glycerol concentrations and reduced maltose levels. Phenolic profiling revealed reduced levels of epigallocatechin gallate and 4-hydroxybenzoic acid, accompanied by increased catechin and syringic acid concentrations, suggesting active microbial biotransformation of phenolic compounds. Correlation analysis indicated associations between specific metabolites and microbial groups. Overall, probiotic-fermented jambolan pulp modulated microbial composition and metabolic activity in elderly microbiota, increasing microbial richness and promoting phenolic compound biotransformation and shifts in short-chain fatty acid production.

S5-FT5: Comparative Evaluation of Anti-inflammatory Potential of Nisin and Gassericins K7

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Bacteriocins, which are antimicrobial peptides produced by lactic acid bacteria, are increasingly being explored as potential therapeutic and postbiotic agents. In contrast to their antimicrobial activity, their direct effects on eukaryotic cells have been much less studied. In this study, the antimicrobial and anti-inflammatory activities of nisin and gassericins K7 were investigated. Nisin Z was produced by an autochthonous Slovene *Lactococcus* isolate using ultrafiltered acid whey permeate (10 kDa cut-off) as a growth medium supplemented with yeast extract and mineral salts, whereas gassericins K7 were produced by *Lactobacillus paragasseri* K7 in a commercial culture medium. The antibacterial activity of purified bacteriocin preparations was confirmed against 18 indicator strains.

Cytotoxicity testing in RAW 264.7 macrophages indicated minimal toxicity of nisin and gassericin K7, with only a modest reduction in cell viability observed at 100 µg/mL. Nisin did not show a significant anti-inflammatory effect in LPS-stimulated macrophages, as it did not reduce nitric oxide (NO) production or downregulate inducible nitric oxide synthase (iNOS) expression. However, IL-6 production was suppressed in a dose-dependent manner, while TNF-α was downregulated with high concentrations of nisin. In contrast, gassericins K7 demonstrated a clear dose-dependent anti-inflammatory effect by reducing NO production, downregulating iNOS expression, and suppressing the secretion of the pro-inflammatory cytokines TNF-α and IL-6. Importantly, exposure of macrophages to either bacteriocin alone did not induce any inflammatory response.

These findings highlight the strong biological activity of both bacteriocins and reveal the anti-inflammatory potential of gassericins K7 in eukaryotic immune cells, supporting their potential development as postbiotic or bacteriocin-based therapeutic agents. Although nisin did not show a pronounced anti-inflammatory effect under the tested conditions, further studies are needed to evaluate its effects on additional inflammatory markers and pathways.

S6 Safe Water and Biofilms Control

S6-KN1: Emerging Chemicals, Emerging Risks: Rethinking Microbiological Safety

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Contaminants of emerging concern (CECs), particularly pharmaceuticals and personal care products (PPCPs), are now routinely detected at residual concentrations (< µg/L) in aquatic environments worldwide. Their widespread occurrence reflects both our societal dependence on these compounds and the limited capacity of conventional wastewater treatment processes to remove chemically diverse micropollutants. While much attention has focused on their ecotoxicological effects on higher organisms, their impact on microbial communities, central to environmental, food, and human health, remains insufficiently understood [1,2].

Among these compounds, synthetic musk fragrances and parabens are extensively used in personal and household care products. Due to their persistence, bioaccumulation potential, and endocrine-disrupting properties, they are increasingly recognized as environmentally relevant contaminants. However, unlike antibiotics, these non-antibiotic CECs are not designed to target microorganisms, yet growing evidence suggests that they may profoundly influence microbial physiology and community dynamics [1,3].

This lecture, framed within the "One Health" perspective, will discuss emerging evidence demonstrating that non-antibiotic CECs can modulate microbial behavior, including biofilm formation, tolerance to disinfection strategies, and susceptibility to clinically relevant antibiotics. Such effects are particularly concerning in food-associated environments, where biofilms and antimicrobial tolerance directly impact food safety, sanitation efficacy, and the dissemination of antimicrobial resistance.

Understanding how low-level chemical exposures shape microbial resilience and resistance is critical for safeguarding water quality, food production systems, and public health. This lecture will highlight key mechanistic insights, methodological challenges, and future research directions at the interface of environmental microbiology, food safety, and antimicrobial resistance.

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S6-RT1: Assessing Microbial Risks in Reuse of Process Wastewater in the Dairy Industry

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The food industry uses large quantities of drinking water. To reduce the water foodprint, a dairy company is looking to treat process wastewater to obtain technical water of drinking quality, which is safe and fit-for-use in the production.

The objectives of this study were to assess (1) the compliance of this technical water with the microbiological parameters for drinking water and (2) the effect of individual steps in the Technical Water Plant (TWP).

The study included a detailed description of the use scenario, along with culture and sequence-based characterization of the microbiology of the incoming source water, which is biologically treated process wastewater. The effect of each TWP treatment step (in order: ultrafiltration [UF], reverse osmosis [RO] and ultraviolet light [UV]) was evaluated regarding the removal of microorganisms. Finally, the risk of detecting microorganisms in the technical water was calculated using Monte Carlo Simulations (10,000 iterations) under different TWP operational scenarios.

Metagenomic sequencing showed the microbiological quality of the source water remained stable over 14 sampling weeks, spanning both winter–spring and summer periods. The microbiome was dominated by *Burkholderiaceae*, *Streptococcaceae*, *Rhodobactereaceae*, *Enterobacteriaceae* and *Flavobacteriaceae* with culturable levels of ~103 CFU/ml, and detectable presence of presumptive *E. coli*, *Enterococcus* spp. and *Clostridium perfringens*. After UV disinfection, 100% of the analyzed samples (n = 28) complied with the extended Danish drinking water parameters. Looking at the Log Removal Values (LRVs) for the individual steps of the TWP, the efficacy of UF depended on the age of the membrane (avg. of 0.4 and 2.0 for older and newer membranes, respectively), while the RO step contributed with an avg. 2.2. LRVs for the UV system (operating at 40 mJ/cm^2) were calculated to be 22, 3.4 and 2.2 for bacteria, endospores and fungi, respectively, based on literature values, since LRVs could not be determined experimentally due to the negligible counts (≤ 1 CFU/ml) in the RO treated water. The robustness of the TWP treatment was tested using Monte Carlo simulations for different UV-output scenarios (0, 50 and 100%). The results demonstrated that the UV-step was crucial for the performance of the TWP, and for the reduction of risk of surviving microorganisms in the technical water.

The microbiological risk assessment showed that process wastewater subjected to biological and advanced treatments can produce technical water that fulfills the microbiological requirements for drinking water. Analysis of the TWP multiple barrier approach showed great dependency on the UV treatment.

S6-RT2: Evaluating the Efficacy of Ozonated Recycled Dairy Process Water Against Spoilage Microorganisms: Focus on *Pseudomonas* spp. and *Enterobacteriaceae*

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Fresh stretched-curd cheeses, such as high moisture mozzarella and burrata cheeses, consume approximately 3 liters of water for kilogram of cheese produced. The development of an innovative approach based on membrane filtration, ozonation, and electrolysis technologies, integrated by fermentation processes, has led to the recycling of dairy process waters. The use of these water bodies reduces the consumption of clean (blue) water enhancing dairy water footprint.

In this work we evaluated the ability of dairy water deriving from a combined filtration/ozonation process to support the survival of nine spoiler strains usually contaminating process water, governing liquid (the low concentrated salt solution used to store high moisture stretched cheeses under cold storage), and fresh cheeses.

These strains, belonging to *Pseudomonas*, *Escherichia*, *Serratia*, *Hafnia* and *Klebsiella* genera, were incubated for 72 h in the filtered/ozonated water or in the governing liquid.

Governing liquid, with or without the inclusion of 1% skim milk powder, supported the growth of spoilage microorganisms, while filtered/ozonated water inhibited the growth depending on the strain. Growth curve parameters, such as average and maximum growth rate, time to max rate, time to change and the half-run absorbance value, for each strain were calculated confirming the ability of filtered/ozonated water to control the growth of spoiler microorganisms. At the end of incubation, viable cell counts for all strains in filtered/ozonated water (+ 1% skim milk) resulted lower than those in governing liquid (+ 1% skim milk) showing an average value of 2.07 log CFU/ml and 4.45 log CFU/ml for *Pseudomonas* and *Enterobacteriaceae*, respectively.

The improved control of spoilage microbial populations by filtered/ozonated water, compared to the control governing liquid, suggests that fresh cheeses cold-stored in these recycled waters could show improved microbial quality and consequently an extended shelf life.

S6-RT3: Diversity, Antimicrobial Resistance and Biofilm Forming Ability of *Aeromonas* spp. Isolated from Hungarian Water Samples

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Aeromonas hydrophila is widely distributed in freshwater environments, which serve as important reservoirs for human exposure and potential contamination of food products. It has recently been recognized as an emerging pathogen associated with increasing numbers of human infections, including gastrointestinal diseases and opportunistic extraintestinal conditions. Similarly, *Aeromonas caviae* and *Aeromonas veronii* have been linked to diseases of the gastrointestinal tract and the occurrence of diarrhoea. Of particular significance is the aquatic origin of these bacteria, which establishes a direct link between environmental waters and food safety, particularly through the contamination of drinking water, fresh produce, and seafood. The escalating incidence of antibiotic resistance within the *Aeromonas* genus further underscores this concern, as isolates are frequently documented to be multidrug-resistant, thereby posing a substantial challenge for public health and infection management.

The aim of our study was to investigate samples from natural waters focusing on the isolation of *A. hydrophila* and other *Aeromonas* species, and the determination of their antimicrobial susceptibility profiles. Bacterial isolates obtained by EPA 1605 “Aeromonas in Finished Water by Membrane Filtration using Ampicillin-Dextrin Agar with Vancomycin (ADA-V)” culturing method were identified using MALDI-TOF MS and Sanger sequencing of the 16S rRNA gene, while RAPD-PCR was used for genotyping of the strains. Antimicrobial resistance was assessed by the Kirby–Bauer disk diffusion method with several antibiotics relevant to human medicine. Biofilm formation was quantified using a 96-well microtiter plate assay based on crystal violet staining, followed by solubilization with acetic acid.

A number of bacterial species were detected in the course of water testing, including *A. hydrophila*, *A. veronii*, *Aeromonas eucrenophila*, *Enterobacter ludwigii*, *Acinetobacter lactucae*, *Stenotrophomonas maltophilia*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Results indicated that the *Aeromonas* isolates exhibited reduced susceptibility to beta-lactam antibiotics, while resistance to glycopeptide was also observed. A variability in biofilm formation was observed among the isolates, with capacities ranging from weak to moderate-to-strong.

Natural water environments serve as important reservoirs of clinically relevant, antibiotic-resistant *Aeromonas* strains. In addition, the observed biofilm-forming capacity of the isolates may further enhance their environmental persistence and resistance to external stresses. Their presence highlights the potential for transmission into the food chain, thereby posing an indirect risk to food safety. These findings underline the importance of routine monitoring from both public health and food safety perspectives.

S6-KN2: Cavitation Bubble Interactions with Biological Structures: From Microscale Mechanisms to Water Treatment Applications

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Cavitation – the formation, growth, and violent collapse of vapor bubbles in liquids – has emerged as a promising non-thermal technology for microbial control and water treatment. Despite decades of research and numerous reported applications in food processing, disinfection, and wastewater treatment, the fundamental mechanisms by which cavitation affects biological structures remain poorly understood. This lack of mechanistic understanding has hindered the optimization, scalability, and reproducibility of cavitation-based technologies.

This keynote lecture will present a journey from the microscale physics of individual cavitation bubbles to full-scale water treatment applications, highlighting recent advances that bridge fluid mechanics, microbiology, and environmental engineering. The lecture will demonstrate how a bottom-up research approach can reveal the biological consequences of cavitation and provide the scientific foundation needed for the next generation of sustainable treatment technologies.

At the cellular level, we have shown that bacterial susceptibility to cavitation strongly depends on cell wall architecture and mechanical properties. Experiments with genetically modified *Escherichia coli* strains revealed that the peptidoglycan layer plays a critical role in determining resistance to cavitation-induced damage. Cells with weakened or absent peptidoglycan exhibit dramatically increased sensitivity, indicating that microbial inactivation is closely linked to the biomechanical properties of the cell envelope.

To further elucidate bubble-cell interactions, advanced numerical simulations and single-bubble experiments have been employed. Fluid-structure interaction models demonstrate that stresses generated by collapsing microbubbles can exceed membrane poration thresholds, suggesting that mechanical effects alone can explain significant levels of microbial damage. High-resolution optical studies, where individual cavitation bubbles are precisely positioned near single bacterial cells, reveal that only microorganisms located in the immediate vicinity of a collapsing bubble are affected. These experiments identify microjets, microstreaming, and intense local shear as the dominant mechanisms responsible for cell detachment, membrane disruption, and cell death.

The lecture will also discuss how these microscale insights translate to practical treatment systems. Examples from pilot-scale and field-scale applications will be presented, including wastewater treatment, sludge disintegration, and resource recovery. These studies demonstrate that cavitation can simultaneously enhance microbial control, improve biodegradability of organic matter, and support circular-economy approaches through increased methane production during anaerobic digestion. By connecting single-bubble dynamics with reactor-scale performance, the lecture will illustrate how mechanistic understanding can guide the development of more efficient and sustainable technologies.

Such knowledge opens new opportunities for food safety, process water treatment, biofilm control, and environmentally responsible microbial management.

S6-RT4: Evolution of *Listeria monocytogenes* to a Strong Biofilm Producer Phenotype

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Listeria monocytogenes is a foodborne pathogen that can cause listeriosis, a severe invasive illness characterised by one of the highest mortality rates of bacterial foodborne infections. Biofilm formation is key in *L. monocytogenes*' transmission and persistence in food processing environments. However, possible strategies for the evolution of *L. monocytogenes* towards higher biofilm formation capacity had not yet been investigated.

To further understand the mechanisms contributing to biofilm formation, an experimental evolution system was used to isolate strong biofilm-producing strains of *L. monocytogenes* EGDe (reference strain) and FBR16 (hypermutator food isolate).

After cycles of plastic surface colonisation, biofilm formation, dispersal and attachment to new surfaces, evolved variants (EV) strains were isolated and found to produce up to seven-fold more biofilm and improved attachment to different surfaces than their respective ancestral (AN) strains. Phenotypic assays revealed an increase in cell surface hydrophobicity as a shared dominant feature of EV isolates. Proteomic analysis showed protein Lmo1799, a putative peptidoglycan binding protein with 226 Ala-Asp tandem repeats, to be the most upregulated protein in EV strains compared to the AN strains. Genomic analysis of the EGDe EV strain identified a single-nucleotide insertion in the upstream region of *lmo1799* and an in-frame deletion of 42 nucleotides in *lmo1799*, conceivably resulting in high-level expression of *lmo1799*. To evaluate the impact of Lmo1799 on the EV phenotypes and the overall biofilm capacity of *L. monocytogenes*, EGDe EV mutants lacking *lmo1799* and/or the upstream insertion were constructed. Notably, both constructed mutants showed reduced biofilm formation and lower surface hydrophobicity compared to the EV strain, indicating the importance of these mutations for the strong biofilm capacity.

Overall, this study demonstrated the ability of *L. monocytogenes* to evolve and adapt its phenotype to a strong biofilm producer and further explored the mechanisms contributing to biofilm formation.

S6-RT5: Effects of Wastewater Discharges on Plastic-Associated Biofilm and Blue Mussel (*Mytilus edulis*) Microbiomes

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Antimicrobial resistance (AMR) and marine plastic pollution are individually major environmental and global health challenges. However, there is limited knowledge about the occurrence of pathogens and antibiotic-resistant bacteria (ARB) in wastewater (WW) effluents from urban areas and how they affect the marine environments into which they are discharged. Furthermore, the potential role of plastic debris as a vector for the spread of pathogens, ARB, and antibiotic resistance genes (ARGs) through marine ecosystems and into the human food chain is also poorly understood. The aim of this study was to investigate how WW discharges influence the microbiomes of plastic-associated biofilms and blue mussels in a field experiment conducted in the Trondheim fjord, Norway. Blue mussels are useful indicator organisms for microbiological and chemical contaminants in marine environments as they filter and concentrate particles from seawater. Three plastic types (polystyrene, polyethylene, polyvinyl chloride), two non-polymeric materials (glass and wood), and blue mussels were deployed for five weeks at three field sites: two near WW effluent outlets and one reference site. Bacterial community composition was analysed using 16S rRNA gene sequencing, complemented by quantification of selected faecal and environmental indicator bacteria. In addition, resistome analysis, antimicrobial susceptibility testing of bacterial isolates, and measurements of potentially toxic metals in blue mussels were performed. Bacterial communities differed significantly ($p < 0.05$) between locations. Faecal and environmental indicator bacteria were detected in biofilms at all locations, but resistance rates differed between locations for specific bacteria-antibiotic combinations. After exposure, blue mussels near WW effluent outlets exhibited higher levels of faecal indicators and specific toxic metals. Analysis is still ongoing, and results will be presented in more detail at the conference. This study provides new insights into how WW discharges shape biofilm formation on marine plastics and affect the microbiome and safety of blue mussels in coastal environments.

S6-FT1: Nanoparticle-Based Antimicrobial Strategies Against Biofilm-Forming *Staphylococcus* spp. from Slaughterhouse Surfaces

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The persistence of bacterial contamination on food processing surfaces represents a significant challenge for food safety, particularly in slaughterhouse environments where cross-contamination events are frequent. Among the microorganisms commonly detected in these settings, *Staphylococcus* spp. are of particular concern due to their ability to form biofilms and persist under disinfection treatments. Therefore, improving antimicrobial strategies targeting surface-associated bacteria remains a priority.

In the present study, we evaluated the antimicrobial activity of different metal-based nanoparticles (Au NPs, Ag NPs, ZnO NPs and CuO NPs), alone and in combination with EDTA or a disinfectant formulation (HLE), against *Staphylococcus* spp. strains isolated from slaughterhouse environmental surfaces. Particular attention was given to differences in response between strains and treatments, in order to better assess their applicability under different contamination scenarios.

The antimicrobial response varied depending on both the nanoparticle type and the strain. While individual nanoparticles showed variable activity, their combination with EDTA or HLE significantly enhanced antimicrobial activity, leading to a greater reduction of both developing and preformed biofilms. Notably, consistent strain-dependent differences were observed across treatments, highlighting the importance of considering the microbial composition when selecting antimicrobial strategies for surface decontamination, and suggesting that no single antimicrobial approach is universally effective.

Overall, these findings highlight the potential of nanoparticle-based combinations to improve current disinfection approaches in slaughterhouse environments. This strategy may contribute to reducing bacterial persistence on food processing surfaces and to optimizing antimicrobial treatments according to specific contamination scenarios.

Keywords: antimicrobial resistance, *Staphylococcus*, metal-based nanoparticles, biofilms, slaughterhouse surfaces, food processing environments

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S6-FT2: Colonization of Native Dairy Facility Biofilms by Persistent *Listeria monocytogenes* Strains

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Listeria monocytogenes is an opportunistic pathogen capable of persisting in food processing environments, posing a serious risk to public health and food safety. Its persistence is frequently associated with biofilm formation, particularly integration into multispecies biofilms, which provide structural and physiological protection against environmental stressors, including sanitizers. While previous studies have demonstrated the protective effect of multispecies biofilms and the potential facilitation of *L. monocytogenes* by co-occurring taxa, the mechanisms governing colonization such as timepoint of integration and community composition remain poorly understood.

In this study, we investigated the integration of a persistent *L. monocytogenes* strain into native multispecies biofilms derived from two Austrian dairy processing facility sites. Biofilms were cultivated on stainless steel under controlled laboratory conditions, and experimental designs included sequential inoculations of *L. monocytogenes* and native communities. Biofilms were harvested at 24 h and 48 h, with quantification of *L. monocytogenes prfA* gene copies and 16S rRNA gene copies to track integration. Full-length 16S rRNA gene sequencing was performed to assess community composition and identify taxa potentially facilitating or inhibiting *L. monocytogenes* persistence.

Both dairy facility-derived communities formed distinct, reproducible multispecies biofilms under laboratory conditions, as confirmed across technical replicates. *L. monocytogenes* was able to successfully colonize both biofilm communities, and colonization occurred independently of the inoculation timepoint.

In biofilm community A, *Hafnia-Obesumbacterium avlei*, *Serratia liquefaciens*, *Pseudomonas fragi*, *Kluyvera intermedia*, and *Acinetobacter johnsonii* emerged as the dominant co-occurring species. In contrast, biofilm community B was characterized by the prevalence of *Citrobacter werkmanii*, *Morganella morganii*, *Hafnia-Obesumbacterium paralvei*, *Lelliottia jeotgali*, and *Pseudomonas poae*. Beta diversity analyses indicated that both the initial community composition and the timing of *L. monocytogenes* inoculation were the primary drivers shaping differences in community structure.

Differential abundance analysis revealed that *Pseudomonas lurida*, *Staphylococcus xylosus*, *P. poae*, *H.-O. paralvei*, *C. werkmanii*, unclassified *Enterobacteriaceae*, and unclassified *Lelliottia* were depleted in biofilms where *L. monocytogenes* was present. In contrast, *L. monocytogenes* co-occurred with *Leuconostoc pseudomesenteroides*, *Raoultella ornithinolytica*, *Stenotrophomonas rhizophila*, *Leuconostoc citreum*, *Pseudomonas synxantha*, and *Serratia marcescens*.

These results indicate that *L. monocytogenes* can integrate into diverse native multispecies biofilms, with specific co-occurring taxa varying between communities. The findings provide insight into ecological interactions that facilitate pathogen persistence and suggest potential microbial targets for interventions aimed at reducing the persistence of *L. monocytogenes* as well as product contamination.

S6-FT3: Biofilm-Mediated Survival of *Campylobacter jejuni* on Microplastics

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Microplastics are defined as plastic particles ranging in size from 1 µm to 5 mm. They persist in the environment and become incorporated into the food chains of living organisms. Recently, increasing attention has been paid to their role as mobile surfaces that can adsorb a wide range of organic and inorganic compounds, as well as microorganisms.

In our previous work, we confirmed the co-occurrence of microplastics and food-borne pathogenic and spoilage bacteria on the surface of packaged chicken meat, including *Campylobacter jejuni*, an important food-borne pathogen. Although *C. jejuni* does not survive at atmospheric oxygen concentrations, it is one of the leading causes of bacterial gastroenteritis worldwide, representing a significant economic burden estimated at €2.4 billion in the EU by EFSA.

We therefore developed a novel method to quantify bacterial biofilms on microplastics and used it to assess whether microplastics can facilitate the adhesion, biofilm formation, and survival of *C. jejuni*. We found that *C. jejuni* forms biofilms on 5 mm PET microplastics, and that its survival under atmospheric oxygen conditions is supported by microplastics precoated with live *Pseudomonas fragi*, a common chicken meat spoilage bacterium, but not by inactivated cells or its extracellular polymeric substance (EPS).

The survival of the oxygen-sensitive *C. jejuni* may be enhanced by biofilm formation or incorporation into multispecies biofilms that provide protective microenvironments. In particular, *Pseudomonas* spp. biofilms on microplastics may function as oxygen-buffered niches, potentially supporting prolonged persistence of *C. jejuni* during food processing and storage. These findings suggest that microplastics may act as biofilm-associated reservoirs that facilitate foodborne pathogens to persist under otherwise unfavourable environmental conditions within food processing environments.

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S6-FT4: Real-Time Monitoring of *Pseudomonas aeruginosa* Biofilm Formation Using a Radiofrequency Sensor for Food Safety Applications

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Biofilm-associated contamination is a major challenge in the food industry, due to its persistence in cleaning procedures and widespread presence on processing equipment. Biofilm detection as early as possible is an interesting way to optimize cleaning and disinfection operations. Recently, multidisciplinary approaches have been increasingly explored to control biofilm formation and to enhance food safety. Among those approaches, radiofrequency (RF)-based sensing exhibits a promising potential for real-time, non-destructive, and in situ biofilm detection.

We aimed to develop a cost-effective RF sensor, composed of an open-ended coaxial probe (OCEP) coupled with a mini vector network analyzer (miniVNA). The sensor responses were evaluated through the measurement of the reflection coefficient, S11, which is impacted by the dielectric properties of the material under test. Indeed, temperature has a direct impact on the medium's conductivity, thereby modifying the S11 response, underscoring the importance of controlling environmental conditions to ensure reliable measurements.

The conditions were chosen to enable significant biofilm formation on stainless steel and polytetrafluoroethylene (PTFE or teflon), the two main materials that constitute the OCEP. Based on this, the real-time monitoring of *Pseudomonas aeruginosa* PAO1 using the radiofrequency sensor was performed in a frequency range from 1 MHz to 1.5 GHz. The results showed an S11 variation of 0.65 dB with three different phases.

We hypothesize that the initial plateau is due to a lag phase during which bacterial activity is limited. Then, the first drop of signal is probably related to the variation of the medium conductivity due to the metabolic activity of bacteria. Actually, nutrient consumption leads to the production of ions, which may modify the conductivity and consequently the S11 responses. Meanwhile, the second drop of signal can be related to the detection of biofilm, as suggested by the difference in S11 response in the presence and absence of biofilm formed on the probe surface.

These findings highlight the promising capability of the RF sensor for biofilm detection. They also emphasize the importance of controlling environmental conditions to ensure reliable measurements. A more systematic and theoretical analysis is required to validate the applicability in the food facilities, particularly in the open circuit system.

S6-FT5: Assessment of Mixed *Salmonella* Biofilms on Stainless Steel and Their Impact on Beef Quality During Storage Using Multispectral Imaging

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Biofilms represent a major challenge in food processing due to their persistence and resistance to sanitation methods. Their complex structure and interactions within mixed communities can affect food quality and safety. Rapid, non-invasive detection remains limited, highlighting the potential of automated alternatives like Multispectral Imaging (MSI). This study investigates the behaviour of mixed *Salmonella* biofilms on stainless steel and their impact on beef quality and safety, while evaluating MSI as a rapid monitoring tool.

Multispecies biofilms composed of *Salmonella* spp., *Pseudomonas* spp. and lactic acid bacteria (LAB) were developed on stainless steel coupons using different strain combinations and species-level monocultures, with sterile coupons as controls. Coupons were incubated at 15°C for 2 days in tryptic soy broth (TSB) for biofilm formation. Microbiological analyses and MSI images were acquired on day 0 and day 2. Beef fillet samples were contaminated using biofilm-inoculated coupons (three-species communities), planktonic cells, or uninoculated as controls and stored aerobically at 4 and 12°C. Microbiological analyses, pH measurements and MSI data were collected throughout storage. Machine learning models were applied to MSI data from both coupons and beef samples to classify contamination states and detect biofilm presence.

Microbiological analyses revealed clear differences in microbial dynamics within mixed-species communities on stainless steel surfaces, indicating active biofilm development over time. In beef samples, higher populations were observed in planktonic compared to biofilm samples at both temperatures. These changes did not significantly affect pH values but were associated with a decline in product quality during storage. MSI data combined with machine learning successfully captured these variations in both coupon and beef samples. Discrimination between control and biofilm inoculated coupons achieved an accuracy of 100%, while intraspecies classification in monoculture datasets reached 60%. In beef samples, distinct spectral patterns were observed between biofilm, planktonic and control cases, with model accuracy reaching 80%.

This study highlights the role of mixed *Salmonella* biofilms in influencing contamination dynamics from processing surfaces to meat products. The integration of MSI with microbiological analysis offers a promising approach for rapid, non-invasive monitoring of microbial contamination in food safety applications.

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S7 Hygiene and Human Interaction in the Food Environment

S7-KN1: Beyond Knowledge: How Human Behavior Shapes Hygiene in Food Environments

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Food environments, whether domestic or professional, are shaped by a complex interplay of frame factors, habits, risk perception and social norms. Food safety may be seen as a technical challenge addressed through food legislation, guidelines and information. However, the decisive determinants behind food safety behavior seems to be beyond knowledge.

Decades of international research show that domestic refrigerators rarely meet recommended safety standards. Similar discrepancies between knowledge and practices appear among students, adults and even health professionals. Sources of food safety knowledge play a central role. University students in Sweden rely heavily on family and friends, creating a fragmented information landscape where evidence based food safety competes with anecdotal advice. The mandatory school subject Home and Consumer Studies reaching all children in Sweden, would be an ideal channel for food safety education. However important risk areas are neglected due to limiting frame factors, non-reflective teaching traditions, or insufficient teacher knowledge. Without European food safety legislation in the domestic setting there is no given control point that ensures safe food handling among consumers.

Structural equation modelling among university students in Sweden demonstrates that food safety behavior is strongly influenced by background factors, knowledge and attitudes, with knowledge functioning as only one component in a broader behavioral system. Such findings align with the social cognitive model Health Action Process Approach (HAPA), which distinguishes between motivational processes (risk perception, outcome expectancies, self-efficacy) and volitional processes (coping self-efficacy; recovery self-efficacy; action planning, coping planning and action control) in predicting health-related behavior.

Effective strategies must therefore address motivational drivers, strengthen action planning, reduce environmental barriers, and foster long-term habit formation. These challenges are particularly pronounced among older adults in the domestic settings, who represent a high risk group for foodborne illness but receive limited targeted communication. Health professionals including dietitians, are cited as trusted sources of food safety information and their roles include food safety communication. Nevertheless, even health professionals express a need for enhanced training to support food safety behavior in vulnerable populations.

The food safety future lies in education that strengthens attitudes and supports behavior. Safe practice is a choreography of motivation, habits and culture. By integrating a theory-driven approach with targeted education and by empowering health professionals as well as teachers in Home and Consumer Studies we can strengthen the communication pathways and design food environments where safe food handling becomes the norm.

S7-RT1: Identification and Characterization of Biofilm Hotspots on Post Sanitation Surfaces

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Biofilms are a major challenge faced by the food industry. They are difficult to remove and lead recuring product contamination with pathogens and spoilage organisms. A large body of research on biofilms is available, but mostly from laboratory conditions. The objective of the present work was to determine biofilm harborage at a large beef fabrication facility, covering equipment surfaces and various locations on the floor, by rapid biofilm scanning test, quantifying the three major components of extracellular polymeric substances (EPS), and profiling of the microbial community with conventional culture-based method and high throughput sequencing method, to better understand their composition and potential impact on food safety. Enumeration of total plate count (TPC) and *Pseudomonas* were determined by plating on appropriate agars and profiling of microbial communities was carried out by 16S rRNA gene amplicon analysis. The surfaces (*n* = 162) were categorized into eight groups: drive and support rollers of conveyor belts, cutting saw blade covers, heads of various sprayers, dip tanks for tools, floor drains, wall corners, and floor mats. Floor mats had the highest median TPC at 6.88 log CFU/10 cm², higher for all but drains and wall corners by up to 4.0 log. *Pseudomonas* was highest for drains at 3.95 log CFU/10 cm². Of the 162 sampling sites, 46.9% harbored biofilms, mostly from floor mats, drains and wall corners. The microbial community structure in biofilms and non-biofilms differed. Most genera (13/15) in non-biofilms were aerobes except for *Escherichia* and *Clostridium*. The biofilms contained 35 genera, including all of those found in non-biofilms except for *Flavobacterium* and additional 10 aerobic bacteria and 11 facultative/anaerobes. A core group of 11 genera (*Acinetobacter*, *Aliihoeflea*, *Chelativorans*, *Dietzia*, *Escherichia*, *Pelagibacterium*, *Aeromicrobium*, *Rhodobacter*, *Pseudomonas*, *Glycocaulis* and *Sphingomonas*) were found in most (≥ 75%) of biofilms. Biofilms had significant fractions of facultative anaerobes, while non-biofilms had higher fractions of aerobes. Notably, *Pseudomonas*, *Acinetobacter* and *Escherichia*, sizable fractions of both biofilms and non-biofilms, were not distinguished by biofilms. The findings on biofilm hotspots suggest that it would be rewarding to direct more cleaning and disinfection efforts to such areas to better control residential microbiota and reduce cross contamination. The findings on differential harborage of facultative anaerobes and anaerobes, some of which are of safety and shelf life importance, in biofilms compared to non-biofilms may benefit biofilm detection method development and control strategies.

S7-RT2: Impact of Environmental Conditions and Hygiene Practices on Microbial Ecology in Meat Processing Environments

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The interaction between hygiene practices and environmental conditions plays a critical role in the microbial ecology of food production environments. In meat processing industries, where contamination risks are high, understanding these dynamics is essential to improve food safety and optimize hygiene strategies. This study aimed to evaluate the impact of environmental conditions and hygiene protocols on the microbial communities in the production processes of pork and poultry industries.

Sampling was conducted across the entire production process in pork and poultry production procedures. Samples were collected at multiple processing stages during different seasons of the year, allowing the assessment of environmental variability, particularly temperature fluctuations. In addition, sampling was performed both before and after the routine cleaning and disinfection procedures to evaluate their effectiveness.

Microbial analysis was carried out using a dual approach. Traditional culture-based methods were employed to quantify microbial loads, while 16S rRNA gene-based metagenomic sequencing was used to characterize the composition and diversity of microbial communities. This combined methodology enabled both quantitative and qualitative insights into the microbiota present in the processing environments.

The results revealed a significant influence of environmental temperature on both microbial load and community composition across sampling points. Seasonal variations led to noticeable changes in microbial populations, with higher temperatures generally associated with increased microbial counts and changes in dominant taxonomy. Furthermore, the study identified specific critical points within the production processes where hygiene practices were insufficient, as evidenced by persistent microbial presence even after cleaning and disinfection.

These critical points were associated with conditions favourable to biofilm formation, highlighting the limitations of standard cleaning and disinfection protocols in certain contexts. The identification of these hotspots enabled a targeted evaluation of cleaning and disinfection procedures, as well as the development and validation of improved strategies for biofilm prevention and removal.

In conclusion, this study demonstrates that environmental factors, particularly temperature, and the effectiveness of hygiene practices significantly influence microbial ecology in meat processing environments. The findings underscore the importance of adaptive and targeted hygiene strategies, taking into account seasonal variability and human interaction, to enhance hygiene control and ensure food safety in the meat industry.

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S7-RT3: A Metagenomic Framework for Rapid *Listeria monocytogenes* Surveillance in Food Production Environments

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Listeria monocytogenes remains a major foodborne pathogen with high mortality and costly persistence in food-processing environments. Established diagnostics rely on selective enrichment and single-colony isolation, which could introduce strong biases by favouring fast-growing strains or those more tolerant to enrichment broth inhibitors, while suppressing slow-growing, viable-but-nonculturable, and other co-occurring strains. This can obscure true pathogen diversity and may contribute to discrepancies between strains detected in food production environments and those associated with disease. To quantify the bias introduced by established culture-based diagnostics and to assess the potential advantage of metagenomics-based pathogen detection directly from the original sample matrix, we developed and evaluated a rapid nanopore sequencing–based metagenomic framework. We designed an artificial metagenomic community of several *Listeria* strains, comprising *L. monocytogenes* lineages I–III (including hypervirulent, persistent, and low-virulence strains), other *Listeria* spp., and a realistic background microbiome representative of food-processing environments. We then used this mock community to spike standard surveillance sponges and compared three workflows: (*i*) direct nanopore metagenomic sequencing of the original sample matrix, (*ii*) quasi-metagenomic sequencing after 4 h, 12 h, 24 h, or 48 h of selective enrichment, and (*iii*) ISO-based culture followed by whole-genome sequencing of a single presumptive *L. monocytogenes* isolate. We found that the culture-based approach recovered only a limited subset of strains, consistently underrepresenting diversity and failing to detect multi-strain contamination. These findings were reflected by the quasi-metagenomic results, where we found relative *L. monocytogenes* enrichment to be strain-dependent, indicating selective enrichment bias favouring specific strains. Metagenomics captured the full spectrum of spiked *Listeria* strains, enabling comprehensive strain-level resolution at all inoculation levels. We only observed relative enrichment of the *L. monocytogenes* strains by quasi-metagenomics compared with metagenomics after 48 h of selective enrichment. While driven primarily by the additional enrichment of *Listeria innocua*, these results suggest that quasi-metagenomics improves *L. monocytogenes* recovery only at the cost of a substantial reduction in speed. We finally showed that the sensitivity and accuracy of metagenomics could be improved by utilising different environmental sampling materials. We did not find any significant performance improvements from nanopore sequencing-based enrichment of *L. monocytogenes* through adaptive sampling approaches. We conclude that integrating long-read metagenomics into routine surveillance shows great promise to improve detection and source attribution in food safety systems.

S7-RT4: Diversity and Source-Tracking of Spoilage Molds in Bakery Products Using MALDI-TOF MS and Molecular Approaches

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Filamentous fungi are significant contributors to global food losses and waste including bakery products. In the baking industry, mold contamination often occurs at the post-baking stage due to exposure to ambient air. The aim of this study was to investigate fungal diversity and to track the environmental sources of spoilage molds in finished products in a French industrial bakery plant, using MALDI-TOF MS and molecular approaches including barcoding and genotyping of microsatellites using next-generation sequencing (SSR-seq). Furthermore, we evaluated MALDI-TOF MS as a rapid tool for the source-tracking of two spoilage molds, i.e., *Cladosporium sphaerospermum* and *Penicillium brevicompactum*. Samples from air, food contact surfaces, work clothes and cakes were collected once every four weeks for 42 weeks on 1–4 consecutive days at 2 times. Results showed that areas with uncontrolled air quality such as the pre-baking area had higher fungal loads compared to air-controlled areas such as post-baking zones. Products not sprayed with ethanol showed significantly higher mold contamination frequency highlighting the importance of surface treatment in reducing spoilage. *C. sphaerospermum* and *P. brevicompactum*, known for their xerophilic and airborne dispersal capabilities, were identified as the main product spoilers. SSR-seq provided insights into the genetic diversity of these species and showed that certain haplotypes, particularly originating from post-baking areas, were more frequently found on spoiled cakes suggesting repeated contamination events from persistent sources. Using both unsupervised and supervised approaches, we showed that there was a good agreement between MALDI-TOF MS and genotyping data analysis at the population level. This study provides new insights into the fungal ecology encountered in the bakery industry.

S7-RT5: In Fridge We Trust – A Mixed Methods Investigation of Consumers Knowledge, Attitudes and Practices (KAP) Related to Refrigeration in Slovenia

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Food safety is a critical public health issue, with a substantial proportion of foodborne outbreaks in the European Union linked to domestic environments. Inadequate refrigeration temperatures are particularly relevant due to their impact on the growth of psychrotrophic pathogens such as *Listeria monocytogenes*. Proper refrigerator temperature is essential for preventing foodborne illness and proper food placement in the refrigerator can help prevent cross contamination. This study investigated consumers' knowledge, attitudes, and practices related to refrigerator food storage in Slovenia using a mixed-methods approach combining a structured questionnaire (n = 1621) and focus group discussions (n = 40).

The questionnaire results show that the participants demonstrated mostly good levels of knowledge, attitudes, and practice. Most participants were aware of the general importance of refrigerator temperature for food safety and appropriate food placement approaches. However, there is a need for improvement in knowledge on the correct refrigerator temperature range (≤ 5°C) and practices of measuring the actual refrigerator temperature with a thermometer as very few participants practice this.

Focus group discussions provided further insight into this topic and enabled a better understanding of participants knowledge, attitudes and practices. Participants often expressed trust in refrigerator technology, manufacturer settings, and reliance on refrigerator displays. Most did not perceive themselves as responsible for active temperature monitoring in their refrigerators. Some also shared some innovative but unsafe approaches to checking the refrigerator temperature.

Together, the quantitative and qualitative findings highlight a discrepancy between perceived and actual control of microbiological risk in domestic refrigeration. Furthermore, insufficient practices of refrigerator temperature measurement and control are due to a combination of factors including trust in technology, ambiguous information, and somewhat lacking feeling of personal responsibility.

These results suggest that there is a need for the development of consumer food safety interventions on the topic of refrigeration, which should not only include the aspect of knowledge, but also address the role of consumers in ensuring food safety, clearer temperature communication, and innovative approaches that could promote active temperature monitoring.

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S7-KN2: Control of *Listeria monocytogenes* in Food Processing: Best Practice and Critical Factors for Implementation

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Listeria monocytogenes remains one of the most challenging foodborne pathogens to control in industrial processing environments. Despite decades of research and guidance, many facilities continue to struggle with persistent contamination while others obtain control. To understand why some food processors succeed while others fail, we conducted an expert elicitation study involving 13 international specialists from industry, service providers, and academia, each with more than 10 years of practical experience in *Listeria* control. Through in depth interviews, we collected more than 70 anonymised, practice based examples of successful and unsuccessful control efforts across meat, seafood, dairy, and mixed ingredient sectors.

The examples were analysed across key thematic areas: preventing entry, eliminating niches, limiting cross contamination, effective cleaning and disinfection (C&D), environmental monitoring and source tracking, and the role of decontamination and preservation technologies. The results confirm that persistent problems most commonly originate from poor hygienic design, inaccessible or worn equipment, inadequate zoning, and deficiencies in cleaning practices. While emerging technologies such as whole genome sequencing increasingly support source tracking, experts emphasized that robust monitoring and C&D routines must first be in place for typing data to have operational value.

A strong food safety culture, leadership commitment, technical competence, and effective cross functional collaboration were identified as critical for success. Conversely, inadequate resources, weak decision making processes, and tolerance of low level contamination undermine long term control. The findings offer practical guidance for industry and address areas for further research.

S7-FT1: Improving Hygiene in Food Processing: Cold Atmospheric Plasma Against Biofilms

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Biofilms formed by hygienically relevant bacteria on food-contact surfaces represent a persistent challenge in the food industry, as they can withstand conventional cleaning and disinfection procedures and contribute to food contamination. Due to the limitations of chemical disinfectants and increasing environmental concerns, plasma technology has emerged as a promising alternative. By generating highly reactive species at low temperatures, plasma enables rapid microbial inactivation without relying on harsh chemicals, offering a promising shift toward greener sanitation strategies. We evaluated the effectiveness of plasma treatment for the inactivation of biofilms formed by *Salmonella enterica* serovar Typhimurium, *Listeria monocytogenes*, and *Escherichia coli* on stainless steel surfaces under varying exposure conditions. Plasma treatment resulted in a statistically significant, exposure-dependent reduction in viable cell counts across all tested biofilms. The extent of inactivation varied among bacterial species, with *L. monocytogenes* showing the highest susceptibility, *E. coli* exhibiting substantial reductions, and *S.* Typhimurium demonstrating the greatest tolerance. Beyond cell inactivation, plasma exposure disrupted bacterial physiology by reducing metabolic activity and inducing intracellular oxidative stress. Interestingly, total biofilm biomass remained largely unchanged, indicating that while plasma is highly effective at killing cells, it does not efficiently remove the extracellular polymeric matrix that maintains biofilm structure. These findings highlight plasma treatment as a powerful complementary tool for biofilm control on food-contact surfaces, with exposure time playing a critical role in antimicrobial efficacy. Overall, this work reinforces the potential of plasma technology as a next-generation, environmentally friendly intervention in food safety.

S7-FT2: Genomic insights into Antimicrobial, Disinfectant, and Heat Resistance, and Biofilm Formation in *Pseudomonas* spp. Isolated from Packaged Pasteurised Milk in South Africa

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Pseudomonas spp. are frequent contaminants in dairy plants and pose a challenge to milk safety and quality due to their metabolic versatility, antimicrobial resistance, and biofilm-forming ability. In this study, whole-genome sequencing was applied to 13 *Pseudomonas* isolates recovered from packaged pasteurised milk in South Africa. Genomes were annotated using Prokka and RAST in KBase to identify genes associated with antimicrobial resistance, disinfectant tolerance, heat resistance, and biofilm formation. Comparative analyses were supported by metadata on milk origin, seasonality, and cleaning-in-place (CIP) regimes. Heatmaps and principal coordinate analyses revealed distinct resistance profiles, with plant-specific clustering linked to differences in CIP chemistry. Isolates from hydrogen peroxide environments (e.g., P11, P42) showed enrichment in peroxide-detoxification and protein-repair functions, whereas isolates exposed to peracetic acid (e.g., P89, P129) carried more oxidative stress regulation and proteostasis genes. Biofilm-associated traits varied, and some isolates (P11, P129) exhibited features consistent with persistence under oxidative cleaning stress. Heat-related survival genes were broadly conserved, and phylogenetics confirmed species-level clustering within the milk-associated *Pseudomonas* population. These findings demonstrate that *Pseudomonas* isolates from packaged milk exhibit diverse genetic strategies to withstand disinfection and processing stresses, often shaped by the local CIP regimes. The study underscores the need for plant-specific hygiene interventions and provides genomic insight into the persistence of spoilage-associated *Pseudomonas* in dairy processing chains.

S7-FT3: Microbiota Dynamics in Packaged Semi-Hard Cheeses: A Pilot Study Combining Culture and NGS Approaches

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Understanding microbial behaviour during storage is essential for evaluating the safety and stability of ready-to-eat dairy products. While classical cultivation provides targeted information on indicator and pathogenic organisms, high-throughput sequencing enables broader insight into community composition. This pilot study aims to assess the feasibility of using both culture-based methods and NGS-based 16S rRNA analysis to evaluate microbial changes in semi-hard cheeses during storage.

Paired samples of originally packed semi-hard cheeses from retail were analysed at purchase and at the end of shelf life. Classical cultivation was performed on 30 samples (12 cheese types, 14 producers), with an average storage duration of 55 days. Quantitative and qualitative analyses targeted *Enterococcus* spp., coliform bacteria, and staphylococci.

In parallel, 16S rRNA gene sequencing (V4 region, Illumina MiSeq) was performed on 12 selected paired samples. DNA was extracted using the E.Z.N.A. Food DNA Kit. Amplicon Sequence Variants (ASVs) were generated and taxonomically assigned using the SILVA database, and data were rarefied to a uniform sequencing depth.

Cultivation revealed group-specific temporal patterns. Enterococci showed the highest stability, being present in 60% of samples at purchase and 63.3% at the end of shelf life, with concordant results in 76% of paired sets. Staphylococci were moderately variable, detected in 60% of samples at purchase and 66.7% at the end of shelf life; *S. aureus* was detected only at purchase and mainly at < 100 CFU/g. Coliforms exhibited the greatest fluctuation, with seven discordant paired results and occasional late-stage emergence.

Sequencing confirmed the dominance of the families *Streptococcaceae* and *Lactobacillaceae* across samples, with relatively stable profiles between paired time points. However, marked variability in microbiota was observed among producers and between batches of the same cheese type, likely reflecting differences in manufacturing practices. Species-level identification was reliable only for selected genera, particularly *Lactobacillus*, *Weissella*, *Pediococcus* and *Leuconostoc*, whereas species resolution within *Streptococcaceae* was limited. The repeated dominance of *Propionibacteriaceae* in samples from one producer may indicate a specific technological approach.

Cultivation offers sensitive detection and quantification of key indicator and hygiene-relevant groups, enabling direct assessment of potential safety concerns. In contrast, NGS reveals the broader community structure, captures non-culturable or slow-growing taxa and highlights manufacturer- and batch-specific microbial signatures. The two approaches address different analytical goals and their outputs are not directly comparable; instead, they provide complementary perspectives on microbial behaviour.

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S7-FT4: Food Safety Assessment of Last Mile Delivered Ready-to-Eat Multi-Ingredient Salad Bowls

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Last mile food delivery from restaurants is becoming increasingly popular, driven by rapid urbanization, innovation in e-commerce, and changing consumer behaviour. The contribution of last mile practices to the emergence of microbiological hazards or the occurrence of foodborne illness is unknown. This research explores the last mile concept from a food safety perspective, focusing on doorstep delivered, ready-to-eat (RTE) multi-ingredient salad bowls, provided through third-party e-commerce platforms.

A survey of RTE multi-ingredient salad bowls, ordered online and delivered to customers via last mile delivery, was conducted in three European cities (Ghent, Belgium; Tulln, Austria; Burgos, Spain) to assess delivery practices and their microbiological quality, hygiene, and safety. Samples were analysed for foodborne pathogens (presumptive *Bacillus cereus*, *Listeria monocytogenes* and *Salmonella* spp.), a hygiene indicator (generic *E. coli*), and general parameters (mesophilic aerobic plate count and *Enterobacteriaceae*). Challenge tests examined the growth potential of *L. monocytogenes* and *B. cereus* during simulated delivery and prolonged storage (up to 24 h) after delivery, including scenarios of temperature abuse (9 or 22°C) at the consumer level.

The prevalence of generic *E. coli*, *Salmonella* spp. and *B. cereus* was similar in last mile delivered salad bowls intended for direct consumption and those present at retail (data from literature review), although *L. monocytogenes* occasionally showed higher prevalence in retail products. Maintaining the cold chain is essential to prevent microbiological proliferation, although short urban delivery times (avg. 35 min) limit microbiological growth, also when foods are kept in the 'temperature danger zone' (5–60°C). Challenge test results align with the two-hour/four-hour rule, which states that food kept in the temperature danger zone *i*) for less than two hours can be consumed or be refrigerated for later consumption; *ii*) for more than two and up to four hours can still be consumed but not be refrigerated again; and *iii*) for more than four hours, must be discarded, to prevent foodborne illness. During delivery, adherence to good practices, including hygiene of delivery personnel, preservation of packaging integrity, and prevention of cross-contamination, remains critical. The development of tailored prerequisite programs and clear guidance documents for delivery personnel in the last mile distribution sector is recommended, alongside efforts to raise consumer awareness of safe food handling practices at home.

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S7-FT5: Beyond Antibiotics: The Impact of Reduced Use and Alternative Interventions on Animal Welfare in Global Livestock Production

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The global rise of antimicrobial resistance (AMR) poses a major One Health challenge, threatening animals, humans, and the environment alike. The use of antimicrobials is considered a key driver of AMR, particularly in high-density settings such as livestock production. Although various strategies to reduce antibiotic use and implement non-antibiotic interventions in food-producing livestock have been proposed, the available evidence regarding their effects on animal welfare remains fragmented and insufficiently synthesized.

To comprehensively map the scope and nature of available literature on reduced antibiotic use and non-antibiotic interventions in livestock production, focusing on how animal welfare is affected and how its outcomes are reported, using a scoping review approach.

This study followed the Joanna Briggs Institute (JBI) methodology for scoping reviews. A systematic literature search was conducted in three databases: MEDLINE, Scopus, and Web of Science Core Collection. In addition, relevant publications from regulatory authorities were considered. Literature published from January 2015 onwards was included.

The search strategies for each database were developed and rigorously validated in close collaboration with professional librarians, in accordance with the PRESS checklist. Screening was performed in two stages: (*i*) title and abstract screening, and (*ii*) full-text screening. All records were independently assessed by two reviewers according to the predefined Population-Concept-Context (PCC) framework.

Study selection and data extraction was managed using DistillerSR, supported by piloted data extraction forms. The extracted data was analyzed descriptively, and a thematic analysis was conducted to organize the findings into key themes and sub-themes. The results were reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses: Extension for Scoping Reviews (PRISMA-ScR) checklist.

This study provides a comprehensive overview of the existing literature on the effects of reduced antibiotic use and the implementation of non-antibiotic interventions in food-producing livestock on animal welfare. By mapping the breadth and characteristics of the available evidence, it identifies key knowledge gaps and supports researchers, veterinarians, and policymakers in designing and implementing sustainable, evidence-informed strategies to mitigate AMR while safeguarding animal health and welfare within a One Health framework.

S8 Microbiological Risk Assessment: Tools and Data Integration

S8-KN1: JEMRA and FAO work on microbiological risk assessment

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Since the 2024 FoodMicro, the Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) and FAO have continued their work on microbiologically-related food safety issues, which includes

- FAO expert meeting on toxigenic clostridia in foodborne disease: characterization, exposure, burden, control and detection
- FAO workshop on the safety and quality of water used in fisheries
- FAO expert meeting on microbiological risk assessment of parasites in foods, part 1 protozoa
- FAO Expert Meeting on microbiological risk assessment of parasites in foods, Part 2 helminths
- FAO workshop on antimicrobial co-selection at the food: environmental interface
- JEMRA expert meeting on the use of omics-based technologies in microbiological risk assessment
- JEMRA expert meeting on infant formula

This presentation will provide an in-depth look at the application, experiences, and ongoing developments of microbiological risk assessment (MRA) at the international level, with a particular focus on the unique needs and challenges faced by low- and middle-income countries. It will also cover the emerging global issues that are shaping the future of food safety, as well as the FAO's plans and initiatives to address these challenges. It will emphasize how science and evidence-based approaches can be leveraged to inform decision-making, strengthen agrifood safety systems, and promote the harmonization of international food trade practices.

From the FAO's perspective, there is significant value in collecting and harmonizing information, frameworks, and tools related to international microbiological risk assessment (MRA). This harmonization allows for a more comprehensive understanding of food safety risks on a global scale, identifying similarities and commonalities between regions and countries. It also provides a critical platform for addressing global food safety issues that transcend national borders, helping to develop cohesive solutions for shared concerns. Furthermore, international MRA offers the opportunity to assess the availability and quality of data on a global scale, pinpointing areas where knowledge gaps exist and where further research or data collection is needed.

By fostering collaboration, aligning global standards, and ensuring that the scientific basis for food safety decisions is robust and universally applicable, MRA can play a key role in enhancing public health protection and facilitating safer, more equitable international food trade.

S8-RT1: From Publication to Practice: How AI Unlocks Food Safety Models for Everyone

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Mathematical models play an increasingly important role in food safety – from predicting microbial behaviour to supporting risk assessments that inform regulatory decisions. Yet a recurring problem limits the impact of published models: they are usually made available in scientific publications in a non-standardized and sometimes incomplete format, which hinders efficient reuse by others. Extracting model parameters, equations, software code and metadata from a publication is notoriously error-prone and labour-intensive. As a result, valuable scientific knowledge often remains locked in journal articles rather than being applied in practice.

The RAKIP Initiative – a collaboration founded by ANSES, BfR, and DTU Food with currently 14 member organisations – has developed a set of solutions to overcome this problem. At the centre stands the FAIR Scientific Knowledge Exchange (FSKX) format, which allows storing predictive and risk assessment models in a standardized way. It packages model code with the metadata necessary to understand and properly apply the model within its range of applicability. Models stored in the FSKX format can easily be shared, e.g., via email, and model repositories can be established that allow sharing, searching and cloud-based execution of models with user-defined simulation scenarios. A reference implementation of such a model repository is the RAKIP Web Portal (https://foodrisklabs.bfr.bund.de/rakip-initiative/).

This talk presents the latest developments to support the efficient conversion of predictive models from publications into the harmonized exchange format FSKX. By customizing frontier AI models, a service could be established that converts a publication into a complete, properly structured FSKX file — including all relevant model code, metadata and documentation. We show that what previously required hours and days of manual work can now be accomplished in minutes.

A crucial element in this AI-driven process is an automated approach for quality control: the AI's implementation of a model is systematically tested by comparing its outputs – numerically or visually – against the results published in the original paper. This gives users and model providers a transparent, reproducible way to verify that the AI-generated FSKX file correctly represents the model described in the paper.

Together, these developments make it substantially easier for scientists, risk assessors, and regulators to share, find, and apply food safety models – regardless of their programming background. This talk will illustrate the workflow with concrete examples and discuss how AI technologies will contribute to the broader goals of transparency, reproducibility, and efficiency in food safety science.

S8-RT2: Assessing Climate-Driven Risk of *Salmonella* Contamination in Poultry Production Using Machine Learning: A Case Study in France

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Climate change is increasingly recognized as a systemic driver of food safety risks, with disproportionate impacts on public health and food systems. *Salmonella* contamination in poultry remains a major concern in Europe, as poultry meat is widely consumed and salmonellosis is the first most frequently identified foodborne zoonosis in Europe in 2024. Although a seasonal effect on salmonellosis has been demonstrated in Western Europe, it has not yet been clearly established which climatic variables could explain this phenomenon. Similarly, risk assessments targeting *Salmonella* rarely incorporate climate variability in an operational and anticipatory manner.

This study proposes a data-driven risk analysis framework that links climatic conditions to *Salmonella* contamination at the food production stage, with the objective of supporting early warning and risk-based surveillance.

Using data from the French national poultry surveillance program, we analyzed 3,784 samples collected in 2018 and 2020 across 18 departments. These data were combined with twelve derived meteorological variables capturing temperature, precipitation, humidity, and their delayed effects. Several machine learning models were developed and compared to predict *Salmonella* occurrence, addressing class imbalance and temporal dependencies. Emphasis was placed on model transparency and interpretability, enabling a clearer understanding of climate-related risk drivers rather than solely predictive performance.

XGBoost demonstrated the highest predictive performance (AUC up to 0.79) and the best ability to identify contamination events. A comparative lag analysis revealed that climatic conditions occurring four weeks prior to sampling were most informative for risk prediction. Results indicate that warm and moderately humid conditions increase contamination risk, whereas frequent rainfall has a protective effect, likely through dilution and environmental washout mechanisms. These findings highlight the nonlinear and delayed nature of climate–pathogen interactions.

By shifting the focus from retrospective analysis to anticipatory risk modeling, this work contributes to advancing risk science at the interface of climate change, food safety, and public health. The proposed framework supports more equitable and proactive risk governance by enabling targeted surveillance and timely interventions, particularly in regions and production systems most vulnerable to climatic variability.

S8-RT3: Quantitative Risk Assessment of *Bacillus cereus* and Cereulide Production in Powdered Infant Formulas

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Bacillus cereus is a spore-forming, toxigenic bacteria which produces cereulide, a heat-resistant and acid-stable toxin that has become a concern to the food industry. Regarding the recent outcomes involving infant formulas, this study aimed to develop a risk assessment model to estimate *B. cereus* concentration and cereulide presence in infant formulas. The model simulates the production of milk-based infant formula using the dry-mix process, applying a stochastic approach to assess *B. cereus* growth and toxin production, and associated risks under four household scenarios (SC1: immediate consumption; SC2: heating at 98°C for 5 min; SC3: microwave heating at 90°C for 30 s; SC4: refrigeration for 24 h), accounting for variability in time–temperature conditions across production stages and household reconstitution scenarios. Monte Carlo simulations estimated contamination levels in infant (PIFI) and toddler (PIFT) formulas, with Risk thresholds of 5 log CFU/g (powder) and 5 log CFU/mL (reconstituted) for *B. cereus* and 0.054 ng/mL (PIFI) and 0.1 ng/mL (PIFT) for cereulide, according to the European Food Safety Authority (EFSA). The probability of *B. cereus* concentrations exceeding 5 log CFU/g in infant formula at the end of the production process was estimated at 0.12, decreasing to 0.09 under the simulated consumption scenarios. Along the processing chain, the highest predicted cell concentrations were observed during the production of the dairy base in the feed tanks before concentration, reaching 9.62 log CFU/mL (P5: 7.54; P95: 9.74). During the dry-mix stage, the estimated concentration decreased to 3.36 log CFU/g (P5: 1.04; P95: 7.86). For the simulated consumption scenarios, the predicted concentrations were: SC1 = 2.46 log CFU/g (P5: 0.97; P95: 5.51), SC2 = 2.44 log CFU/g (P5: 0.90; P95: 5.20), SC3 = 2.42 log CFU/g (P5: 0.90; P95: 5.04), and SC4 = 1.77 log CFU/g (P5: −0.37; P95: 5.57). Regarding cereulide levels, the estimated concentration throughout most of the process was 0.01 ng/mL. However, during the concentration step of the dairy base, levels increased to 0.0734 ng/mL (P5: 0.04; P95: 135,00). remaining stable until the drying stage. In the final dry-mix stage, cereulide concentrations reached 1.65 ng/mL (P5: 0.04; P95: 138.18). Under simulated household scenarios, the mean concentrations were similar for PIFT (mean: 1.65 ng/mL; P5: 0; P95: 17.49) and PIFI (mean: 1.59 ng/mL; P5: 0; P95: 18.09), with no significant effect of preparation conditions. Overall, the probability of exceeding unsafe cereulide levels was estimated at 0.09, regardless of preparation scenario or formula type.

S8-RT4: European Preparedness for Changes in *Listeria monocytogenes* Regulations

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Recent years have seen a significant evolution in the European regulatory framework for *Listeria monocytogenes*, driven by persistent public health concerns and an increasing incidence of listeriosis across the EU, one of the most severe foodborne diseases in terms of hospitalization and mortality.

Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs has been revised through Commission Regulation (EU) 2024/2895, extending the application of "not detected in 25 g" criterion to foods where food business operators (FBOs) cannot demonstrate that the level of *L. monocytogenes* will not exceed the limit of 100 CFU/g throughout shelf-life.

To support FBOs in understanding *L. monocytogenes* growth in the ready-to-eat (RTE) foods they produce and setting a safe shelf-life, the European Commission mandated the EURL for *L. monocytogenes* (EURL *Lm*) with leading a working group to revise the draft "Guidance Document on *Listeria monocytogenes* monitoring and shelf-life studies for ready-to-eat foods". Following review by DG SANTE and Member States, the updated version of the guidance document was published last December.

The new guidance document provides practical recommendations for classifying RTE foods; selecting which *L. monocytogenes* food safety criterion applies; decide on when and which shelf-life studies are needed; demonstrate that food products will comply with the *L. monocytogenes* criteria until the end of the shelf-life, and how to validate, verify and document that such shelf-life studies are adequate to respect the applicable *L. monocytogenes* food safety criterion.

Together with the EURL *Lm* guidance documents, the EU now offers a comprehensive set of guidelines designed for different stakeholders, including laboratories, FBOs, and competent authorities. Complementing these regulatory updates, Commission Implementing Regulation (EU) 2025/179 introduces mandatory WGS requirements for isolates associated with foodborne outbreaks, including *L. monocytogenes*, requiring prompt data submission to EFSA and EN ISO 17025 accreditation for reporting laboratories. In this context, an inter-EURL working group on NGS has been supporting EU laboratories in implementing these requirements through training programs and several guidelines to strengthen capacity building.

These coordinated developments reflect a shift toward a proactive, integrated, and data-driven approach to *L. monocytogenes* control in Europe. Our work directly addresses the operational and analytical challenge posed by these new requirements, providing technical guidance and training to ensure compliance and protect consumers. Presenting those activities allows for discussion, exchange, and dissemination of best practices to stakeholders across Europe, accelerating the adoption of harmonized, science-based approaches that reduce the burden of listeriosis.

S8-RT5: Risk Ranking of Microbiological Hazards in Food in Ireland

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Microbiological food safety risks arise from a wide range of pathogens that can contaminate foods at various points across the food chain. Exposure to these hazards can result in illnesses that vary widely in severity and duration, depending on factors such as the specific pathogen involved, the amount of the pathogen ingested, and the susceptibility of the individuals who become exposed. Risk ranking is a key component of risk-based food safety management, enabling prioritisation of microbiological hazards based on their relative public health impact and guiding more targeted and effective risk mitigation efforts.

The Food Safety Authority of Ireland requested its Scientific Committee to: (*i*) undertake the first national risk ranking exercise of major microbiological hazards transmitted through food in Ireland, and (*ii*) identify data gaps that contribute to uncertainty within the evaluation.

Seven hazards were selected using established inclusion and exclusion criteria, such as nationally reported disease incidence and clinical severity. The hazards assessed were *Campylobacter* spp., *Listeria monocytogenes*, *Salmonella* spp., Shiga toxin-producing *Escherichia coli*, norovirus, hepatitis A virus and hepatitis E virus. For each hazard, the burden of disease was calculated in disability adjusted life years (DALYs) using nationally reported disease incidence data from 2015 to 2019 obtained from the Health Protection Surveillance Centre of the Health Service Executive. To address probable underestimation in the surveillance data, the reported incidence figures were adjusted using multiplication factors provided by an expert panel. Disease burden estimates for each hazard were generated through disease model simulations, using either the European Centre for Disease Prevention and Control's BCoDE toolkit or bespoke models built in R software. To estimate the proportion of each disease burden attributable specifically to foodborne transmission, an expert elicitation was conducted using a two-round Delphi method with experts from relevant disciplines. Mean annual foodborne DALYs and associated 95% uncertainty intervals were quantified and used to generate a ranked list of hazards. The ranking highlighted foodborne pathogens of greatest public health concern in Ireland due to high incidence (e.g., norovirus), severe outcomes (e.g., *L. monocytogenes*), or both (e.g., *Campylobacter* spp.).

The work also revealed substantial data gaps which can affect robustness, such as the absence of Ireland specific underestimation values, limited national source attribution data, and reliance on model parameters derived from studies conducted in other countries. Addressing these gaps will strengthen future risk ranking exercises and support evidence-based prioritisation within Ireland's food safety system.

S8-KN2: Using Predictive Models to Stay Ahead of Risk

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Predictive models have become indispensable tools for anticipating and mitigating food-safety risks across increasingly complex “farm-to-fork” systems. As food supply chains expand, diversify and operate under dynamic environmental conditions, reactive approaches are not sufficient to prevent contamination events or emerging hazards. Predictive microbiology, quantitative risk assessment and newer AI-driven modelling technologies can be strategically used to stay ahead of risk, provided that users understand their assumptions, limitations and integration requirements within real world food systems. A number of outbreak case studies highlight several recurring challenges, including failures in adoption and knowledge transfer. Other cases reflect incorrect or overly narrow modelling assumptions, highlighting that effectiveness requires alignment with real world operational environments and recognition of system complexity. Conversely, several success stories underscore the transformative potential of predictive models in a risk assessment context when appropriately implemented. National risk-assessment strategies, bespoke modelling tools integrated into HACCP systems and recent advances in AI-enhanced frameworks demonstrate how predictive models can guide evidence-based decision-making, reducing foodborne illness and minimising costly product recalls. In parallel, structured education is required to enhance user competency and support wider adoption across industry and regulatory sectors. Staying ahead of risk requires not only advanced predictive tools but also an understanding of their assumptions, active integration into decision systems and continuous refinement to reflect emerging data, diverse food matrices and evolving supply-chain environments. When these elements are aligned, predictive models provide a powerful, proactive foundation for modern food-safety risk assessment.

S8-FT1: Predictive Modelling to Assess Growth Risk of Mastitis-Derived Enterotoxigenic *Staphylococcus aureus* in Milk

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Bovine mastitis represents an important source of *Staphylococcus aureus* in the dairy supply chain, a pathogen capable of causing food poisoning through the production of heat-stable enterotoxins. Temperature abuse during milk handling and storage may permit rapid growth, increasing the likelihood of enterotoxin production once concentrations approach or exceed 5 $\log_{10}$ CFU/mL. Although predictive growth models for *S. aureus* in milk exist, none have been developed using genomically characterised, mastitis-derived isolates with confirmed enterotoxigenic potential.

To address this gap, two *S. aureus* isolates, SA_587 (ST1074) and SA_901 (ST124), were selected from a collection of 20 strains recovered from raw milk of cows with bovine mastitis. Selection prioritised isolates representing the two predominant sequence types in the collection, with enterotoxin production confirmed by two independent immunoassays (SET-RPLA and RIDASCREEN® SET Total). Enterotoxigenic potential was further supported by toxin gene profiling using whole genome sequencing. Growth kinetics were determined in reconstituted skimmed milk (10% w/v) at 12, 15, 18, 20, 25, 30 and 37°C using the Baranyi-Roberts model, while the temperature dependence of maximum specific growth rate was described using the Ratkowsky square-root model.

All primary growth models showed good fit (RMSE < 0.33). For both isolates, maximum specific growth rate increased from 0.037–0.039 h^{-1} at 12°C to 0.562–0.676 h^{-1} at 37°C, while lag phase duration decreased from 75.5 h (SA_587) and 60.9 h (SA_901) at 12°C to < 2 h at 37°C. The two isolates showed similar responses at different tested temperatures. The 5 $\log_{10}$ CFU/mL threshold associated with enterotoxin risk was reached within approximately 66 h at 15°C and 120–144 h at 12°C, indicating that even mild temperature abuse may be sufficient for *S. aureus* to reach concentrations associated with enterotoxin production. At temperatures ≥ 20°C, this threshold was reached within 48 h. Ratkowsky modelling yielded theoretical minimum growth temperatures of 7.1 ± 1.2°C and 7.3 ± 1.0°C for SA_587 and SA_901, respectively (RMSE: 0.24 and 0.28).

This study presents the first predictive growth model in milk parameterised using *S. aureus* of bovine mastitis origin. The results provide a quantitative basis for establishing temperature control targets during on-farm storage and cold chain management. The kinetic parameters generated will support dynamic modelling and toxin risk assessment under fluctuating temperature conditions relevant to milk storage, handling, and temperature-abuse scenarios.

S8-FT2: Mechanistic Individual-Based Modeling of Spatiotemporal Bacterial Dynamics: Integrating Fickian Diffusion and Cellular Automata in Structured Food Matrices

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Traditional predictive models in food microbiology largely rely on data from homogenous liquid cultures. However, these models frequently diverge from empirical observations in real food systems, where solid-like matrices impose physical constraints that limit the mobility of nutrients and bacteria. These constraints create spatially heterogeneous microenvironment that are fundamental to microbial behavior but remain largely absent from current predictive frameworks. This study developed a novel Individual-Based Modeling (IBM) framework using a cellular automaton (CA) to explicitly account for environmental heterogeneity.

The framework was implemented in Python as a discrete grid-based computational model. Each microbial cell was assigned to a single 1 μm² grid cell. The simulation integrated two mechanistic modules: bacterial growth dynamics, incorporating metabolism and division at the single-cell level, and numerical modeling of compound diffusion based on Fick's second law. To manage computational complexity, the 40,000 × 40,000 grid (representing a 16 cm² physical area) utilized coarse-grained units of 100 × 100 cells for diffusion calculations. Simulations were performed at a 1-min temporal resolution for 3000 iterations, capturing 50 h of emergent growth dynamics. Bacterial population size and spatial distribution were recorded every 60 iterations. To complement the simulations, the growth kinetics of *Listeria monocytogenes* ATCC 19111 were investigated at 20°C on MRS (de Man, Rogosa and Sharpe) agar under both monoculture and co-culture with *Latilactobacillus sakei* NBRC 15893 for comparison with simulation results.

The results demonstrated that the integration of diffusion mechanisms successfully simulated the complexities of environmental heterogeneity. The model accurately captured emergent population dynamics across all characteristic microbial growth phases, including the lag phase, exponential growth, and the transition to the stationary phase. Temporal changes in bacterial population size were reproduced with a root mean square error (RMSE) of 0.208 compared to experimental data. Additionally, the simulation elucidated that environmental heterogeneity significantly influenced growth dynamics during inter-species competition, a finding validated by corresponding experimental growth data. Furthermore, the framework enabled the high-resolution visualization of bacterial colony expansion and spatial distribution through sequential imaging—a distinctive capability missing from conventional population-level models.

This framework provides a robust "Digital Twin" approach for predicting microbial behavior in structured food systems, offering a sophisticated tool for more realistic food safety assessments.

S8-FT3: Exploring Heat Resistance Determinants Across the *Salmonella* Senftenberg Pangenome: A Pipeline Optimization for Functional Annotation Enrichment

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Salmonella is a well-known food-borne pathogen that causes hundreds of outbreaks yearly all over the world. Even though food safety protocols have been improved during the last twenty years, the easy access to genomic data and the constant improvement of bioinformatic tools can make a difference in the design of novel food safety protocols. Pangenome analysis allow the study of the complete set of genes present in a taxon and it can be used for bringing new gene groups associations to light, tracking horizontal gene transfer or assessing the selective pressure in specific groups of genes.

Our aim is to apply these powerful tools to improve the annotation and characterise the evolution and distribution of the genetic determinants responsible of food safety stresses resistance.

As a case of study, the increased heat resistance of the strain *Salmonella* Senftenberg 775W has been related with two "transmissible loci for protein quality control" (TLQPC). Highly similar regions were previously reported in different genera but any other *Salmonella* strain.

We have stablished a novel pangenome for *S.* Senftenberg using a curated collection of complete genome sequences. The genomes were filtered, pre-processed, and annotated using three complementary annotation strategies to maximize gene discovery and functional accuracy. This approach significantly expanded the annotation of genes present across the analysed strains. This analysis revealed novel information related to the TLQPC gene set, as well as previously unreported orthologs associated with heat-stress resistance. The distribution of these genes among phylogenetically distant strains suggests a potential role for horizontal gene transfer in the adaptation of *S.* Senftenberg to harsh thermal conditions. Additionally, two different evolutive lineages have been clearly identified in the taxon based on their genomic profile.

Overall, these novel results contribute to a better understanding of the serovar evolution dynamics. This work provides new genomic insights into the unique stress tolerance of some strains of *S.* Senftenberg and highlights genetic features that may contribute to its persistence in food-related environments, reinforcing its relevance as a foodborne pathogen. From our perspective, the integration of bioinformatic tools in food science will certainly impact positively in the development of food safety protocols and microbiological risk assessments.

S8-FT4: Quantitative Microbial Exposure Assessment of Uropathogenic *Escherichia coli* in Ready-to-Eat Beef: Implications for Risk-Based Interventions in Taiwan

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Uropathogenic *Escherichia coli* (UPEC) is increasingly recognized as an emerging foodborne pathogen associated with urinary tract infections, with meat products being considered a potential reservoir of UPEC. Ready-to-eat (RTE) beef, which is often consumed without additional heat treatment, may be susceptible to microbial contamination during retail. In this study, we conducted a quantitative microbial exposure assessment to evaluate consumer exposure to UPEC associated with the consumption of RTE beef retailed in Taiwan and identify feasible risk intervention strategies. In total, 50 RTE beef samples were purchased from various retail sites in Taiwan. Enumeration of *E. coli* was performed using culture-based methods, and UPEC isolates were identified by polymerase chain reaction targeting UPEC-specific genes (*c3509*, *c3686*, and *chuA*). These microbial sampling data were integrated into a retail-to-consumption exposure model incorporating UPEC prevalences, contamination levels, predictive growth kinetics for UPEC in RTE beef, time and temperature profiles during retail and post-purchase handling, and consumption data derived from a national dietary survey. Monte Carlo simulations with 10,000 iterations were conducted to estimate the probability of consumer exposure exceeding UPEC thresholds (10^5–10^8 CFU/serving). The detected prevalence of UPEC in RTE beef was 4.0% (2/50). Model simulations indicated that the probability of high-dose exposure (≥ 8 log CFU/serving) was low (approximately 0.9%–2.2%) across the evaluated scenarios. A sensitivity analysis revealed that the retail display duration and retail storage temperature were the most influential factors in exposure probabilities. An intervention scenario analysis revealed that reducing the retail display time (2, 4, or 6 h) or lowering the storage temperature (18 or 25°C) decreased predicted exposure, with combined time and temperature interventions showing the greatest reduction (98.8%). Similar risk reductions could be achieved through multiple intervention combinations, allowing flexibility based on retail feasibility. This study illustrates the first QMEA framework for assessing UPEC exposure from RTE beef in Taiwan, highlighting the importance of retail-stage handling practices in mitigating foodborne UPEC exposure.

S8-FT5: Ranking Microbial Risks in Plant-Based Meat Analogues: Insights From a Systematic Review

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Plant-based meat alternatives (PBMA) are emerging as sustainable solutions to replace meat products. The recent evolution of dietary habits, with an increase of 20.5% of sales in France (2022–2024) exposes consumers to emerging microbiological risks. Indeed, these products are primarily produced by extrusion, a thermomechanical process that is heterogeneous in terms of microbial inactivation. Recent outbreaks and recalls raised the need to secure this food production chain.

This work performed a systematic review of biological hazard following the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) method to identify all potential hazards of concern and collect exhaustive data on their prevalence and concentration. Then they were prioritized with a risk ranking approach, using a semi-quantitative decision tree combing probability of occurrence and potential severity to guide food safety actions and inform public health priorities.

A total of 4641 articles (after duplicates removals) were screened based on title, abstract and full text to lead to the selection of 26 articles. The geographical distribution of publications identified the United States, China, and Germany as the leading contributors. Marketed products were the most studied, dominated by hached products, followed by sausages. Soy and pea were the main protein sources. Physicochemical parameters, particularly high-water activity and near-neutral pH, indicated conditions favorable to microbial growth.

A total of 889 samples were analyzed with cultural method and 160 with genomic. The level of microorganisms is very variable and high with the total aerobic mesophilic flora ranging from 0.1 to 8.7 log CFU/g, and the spore forming bacteria ranging from 1.0 CFU/g to 4.10 CFU/g. In addition, *Staphylococcus aureus* was detected in 18 of 262 products (1.0–3.9 log CFU/g), and presumptive *Bacillus cereus* in 6 of 10 (1.1–3.1 log CFU/g). Yeasts and molds were present in all analyzed products (1.2–6.2 log CFU/g), and *Pseudomonas* spp. at 4.2 log CFU/g. Significant gaps remain, particularly regarding *Clostridium perfringens* and spores of *B. cereus*. The use of genomic method enabled to identify additional potential hazard of concern including *Clostridium* spp., *Cronobacter* spp. and *Staphylococcus* spp. These results confirm the presence of pathogenic and spoilage hazards and will provide a ranking to prioritize future actions.

To conclude, PBMAs offers opportunities of sustainable alternatives to meat but the use of novel product formulation and processes requires careful microbiological risk assessment to ensure consumer safety and support a strong development of this emerging market.

S9 Managing Food Safety, Stability and Security in the Context of Global Megatrends

S9-RT1: History, Current Projects and Future Activities of the ICMSF

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The International Commission on Microbiological Specifications for Foods (ICMSF) was formed in 1962 through the action of the International Committee on Food Microbiology and Hygiene (ICFMH), a committee of the International Union of Microbiological Societies (IUMS). The Mission of ICMSF is to be a leading source for independent and impartial scientific concepts that, when adopted by governmental agencies and industry, may help facilitate fair trade and reduce the incidences of microbiological food-borne illness and food spoilage. The Approach of the Commission is to provide basic scientific information through extensive study and makes recommendations without prejudice based on that information. Results of the studies are published as books, discussion documents or referred papers and presented in conferences, symposia, workshops, webinars and knowledge clips. The Commission has worked to improve the microbiological safety of food by focusing on aspects like microbiological methods, recommending sampling plans and microbiological criteria, and defining and promoting the use of Good Hygiene Practices (GHP) and Hazard Analysis and Critical Control Point (HACCP) systems and other systems of managing food safety, like Food Safety Objectives. Currently, ICMSF is developing advice and tools for managing food safety in conjunction with food security and sustainability, which in the essence examines the anticipated impacts of global “megatrends” on microbial food safety and food availability.

S9-RT2: Overview of the Upcoming ICMSF Book 9: Managing Food Safety and Stability in the Context of Global Megatrends

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Our global food system is in a period of transition, due to the relation of food security and sustainable food production regarding global megatrends such as global warming, population growth, geopolitical instability, resource constraints, rapid urbanisation, and technological disruption. These aspects need to be assessed in terms of their impact on food safety management approaches, assuring the challenge of feeding a projected ten billion people by 2050. Ensuring that food is not only available but also safe is fundamental to the health and wellbeing of people and societies. Since its establishment in 1962, the International Commission on Microbiological Specifications for Foods (ICMSF) has played a pivotal role in advancing the science and practical application of food safety risk management worldwide, bridging the gap between scientific understanding and practical implementation. Book 9 builds on this legacy by situating food safety within the broader context of global megatrends and food system resilience. It recognises that the future of safe food cannot be secured by technical measures alone. Instead, it requires integrated thinking that connects microbiological science with global economic, environmental, and social drivers. This volume explores how shifting demographics, climate impacts, water and energy scarcity, digitalisation, and evolving consumer expectations are reshaping risk profiles across the food chain. It examines how emerging technologies – from precision fermentation to data-driven surveillance – can both mitigate and create new types of risk. It also considers how governance structures, trade frameworks, and public trust interact to influence global food security.

S9-RT3: Role of Technology in Food Safety and Stability

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In order to sustain growth, the food industry is continually looking to innovate, leading to increased efficiency and productivity. Therefore, this presentation focuses on the main drivers leading to the development of new intervention and manufacturing/processing technologies. It will also explore their impact on food safety and spoilage. For intervention technologies, i.e. those that target reduction of microbiological contaminants from raw materials or coming from environments where these are handled, these will include non-thermal processes and will cover those that are commercially available and those in development (pre-industrial). Manufacturing/processing technologies are those that are not considered to be intervention technologies innovations but reflect advances in food processing that may have indirect consequences on food safety and spoilage. For these new technologies the likely impact on microbiological safety/quality of products, the mechanism of action, the range of microorganisms affected and their relative susceptibility, where these technologies are currently applied and pros/cons of the technologies, will be discussed.

S9-RT4: Big Data Impacting Dynamic Food Safety Risk Management in The Food Chain

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There is no shortage of food safety-related data within food business operations and across the wider food safety stakeholders. Recent years have seen the emergence of terms such as data lakes, data ecosystems and data trusts, all aimed at collating internal and/or external sources of food safety and quality data. When these large and diverse datasets are integrated, analysed and updated continuously, they create the basis for more dynamic food safety management – enabling organizations to anticipate/forecast emerging risks, adjust controls in near real time and move from reactive compliance toward proactive prevention. Examples include measuring meteorological data during agricultural production, using cold-chain temperature and humidity data to trigger corrective actions before spoilage/safety issues occur; combining production facility environmental monitoring, sanitation and pest control data to identify process drift before contamination risks increase; applying supplier, audit and testing data to dynamically adjust verification intensity; and integrating consumer complaint, social media, regulatory alert and traceability data to accelerate issue detection. The presentation will focus on various datasets and tools across the supply chain that can be used by the food business operator to help make real time adjustments and instigate mitigation measures to prevent food safety/quality issues.

S10 Different Perspectives on Challenging Topics of Food Microbiology in Changing World

S10-RT1: Perspectives from Africa: Out of Africa

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Whilst food safety remains a challenge in Africa, it has emerged as a strategic priority for the continent, underpinning public health, food security, nutrition, economic development, and international trade. The continent bears a disproportionate burden of foodborne disease, with millions of illnesses and thousands of deaths occurring annually, while the associated economic losses continue to constrain development. Rapid urbanisation, population growth, climate change, expanding informal food markets, and increasingly complex food supply chains are placing additional pressure on already fragmented food safety systems.

Despite these challenges, progress is being made across the continent. The adoption of the African Union Food Safety Strategy, increasing political recognition of food safety as a development priority with associated benefits, strengthening laboratory and surveillance capacity, and greater regional collaboration provide important opportunities to modernise food control systems.

This presentation will provide an overview of the current state of food safety in Africa, highlighting the disparity between the formal and informal sectors, the need to strengthen scientific capacity to encourage risk-based approaches and opportunities for strengthening national and regional food control systems.

As Africa continues to strengthen intra-African trade under the African Continental Free Trade Area (AfCFTA) and expands its participation in global food markets, robust food safety systems will become increasingly critical for protecting consumers, enhancing public confidence, improving market access, and driving sustainable economic growth.

S10-RT2: Perspective from Oceania: Application of One Health for Food Safety and Security in Oceania

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Oceania is a very diverse collection of island nations spanning the south and western regions of the Pacific Ocean. Oceania is further divided into four regions: Polynesia, Micronesia, Melanesia and Australasia. It comprises 14 independent nations including a USA state (Hawaii) and USA territories (Guam, American Samoa and Northern Mariana Islands). The most populous of these island nations are Australia, Papua New Guinea, New Zealand and Fiji. This diversity and the relative isolation of each island country, requires a somewhat different approach to One Health, in which biosecurity and minimisation of the ingress of diseases not endemic to those countries becomes as much of as concern as is management of potential zoonoses from domestic and native animal populations. Many of these island nations are currently free of zoonotic diseases that are endemic elsewhere which provides both human health protection and financial advantage in international trade in food.

Sources of introduction of 'new' microbial hazards into island nations include the importation of live animals, human travellers that have been to regions where other diseases are endemic, and the flight of birds and insects from regions where certain diseases that are established can bring those diseases into island nations in which they are not currently established.

In Australia One Health approaches are most overtly applied for control of domestic emergence and transfer of antimicrobial resistance in farmed animals (including fish) to humans or native animal populations. However, other disease incursions that could affect farmed animal populations are also actively monitored and managed because of the potential for food security threats.

For example, at the time of writing, migratory birds that are able to fly from Antarctic regions to continental Australia have been discovered as dead or dying in Australia's southern coastal regions. They have also been shown to be carrying the avian influenza H5N1 virus that is now apparent in populations of Antarctic wildlife and more recently Australian mammals (seals). Monitoring and management of that potential threat to Australian commercial bird populations and Australian native animals is currently a major biosecurity issue in Australia.

This presentation will review how the One Health concept is being applied in Oceania, both in individual nations, but also between nations, including through co-ordination by UN organizations. The review will be based on information that is publicly available, and from discussions with people actively involved in the application of One Health principles in major nations in Oceania.

S10-RT3: Perspectives from Latin America

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Latin America is known by its high potential for producing foods to support the worldwide demand. This macro-region is mostly placed in the tropical area of the globe, characterized by rich volcanic soils and large flat areas, favoring crops and animal production. Also, the Andean Mountain Range determines different climatic conditions, allowing the production of a variety of crops. As consequence, agriculture and livestock are some of the most important economic activities for Latin America countries. Based on the 2024 database of the Food and Agriculture Organization of the United Nations (FAO), Latin American countries are among the world main producers and exporters of soybeans, corn, beef and poultry (Brazil and Argentina), as well as fruits (Mexico and Chile), with a positive trade balance of US$217,7 billion in 2023. Considering the relevance of these food commodities for the economy of Latin America, a natural movement by the producing countries is their active participation and discussion on agreements to allow the international trade of such foods, including their safety and quality requirements for trade and consumption. This global trade reality leads to discussions in agriculture and health bodies of Latin American countries, to harmonize the safety and quality parameters of the produced and exported foods. Many challenges and trends are relevant in those discussions, guided by holistic approaches like One Health and Food Security. Based on the producer perspective, the control of foodborne pathogens and zoonotic agents is a continuous concern, especially with the increasing demand to reduce and/or eliminate the use of antimicrobials and agrochemicals. Inspection and regulatory bodies must harmonize guidelines and safety parameters for international trade, and adopt tracking procedures to identify the sources of contamination and to investigate foodborne outbreaks. Finally, consumers must be aware of the potential hazards linked to foods, even more with the increasing demand for organic and artisanal foods, and be educated on safe food handling. Sustainability is a key factor across all those challenges, and it is imperative to produce sufficient and safe food considering the need to preserve the environment. In this lecture, those points will be connected, facilitating the understanding of the current scenario Latin America is facing to maintain its position as food producer and exporter.

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S10-RT4: Perspectives from Europe: European Food Safety in the 21st Century: What Was Done Well and What Could Be Done Better?

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The 21st century has witnessed a profound transformation of European food safety (EFS), resulting in one of the world's most advanced systems for consumer protection. Triggered by major food crises, including bovine spongiform encephalopathy (BSE) and dioxin contamination, and supported by EU enlargement, European food safety evolved from a reactive approach toward the preventive farm-to-fork concept. This transition was enabled by the establishment of the European Food Safety Authority (EFSA), harmonized legislation, mandatory traceability, and the Rapid Alert System for Food and Feed (RASFF). In parallel, advances in molecular diagnostics, whole-genome sequencing, analytical chemistry, and digital surveillance have substantially strengthened hazard detection and evidence-based risk assessment.

Despite these achievements, harmonized legislation has not always translated into harmonized implementation. Differences in regulatory capacity, laboratory infrastructure, investment, digitalization, and food safety culture continue to generate uneven levels of protection across Member States. Moreover, the current system remains predominantly compliance-driven, whereas emerging risks increasingly require adaptive, predictive, and resilience-oriented approaches. Food safety should therefore be viewed not only as regulatory compliance but also as a socio-technical system in which organizational culture, professional competence, transparent communication, and responsible human behaviour are essential determinants of effective risk management.

The widespread implementation of Hazard Analysis and Critical Control Point (HACCP) principles and voluntary private standards has reduced many traditional foodborne risks and strengthened consumer confidence. However, these tools were primarily developed for relatively stable production systems and are increasingly challenged by globalized supply chains, climate change, antimicrobial resistance, food fraud, novel foods, and the revalorization of traditional foods. At the same time, fragmented digital infrastructures, limited interoperability of surveillance data, and inconsistent enforcement may reduce the responsiveness and resilience of the European food safety system.

Future progress requires more than incremental technological improvements. Artificial intelligence, whole-genome sequencing, blockchain-based traceability, and integrated data platforms should be combined with stronger investment in human expertise, interdisciplinary collaboration, and institutional resilience. A future European food safety system should become predictive rather than reactive, integrating real-time data, systems thinking, and food safety culture into decision-making at every level. Sustainable food safety will ultimately depend not only on scientific excellence and technological innovation but also on governance capable of anticipating emerging biological, environmental, technological, and societal risks rather than merely responding to them.

S10-RT5: Ranking Consumer Practices Related to Climate Change from a Microbiological Risk Perspective

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Climate change is driving shifts in human dietary habits. On the one hand, many practices are emerging as a result of consumer choices in response to environmental challenges. On the other hand, climate-related hazards are forcing populations to adapt by adopting various practices that are imposed by climatic conditions. This heterogeneity of practices poses a challenge for health authorities but also for consumers, raising a crucial scientific question: How can we identify, among a wide variety of new and diverse behaviors, those that require special attention? The objective of this study is to provide a method for prioritizing food practices based on their microbiological risks from a population-based perspective to support public decision-making.

To develop this method, a scoping review was conducted in Scopus, PubMed, and the grey literature. Articles were initially selected based on their titles and abstracts, and a second screening was performed after reading the full texts. The framework published by the EFSA BIOHAZ Panel in 2012 was used to analyze articles presenting methods for ranking microbiological hazards. Based on this, a semi-quantitative method was developed considering:

1. The prevalence of the practice, as measured by the number of people involved and the frequency of practice,
2. The number of food items consumed as part of this practice,
3. The number of microbiological hazards and their respective risk, assessed via *i*) severity expressed in DALYs and *ii*) occurrence derived from a probabilistic decision tree incorporating the physicochemical characteristics of foods, consumer behaviors and potential growth of pathogens.

This method enabled the prioritization of about 30 food practices related to climate change. Each practice included up to 72 food categories according the EFSA's FoodEx2 catalog and up to 11 bacteriological hazards according to our analysis. The three variables practice prevalence, food items and microbiological risks, were computed and standardized to compare heterogeneous practices on a single scale. The method clearly distinguishes high risk practices such as cooking without running water during a flood or vegetarianism from practices presenting a more moderate risk, such as consumption of insects.

This method is intended to become a decision-making tool for public authorities and consumers. Until now, developed and validated for bacterial hazards, it will be expanded to viruses and parasites, and ultimately implemented into a user-friendly interface that facilitates decision-making and communication.

S10-RT6: Consumers' Perceived Risk and Worry About Microbial Food Safety

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While addressing consumers' perceived risk and worry about foodborne pathogens is essential for effective risk management and communication, the determinants underlying these cognitive and affective dimensions of consumer concerns remain poorly understood.

A comprehensive literature review and formal group discussions informed the identification of 50 potential determinants and the development of a questionnaire measuring these determinants. The questionnaire also assessed the intensity and interrelationship of consumers' perceived risk and worry about foodborne pathogens. As a case study, the instrument was administered online among 420 Dutch-speaking adults in Flanders.

The case study revealed high levels of perceived risk and worry (median = 6 on a 7-point Likert scale). Penalized multivariate variable selection followed by generalized ordinal logistic regression indicated that perceived risk was primarily associated with the importance consumers attach to the absence of foodborne pathogens and remembrance of foodborne outbreaks and recalls. In contrast, worry was primarily associated with the importance consumers attach to food healthiness, safety, and sustainability; distrusting the safety of unfamiliar foods; perceived dread regarding the health consequences of food poisoning; and zero-risk beliefs.

A moderate positive correlation ($r = 0.42$, $p < .001$) and differences in generalized ordinal logistic regression determinants indicated that perceived risk and worry represent positively related yet distinct dimensions of consumer concerns about foodborne pathogens.

Finally, hierarchical cluster analysis — based on perceived risk and worry — revealed three consumer segments which differed mainly in value orientations and food safety beliefs, rather than in sociodemographics, health status, or involvement with food purchase and preparation. Nevertheless, opportunities for more tailored risk communication were revealed, with highly concerned consumers requiring contextualisation of microbial risks and reassurance to mitigate disproportionate worry, while moderately concerned consumers would benefit from practical, action-oriented guidance to strengthen hygienic practices.

The questionnaire developed in this study, together with the analytical methods applied, offers a framework that food safety experts can replicate within their own cultural and demographic settings. Applying this framework and building on the results obtained may enable these experts to design more effective strategies for addressing consumers' perceived risk and worry — as well as the underlying factors shaping them — through improved risk management and tailored, two-way risk communication. In the long term, this approach could foster greater consumer trust in the food chain, reduce consumers' reliance on unreliable information sources, and mitigate risky behaviours such as unsafe food handling practices.

S10-RT7: Transforming Food Systems Through a One Health Approach: A Systematic Review of integrated Solutions for Health, Sustainability and Resilience

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Global food systems are central to major 21st-century challenges, including climate change, biodiversity loss, antimicrobial resistance (AMR), zoonotic diseases, and diet-related non-communicable diseases. These interconnected issues highlight the need for systemic approaches that simultaneously address human, animal, and environmental health. In this context, the One Health framework offers a comprehensive paradigm for transforming food systems.

This systematic review, conducted following PRISMA guidelines, aimed to identify and synthesize evidence on strategies for food system transformation within a One Health perspective. A total of 7,001 articles published between 2010 and October 2025 were retrieved from four databases. After screening and eligibility assessment, 264 studies were included for analysis.

The results reveal that research is dominated by three main themes: sustainable diets (25.2%), antimicrobial resistance (24.39%), and zoonoses (18.26%), while climate change and agriculture remain comparatively underexplored. Current food systems are characterized by strong interdependencies across sectors but are still predominantly addressed through human-centered approaches.

Across themes, several key challenges were identified. These include the overuse of antimicrobials in agriculture driving AMR emergence and spread across the food chain, persistent zoonotic risks linked to food production and consumption practices, and the high environmental and health burden of current dietary patterns. In addition, structural barriers such as fragmented surveillance systems, limited cross-sectoral coordination, and socio-economic constraints hinder effective implementation of integrated strategies.

The review highlights a wide range of solutions, including the development of antimicrobial alternatives, integrated surveillance systems, climate-smart agriculture, dietary transitions toward plant-based patterns, and strengthened governance frameworks. However, no single intervention is sufficient. Effective transformation requires coordinated, multi-sectoral action combining technological, behavioral, and policy-based approaches.

Overall, this review demonstrates that transitioning toward sustainable and resilient food systems necessitates a fully integrated One Health strategy. Bridging disciplinary silos and reinforcing collaboration across sectors are essential to address complex global challenges and ensure long-term health and sustainability.

Plenary Session

PL-4: Defining the Impact and Path Forward - Where Everything Comes Together

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FoodMicro2026 has brought together new science, established knowledge, discussion, debate and the countless exchanges that happen beyond the lecture hall. Together, they remind us that in food microbiology, some facts do not age well, while some safe harbours remain.
Our responsibility is both to seek out and acknowledge the facts that no longer hold, to test and challenge them, and to establish where we stand today; and, at the same time, to recognise, preserve and pass on the knowledge that has stood the test of time. Both create a continuing need for research and education—for discovering what we do not yet know and ensuring that valuable knowledge is not lost as generations change.
Looking back at what we have heard and learned in Ljubljana, this closing perspective will reflect on where food microbiology stands today, what we need to carry forward, and where science, education and our community should take us next.

POSTERS

S1 Microbial Innovations in Food Fermentation Technologies

S1-P1: Bioconversion of Chickpea Flour by Lactic Acid Bacteria Fermentation: Impact on Phytic Acid Reduction and Protein Functionality

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Phytic acid is one of the antinutritional factors limiting the nutritional quality and use of legumes in food products, as it reduces mineral bioavailability and can decrease protein functionality. Chickpea (*Cicer arietinum* L.) has attracted attention due to its high protein content, balanced amino acid composition, dietary fibre, and bioactive compounds; however, its application is limited due to the presence of antinutritional factors (ANFs), particularly phytic acid. Therefore, strategies that reduce antinutritional factors and improve technofunctional performance are getting attention. There are traditional methods to reduce phytic acid content, including soaking, germination, and thermal processing, but they are often associated with nutrient losses or higher processing costs. Alongside these traditional methods, fermentation has gained increasing attention due to its environmentally sustainable bioprocessing advantages. In this study, phytase and protease activities of six lactic acid bacteria isolated from local sources in Türkiye were screened. *Lacticaseibacillus rhamnosus* DD_B_B65 exhibited the highest protease activity, with a measured value of 3.754 ± 0.102 U/mL. In addition to its proteolytic capacity, phytase activity – an important factor in the reduction of phytic acid during fermentation – was evaluated using the semi-quantitative Pz index assay and was determined to be at a moderate level. Subsequently, chickpea flour was fermented using *L. rhamnosus* DD_B_B65, and the effects of fermentation on both the phytic acid content and the functional properties of chickpea protein isolates were investigated. While fermentation reduced the phytic acid content (up to a 33% reduction) in chickpea flour, several technofunctional properties of chickpea protein were enhanced, including water- and oil-holding capacity (1.410 g water/g protein and 6.945 g oil/g protein) and foaming capacity (146.5%). Differences in amino acid composition, reduced surface charge, and partial degradation of phytic acid were associated with these changes. These findings demonstrate that fermentation enhances the functional properties of chickpea proteins, modifies baking performance, and influences shelf-life-related parameters. Therefore, food formulations incorporating fermented chickpea flour should be further investigated, and fermentation should be emphasised as an alternative strategy to enhance the technological value of chickpea flour in gluten-free bakery products.

S1-P2: From Fruit Residues to Functional Beverages, Sustainable Water Kefir Fermentation.

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The global food system is under increasing strain due to population growth, inefficient distribution chains, and, most critically, rising levels of food waste. Nearly one-third of all food produced worldwide is lost or wasted annually, contributing to food insecurity, greenhouse gas emissions, and inefficient use of natural resources. Reducing food waste is therefore essential to building a more sustainable and equitable food system. Upcycling food by-products into value-added fermented foods represents a promising strategy within a circular economy framework.

Water kefir is a non-dairy fermented beverage rich in live microorganisms, offering potential probiotic benefits and supporting gut health. It is naturally carbonated and typically contains lower sugar levels than conventional soft drinks, making it an attractive alternative for health-conscious consumers, including vegans and individuals with dairy intolerance. The combination of water kefir cultures with fruit juices and fruit processing by-products has emerged as a promising approach for developing probiotic and biofortified beverages.

This study aims to evaluate selected microbial strains and consortia for the fermentation of fruit waste substrates to produce a nutritious and marketable water kefir beverage. Fifteen strains previously isolated from different water kefir sources were selected: two *Bifidobacterium*, four *Lactobacillus*, four acetic acid bacteria, four yeasts, and one *Zymomonas*. Three abundant fruit by-products in Ireland – apple pomace, strawberry, and raspberry residues – were chosen as substrates. Fruit juices were prepared by blending the by-products and diluting them 1:10 (w/v) with water prior to fermentation.

Each juice was fermented separately with individual isolates for 48 h. A consistent decrease in pH and an increase in microbial counts were observed across substrates, indicating successful fermentation and microbial adaptation. Based on these preliminary findings, strains with Qualified Presumption of Safety (QPS) status were selected to formulate microbial consortia. These consortia were used to ferment the fruit-based substrates, monitoring pH, viable cell counts, sugar consumption, and ethanol production.

The results will guide the selection of an optimal microbial consortium and fruit substrate for industrial-scale production. This approach offers a sustainable solution for fruit waste valorisation while contributing to the development of functional, non-dairy fermented beverages aligned with current environmental and health priorities.

S1-P3: Development of a Synbiotic Yogurt Enriched with Pomegranate By-Product and Probiotic *Lactobacillus rhamnosus* SP1

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Agro-industrial by-products represent an important source of bioactive compounds that can be exploited as functional ingredients in fermented foods, contributing to products with improved nutritional value while supporting circular economy strategies. The aim of this study was to evaluate different agro-food by-products as potential sources of bioactive compounds and to select the most suitable one for the development of a synbiotic yogurt.

Three materials were evaluated: the residue generated during pomegranate juice production (PB), coffee fruit peel (CP), and the residue from quince paste production (QB). These materials were characterized in terms of nutritional composition and bioactive properties. PB showed significantly higher ($p < 0.05$) total phenolic content (62.89 ± 3.32 mg GAE/g) compared with CP (14.63 ± 0.59 mg GAE/g) and QB (6.17 ± 0.32 mg GAE/g). Likewise, PB exhibited the highest antioxidant activity, reaching 2025.22 ± 199.68 µmol TE/g (ABTS) and 477.34 ± 21.37 µmol TE/g (ORAC-FL). This by-product also showed a favorable nutritional profile for incorporation into fermented dairy matrices, highlighting its soluble fiber content, mineral compounds, and low lipid levels.

Based on these results, PB was selected for incorporation into synbiotic yogurt formulations produced using a yogurt starter culture and the probiotic strain *Lactobacillus rhamnosus* SP1. Six formulations were developed: yogurts containing PB combined with the starter culture, the probiotic strain, or both cultures, together with three corresponding control formulations prepared with the same cultures but without PB. The products were characterized in terms of proximate composition, technological parameters, and bioactive properties. The incorporation of PB reduced ($p < 0.05$) syneresis by 10–15% compared with control yogurts. PB also increased dietary fiber content, while protein levels remained within typical yogurt values. Furthermore, PB significantly ($p < 0.05$) enhanced phenolic content and antioxidant capacity compared with control formulations, reaching antioxidant activity values close to 25 µmol TE/g (ABTS), compared with approximately 1.4 µmol TE/g in control yogurts.

These results demonstrate the potential of PB as functional ingredients for synbiotic dairy products development with enhanced bioactive properties and dietary fiber content. The valorization of agro-industrial residues in fermented matrices represents a promising strategy for the development of sustainable functional foods integrating lactic fermentation and probiotics.

S1-P4: Coffee Bean Microbiota as a Potential Source for New Microbial Starter Cultures: Insights from Brazilian *Coffea arabica* and *Coffea canephora* Processing

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Coffee is one of the most widely consumed beverages in the world. While genetics and environment play a role, post-harvest processing is a critical stage of the final beverage's biochemical and sensory profile. During this stage, coffee cherries undergo spontaneous fermentation mediated by complex microbial consortia. While Brazil is a global leader in coffee production, there remains a need for systematic quantitative data on microbial ecology and the functional characterization of indigenous strains for *Coffea arabica* and particularly *Coffea canephora* (Conilon). In this project we investigated the microbial population dynamics during dry processing (Arabica and Conilon) and wet processing (Arabica) at a farm in Espírito Santo, Brazil, with the primary objective of identifying native strains with the potential to serve as tailored starter cultures.

Throughout the whole processing, we monitored total viable counts, lactic acid bacteria (LAB), yeasts, and *Enterobacteriaceae*, alongside pH changes. The research progressed from quantitative monitoring to the isolation and taxonomic characterization of representative LAB and yeast strains. Beyond taxonomic identification, these isolates were subjected to metabolic and enzymatic activity assays to evaluate their technological potential as starter cultures, focusing on traits that enhance fermentation efficiency and flavor precursor development.

Our results highlight distinct microbial successions influenced by both the coffee species and the processing method. The monitoring of *Enterobacteriaceae* and pH provided critical insights into the hygiene and safety of the fermentation environment, while the high counts of yeasts and LAB highlight their central role in the fermentation process. By combining quantitative monitoring with the establishment of a regional strain collection, this research provides new insights into the microbial ecology of Brazilian coffee. Furthermore, it establishes a vital scientific basis for the development of starter cultures designed to improve consistency and enhance the cup quality of Arabica and Conilon coffees.

S1-P5: Understanding Yeast Fermentations of Plant-Based Substrates

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Global population growth and limited resources call for a protein transition towards more sustainable alternatives. Plant-based foods are a promising alternative to animal-based products, given their lower greenhouse gas emissions. However, the shift towards plant-based foods is hampered due to the presence of anti-nutritional factors and characteristic off-flavours, often described as beany or grassy.

Yeasts have been used extensively for centuries in fermentation processes to improve the quality of fermented food products, contributing to texture, preservation, and taste. Despite these promising results, there are at least two key challenges with respect to plant-based fermentations. First, there is a limited fundamental understanding of the conversion of plant-food raw material by yeast species, and secondly, optimization of plant-based fermentation mostly relies on trial and error approaches.

In this project, we aim to expand our understanding of the role of enzymatic processes and metabolic pathways of selected food-grade yeast species. We intend to use high-throughput yeast fermentation with different plant-based substrates to explore their capabilities to degrade off-flavours and antinutritional factors in plant-derived raw food materials. In conclusion, we will combine high-tech analytical methods with artificial intelligence to predict and validate the currently unknown functions. This will allow us to rationally design optimal fermentations to make more tasty, healthy, and sustainable plant-based foods.

S1-P6: Division of Labour in the Olive Microbiome: Genomic and Functional Insights into Microbial Community Interactions

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Lactic acid bacteria (LAB) are key microorganisms driving olive fermentation, yet their interactions within the olive microbiome remain poorly understood. We characterised the olive microbiota, comprising LAB, other bacteria, and yeasts, using amplicon sequencing and shotgun metagenomics, and isolated ~600 microbial strains representing all three groups. Our results reveal highly diverse microbial communities with division of labour in vitamin metabolism. Based on metagenomic data, LAB lack biosynthetic genes for certain vitamins, such as B3, B5, and B7, while certain *Gammaproteobacteria* carry diverse genes for the synthesis of these vitamins, suggesting potential dependence of LAB on co-occurring bacteria to meet their nutritional requirements. To test this, we will screen bacterial isolates for vitamin production and secretion, and study their capacity to stimulate the growth of LAB in a co-culture as well as in lab-scale fermentations. These findings could have practical implications for the rational design of starter cultures, more consistent fermentation, and the development of fermented products with enhanced nutritional value.

S1-P7: Genomic and Metagenomic Insights into Sourdough Microbiota: From Community Profiling to Strain-Level Reconstruction

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Sourdough represents a complex microbial ecosystem where lactic acid bacteria (LAB) and yeasts interact to drive fermentation performance and impact final product quality. Understanding the structure and dynamics of this microbiota throughout the production chain is essential to ensure process consistency, reproducibility and desired product attributes.

This study aimed to characterize the microbiota (bacteria and yeasts) of fresh sourdough, over two production campaigns (winter and summer), throughout all stages of the production process, including mother dough, intermediate propagation steps (third refreshment) and preferments ("biga" at 8 and 18 h). By including samples across different seasons and production steps, we sought to capture both temporal and process-driven variations in community composition and strain prevalence. A combined approach was employed, integrating the isolation, phenotypic characterization and whole-genome sequencing of isolated LAB and yeast strains, with shotgun metagenomic profiling of the samples, allowing strain-level resolution and precise tracking of isolate genomes within the metagenomic datasets. Comparison of Metagenome-Assembled Genomes (MAGs) and isolate genomes confirmed that the isolated strains, particularly those of *Fructilactibacillus sanfranciscensis* are consistently detected across sourdough production stages and final products, with high genome coverage and relative abundance, supporting their role as core community members. Based on the results, a strategy for sourdough microbiome reconstruction was developed by combining representative isolates, followed by an evaluation of the technological functionality of the reconstructed ecosystem.

The reconstructed sourdough demonstrated functional properties comparable to the original system, supporting the reliability of the selected strains in reproducing key fermentation traits.

This approach allowed the reconstruction of the sourdough starter from characterized isolates and provided insights into strain-level dynamics, linking individual genomes to their occurrence and persistence throughout the production process.

S1-P8: Application of Native Cocoa Microbiome in Novel Substrates for Flavour Creation

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Yeasts associated with cocoa fermentation are critical for inducing biochemical changes and producing metabolites which are crucial flavour and aroma precursors. These are key for determining chocolate quality and sensory profile. Given the volatilities in cocoa commodity prices in the recent decade, due to systemic issues like persistent plant diseases, unpredictable weather patterns and global warming reducing cocoa supply, there is a need to explore sustainable solutions to combat the deficit and build resilience in the supply chain. Carob is a well-known cocoa substitute with some cocoa-like flavour notes, yet significant sensory differences remain.

This study aimed to evaluate whether native cocoa-derived microrganisms from West Africa could be used to enhance flavour development in carob and reduce the sensory gap relative to cocoa. Fermentations were carried out in duplicates for 72 h using parameters of green processing with minimal water and energy input and no synthetic additives at ambient temperatures of 25°C, with uninoculated carob included as a control. The fermented material was then subjected to typical cocoa-like post-fermentation processing steps including drying, roasting and grinding with fat and sugar, and used to develop 70% dark chocolate equivalent prototypes.

The resulting products were analysed using an electronic nose (e-nose), and principal component analysis (PCA) was applied to the volatile profile data and compared with sensory data generated by a panel of six professional assessors using unstructured descriptive analysis to develop a sensory lexicon and characterise sample differences. PCA revealed clear separation between different samples, indicating distinct volatile profiles, with some fermented samples associated with increased alcohol-related compounds and others with ester-dominated notes. These differences were reflected in the sensory evaluation, where some fermented samples demonstrated enhanced fruity, floral, and chocolate-like attributes relative to unfermented carob.

Overall, the findings indicate that cocoa-associated microbiota can drive targeted flavour development in non-cocoa substrates, demonstrating their potential as functional starter cultures for alternative chocolate product development. While preliminary, these results suggest a promising strategy to improve the sensory quality of cocoa substitutes and contribute to more sustainable chocolate production. Future work will focus on detailed volatile compound identification and quantification, as well as optimisation of fermentation parameters and scale up to validate findings.

S1-P9: Adapting Starter Cultures for Fermentation of Plant-Based Milks Using Adaptive Laboratory Evolution

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The rapid expansion of plant-based dairy alternatives has created a need for starter cultures that are better suited to plant-based substrates. While plant-based milks account for approximately 14% of the liquid milk market, plant-based yogurts represent only about 3.5% of the yogurt sector, underscoring a major gap in consumer acceptance. A key challenge is that traditional yogurt starter cultures are naturally optimized for lactose-rich milk and therefore perform poorly in plant-based matrices rich with non-lactose sugars.

Here, we addressed this limitation using oat milk as a model substrate because it is a widely consumed and has favorable sustainability profile relative to almond milk, particularly with respect to water use. Oat milk is rich in starch-derived sugars, mainly maltose, and the conventional yogurt starter bacterium *Streptococcus thermophilus* has no capacity to utilize maltose. To improve its performance, we applied adaptive laboratory evolution (ALE) under carbon-limited, maltose-enriched conditions to select *S. thermophilus* variants with enhanced maltose utilization.

The evolved strains showed a markedly improved ability to ferment maltose relative to the ancestral strain. When combined with *Lactobacillus delbrueckii* subsp. *bulgaricus* for oat milk fermentation, the evolved *S. thermophilus* strains exhibited superior fermentation performance, including shorter acidification times, higher lactic acid production, and increased final viable cell counts. These findings demonstrate that ALE can provide a practical, non-GMO route for adapting conventional dairy starter cultures to plant-based fermentation matrices. In addition, ALE offers an efficient alternative to extensive screening for naturally occurring strains with the desired traits, thereby accelerating starter culture development for new substrates.

Overall, our work positions ALE as a powerful food fermentation technology for the development of next-generation starter cultures tailored to alternative raw materials. This strategy may help overcome key bottlenecks in the development of plant-based fermented foods and support the production of products with improved functionality, stability, and industrial relevance.

S1-P10: Fermenting the Future: Optimizing Lactic Acid Bacteria Fermentation to Develop New Plant-Based Products

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Over the past few decades, significant shifts in consumers' dietary habits have prompted both the food industry and the scientific community to seek more sustainable and health-promoting alternatives. In this perspective, plant-based products (PBPs) have gained mounting attention thanks to their nutritional benefits and reduced environmental impact. PBPs are widely recognized as major sources of health-promoting constituents (such as vitamins, minerals, antioxidants, phenolic compounds and dietary fiber) but they may also contain varying concentrations of anti-nutritional factors (like oxalates, protease and α-amylase inhibitors, lectins, condensed tannins, and phytic acid) which can influence nutrient bioavailability, digestibility and the overall nutritional quality. To overcome these limitations, microbial fermentation with lactic acid bacteria (LAB) has emerged as an effective strategy. This study aims to evaluate the growth performance of selected strains, isolated from traditional fermented foods, in individual plant-based matrices (such as rice, chickpea, and yellow pea flours) and their combinations to develop an innovative plant-based fermented product.

Microbial strains isolated from table olives, cheeses and African fermented cereals were tested in both single and mixed flours. Growth performance and metabolic activity were evaluated by measuring pH and performing microbial counts at time zero and after 48 h of incubation under agitation at 30°C. Results show that *Lactiplantibacillus pentosus*, *Lactiplantibacillus plantarum* and *Limosilactobacillus fermentum* grow in both single and mixed flours, whereas *Limosilactobacillus rhamnosus* showed reduced ability. Chickpea and yellow pea flours were found to be the preferred matrix for most of the strains. Tests with *Lactococcus lactis*, *Leuconostoc* spp. and *Pediococcus pentosaceus* are still ongoing.

In conclusion, the present study shows that LAB exhibit differential growth performance depending on the plant-based matrix, highlighting the critical role of substrate composition in fermentation processes. Legume-based substrates seem to be more favorable environments for microbial growth compared to cereals. These findings support the potential of targeted microbial fermentation as a strategy to enhance the nutritional and functional quality of plant-based products. Future research will further investigate the impact of these fermentations on the reduction of anti-nutritional factors and the generation of bioactive compounds, ultimately contributing to the development of innovative, sustainable, and health-promoting food products.

S1-P11: Production of a GABA-Enriched Fresh Dairy Product via Autochthonous Lactic Acid Bacteria

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Traditional dairy productions are characterized by unique microbial biodiversity. As the outcome of an extensive sampling effort, including raw milk, curds, natural whey cultures, and different cheeses, more than 140 microbial isolates were preliminarily evaluated, resulting in a collection of 98 bacterial strains. Among these, 18 strains belonging to *Streptococcus thermophilus*, *Enterococcus faecalis*, *Enterococcus gallinarum*, *Lacticaseibacillus paracasei*, *Limosilactibacillus fermentum*, *Lactococcus garviae*, *Lactococcus lactis* species, were selected for their possible production of gamma-aminobutyric acid (GABA).

In UHT whole milk, supplemented with sodium gluconate (Glu-Na) at 20 mM, six strains were found to be GABA producer. Three strains, namely *S. thermophilus* ST3 and *L. lactis* 5pre and 7pre, were found to release GABA at approximately 300 mg/L, while other three *L. lactis* strains produced GABA concentrations at 28–32 mg/L. Viable cell populations of these strains ranged from 7,13 ± 0,30 log CFU/ml (ST3) to 9,15 ± 0,05 log CFU/ml (7pre).

Further assays with *L. lactis* 5pre and 7pre strains cultivated in presence of 0, 0.5, 2.5 and 5 mM Glu-Na demonstrated that the release of GABA is appreciable from 2.5 mM Glu-Na and after 24 h of incubation.

At a local dairy, full-fat milk, pasteurised and cultured with *L. lactis* 5pre, was mixed with low-moisture ricotta cheese, sodium chloride and texturizing agents, to obtain a fresh dairy product containing GABA at 151,5 ± 9,3 mg/kg FW.

This concentration is higher than that already reported to produce benefits from a diet including GABA-enriched foods, and therefore this new dairy product with autochthonous lactic acid bacteria represents a possible support in a diet for people with low to mild cardiac pressure imbalances.

S1-P12: Fermented Foods Select Their Microbes: Strain-Level Evidence from Lactic Acid Bacteria

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The gradual decline in microbial exposure associated with Westernised diets has been linked to reduced gut microbiome diversity and increased rates of chronic diseases. In this context, this study aims to explore the potential of traditional African fermented foods (FFs) as sources of live microbes that can modulate the gut microbiome and restore microbial diversity. A crucial step towards this goal is to characterise culturable microbial diversity across different FF matrices and to identify strain-level patterns associated with specific food sources. In this research, we performed a cultivation-based screening of lactic acid bacteria (LAB) in Kenyan FFs, including plant-based products (e.g., almond, soya, and peanut), fermented milk, and traditional cereal-based fermentations. Isolates were recovered on MRS and M17 media and processed through a stepwise selection pipeline. Species identification was conducted using MALDI-TOF mass spectrometry, revealing a high prevalence of *Lactococcus lactis*, *Weissella cibaria* and *Pediococcus pentosaceus* across all matrices. To examine intra-species diversity and potential source-dependent structuring, strain deduplication was carried out using (GTG)5-REP-PCR fingerprinting. Comparative analysis of the profiles revealed high genetic diversity and clustering patterns associated with the source of isolation. While some strains clustered according to their original matrix, indicating ecological adaptation, others exhibited cross-source similarities, suggesting the presence of broadly distributed, functionally resilient LAB lineages. Results were further confirmed by whole genome sequencing and comparative analysis with publicly available genomes. These results support the hypothesis that certain LAB possess specific microbial traits that enable them to thrive across diverse niches, while others retain niche-specific signatures. Integrating genomic and functional analyses will be vital for identifying candidate strains with the greatest potential to influence the gut microbiome and promote human health.

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S1-P13: Fermentation and Bitter Leaf Enhancement Improved the Antioxidant Potentials and Mineral Composition of Fermented Soybean Products

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Soybean (*Glycine max*) is an integral component of traditional diets in many African countries. Bitter leaf (*Vernonia amygdalina*) is widely consumed as a leafy vegetable flavoring herb in local soups, which aids in the reduction of total cholesterol, low-density lipoprotein, and triglyceride levels. Soy-iru and soy-wara are fermented soybean products used as meat substitutes in rural Nigeria. This is a novel product development, where bitter leaf extracts (aqueous and ethanolic) were employed in enhancing fermented soybean products.

Phytochemical and antioxidant potentials of the extracts were determined. Probiotics were previously isolated from the fermentation of soy-iru and soy-wara. Bitter leaf extracts (1, 3, and 5%) were employed to enhance the controlled fermentation of soy-iru and soy-wara using probiotic microorganisms. Total phenolic content (TPC in ascorbic acid equivalent [AAE]), total flavonoid content (TFC in quercetin equivalent [QE]), 1, 1-diphenyl-2-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP in [AAE]) using an *in vitro* assay, and the mineral contents were determined.

Bitter leaf extracts indicate an abundance of saponins (3.40 mg/g) and alkaloids (2.75 mg/g) in aqueous extracts, while terpenoids (2.50 mg/g) and flavonoids (3.72 mg/g) are present in ethanolic extracts. There were significant differences at $p < 0.05$ in DPPH scavenging activities (56.1% and 62.8%) at 250 mg/mL and FRAP contents (3.47 µg/mL and 4.02 µg/mL) at 100 µg/mL for aqueous and ethanolic extracts, respectively. *Priestia flexa* and *Weissella confusa* were employed to ferment soy-iru and soy-wara, respectively. Fermented soy-iru supplemented with 3% aqueous extract had significant differences at $p < 0.05$ for TPC (2.08 µg/g AAE) and FRAP (13.82 µg/g AAE), while 1% supplementation of ethanolic extract was for TPC (1.87 µg/g AAE) and FRAP (9.34 µg/g AAE) at 200 mg/mL concentration. Fermented soy-wara supplemented with 3% of the aqueous extracts had significant differences at $p < 0.05$ for TFC (3.83 µg/g QE), TPC (1.46 µg/g AAE), and DPPH (40.76%), while 3% supplementation of the ethanolic extracts exhibited significant differences for FRAP (6.82 µg/g AAE) and 1% and 3% for TFC (3.37 µg/g QE), respectively, at 200mg/mL concentration. There was a significant difference at $p < 0.05$ for 1% supplementation of aqueous extract in DPPH (48.67%) of fermented soy-wara. Fermented soy-iru and soy-wara supplemented with 5% aqueous extract exhibited significant differences at $p < 0.05$ for potassium (13.2 mg/kg and 12.8 mg/kg), respectively.

Bitter leaf enhancement and fermentation of soy-iru and soy-wara with probiotic microorganisms (*P. flexa* and *W. confusa*), respectively, improved the antioxidant and mineral contents of the fermented soybean products.

S1-P14: Lactic Acid Bacteria in Plant-Based Fermentations: Strain-Substrate Combinations Governing Acidification Outcomes

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Fermentation with lactic acid bacteria (LAB) has emerged as a promising strategy to improve the safety, sensory quality, and nutritional value of plant-based foods. Rapid and effective acidification is a central fermentation outcome, providing a safety hurdle against pathogens while driving texture and flavour development. Yet the factors governing LAB acidification performance across diverse substrates remain poorly understood. We therefore characterised acidification kinetics of various LAB species, plant-based substrates and sugar supplementations, to identify the strain-substrate interactions underlying rational starter culture selection.

Thirty-nine LAB strains from four species (*Lactiplantibacillus plantarum*, *Lactococcus cremoris*, *Lactococcus lactis*, and *Leuconostoc mesenteroides*), of both dairy and plant origin, were fermented on five substrates (rice flour, pea protein, oat protein, amaranth flour, and quinoa flour) under four sugar conditions (no sugar, glucose, sucrose, or raffinose [2.5% w/v]). Substrates were characterised for composition and buffering capacity prior to fermentation. Acidification kinetics were monitored via fluorescence-based pH measurements over 24 h at 30°C.

Acidification performance varied substantially across strain-substrate combinations, reflecting the interplay between substrate composition and strain-specific metabolic capabilities. Amaranth and quinoa flours supported rapid acidification across multiple strains, with pH values reaching a pH ≤ 5.0 within 8 h, while rice and oat protein exhibited minimal acidification without supplementation regardless of strain. These differences were linked to substantial variation in buffering capacity, with yellow pea protein concentrate requiring 18-fold more lactic acid to achieve equivalent pH reduction compared to rice flour. Carbohydrate availability was a primary limiting factor, with glucose supplementation enabling previously inactive strains to achieve pH reductions exceeding 2.5 pH units within 24 h, while raffinose utilisation was restricted to specific strain-substrate combinations. Substantial within-species heterogeneity was evident across all four species, confirming that species identity and isolation origin is insufficient to predict fermentation performance. Notably, *L. cremoris* AK25 and *L. lactis* AK39 emerged as the most consistent high performers, maintaining robust acidification across compositionally diverse substrates while conspecific strains showed variable performance. Together, these findings demonstrate that strain-level metabolic compatibility, rather than species identity or substrate composition alone, determines fermentation success in plant-based systems.

This systematic characterisation provides an empirical foundation for rational starter culture selection in plant-based fermentation, moving beyond dairy-optimised cultures and species-level assumptions towards data-driven strain selection tailored to the metabolic demands of compositionally diverse plant ingredients.

S1-P15: KOMBREAD, a Functional Bread Prepared with a Kombucha-Based Sourdough: Physicochemical Properties, Microbiological Characterization, and Quality Evaluation

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This study investigated kombucha as an innovative functional starter for sourdough breadmaking. A kombucha-based sourdough was prepared and reached technological maturity in just five days, significantly faster than conventional spontaneous fermentation. Its performance was compared against a traditional artisanal sourdough control. Bread quality was comprehensively evaluated through physicochemical, structural, and sensory assessments. Results demonstrated that kombucha breads exhibited specific volume, crumb structure, texture, and staling behavior comparable to traditional sourdough bread. Sensory analysis confirmed high overall acceptability and similarity between the products. Notably, kombucha breads displayed significantly higher total phenolic content and antioxidant capacity. These findings highlight kombucha as a highly promising alternative starter capable of accelerating sourdough production while enhancing the functional and nutritional properties of bread.

S1-P16: Cutting Edge: Leveraging Edge-Detection in RNA-Seq Data to Characterize Cobalamin Riboswitches in Food-Relevant Bacteria

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Cobalamin (vitamin B_{12}) is naturally absent from plant-based foods, representing a key nutritional challenge in the transition toward sustainable food systems. Vitamin B_{12} is exclusively synthesized by certain microbes, including the food-relevant bacteria *Priestia megaterium* and *Propionibacterium freudenreichii*, providing a platform for in situ fortification through fermentation.

However, because the biosynthesis of B_{12} is among the most complex pathways in nature, involving over thirty enzymatic steps, it requires tight regulatory control to prevent unnecessary metabolic costs. This regulation is mediated by cobalamin-responsive riboswitches, which control the expression of genes involved in biosynthesis, transport, and B_{12}-dependent metabolic processes.

Riboswitches are cis-regulatory RNA elements located in the 5' untranslated regions (5' UTRs) of mRNAs. Upon ligand binding, they undergo conformational changes that modulate downstream gene expression at the transcriptional or translational level, either by leading to premature termination of transcription or by masking the ribosome-binding site, respectively. Traditionally, their functional characterization has relied on *in vitro* assays or in silico predictions, leaving their *in vivo* regulatory dynamics less explored.

In this study, we use bioinformatic pipelines to map the global landscape of cobalamin riboswitches in these two bacterial species and predict their affinities for different vitamin B_{12} analogs, identifying several previously undercharacterized riboswitches in *P. freudenreichii* and *P. megaterium*.

Condition-dependent premature termination creates a 3' edge, an asymmetric pile-up of read termini, in population-level RNA-seq data. Using edge-detection tools, we recover important aspects of riboswitches *in vivo* regulatory behavior. Our results reveal that, contrary to the prevailing assumption that the riboswitches of *P. freudenreichii* predominantly regulate gene expression at the translational level, there are clear termination signals within the ORFs of some of the riboswitch-associated operons. These findings suggest a more diverse mechanistic repertoire than previously recognized and provide deeper insight into the regulatory barriers to enhancing in situ fortification of vitamin B_{12} in plant-based foods.

S1-P17: A Polyphasic Approach Combining Culture-Dependent Microbiology and 16S Metabarcoding to Compare Traditional Senegalese Fermented Fish and Shellfish Products

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Traditional fermented fish products in Senegal are produced through diverse artisanal practices involving differences in raw materials, salting, fermentation and drying conditions, which are expected to shape their microbial ecosystems. Within the SENBICPha project, 60 samples of fermented fish (guedj made from 3 fish species) and shellfish (yeet made from *Cymbium* spp.) have been collected, in various regions and their microbial and physico-chemical characterization was performed. The microbial ecosystems of the samples were compared using a polyphasic approach combining culture-dependent microbiology and 16S rRNA gene metabarcoding (V3–V4 region), in order to assess the influence of product matrix, process and production context on microbial profiles. Physico-chemical characterization included NaCl concentration, % humidity, pH and biogenic amine quantification.

Physico-chemical analysis revealed variable NaCl content around (0.65 ± 0.20 – 13.57 ± 1.60)% and (4.88 ± 1.95 – 13.20 ± 1.84)% for guedj and yeet, respectively. Biogenic amine quantifications showed higher amounts of cadaverine, putrescine, and histamine in guedj made from sole fish. Culture-based analyses showed aerobic mesophilic counts ranging from 2.31 to 7.03 log CFU/g, spore-formers from 1.18 to 4.53 log CFU/g, lactic acid bacteria from 1.70 to 5.65 log CFU/g, yeasts and moulds from 0.70 to 2.41 log CFU/g, and presumptive *Enterobacteriaceae* from 0.70 to 2.38 log CFU/g. *Escherichia coli* remained below the detection threshold (< 1 log CFU/g) in all tested samples, except for 3 yeet samples where counts ranged from 1.00 to 1.18 log CFU/g. Preliminary 16S analyses of a first dataset retained 213 ASVs and showed a tendency toward clustering by sample type. The dominant genera included *Clostridium*, *Staphylococcus*, *Alkalibacillus*, *Tetragenococcus*, *Peptostreptococcus*, *Salinicoccus*, *Sarcina* and *Bacillus*, with clear differences in relative abundance patterns between products. At this stage, microbial composition appeared relatively close between Kong and Sole samples, while differing from Gaïdé guedj (shark). Yeet samples microbiota were clearly different from fermented fish samples.

Overall, these preliminary results support the relevance of combining culture-dependent and 16S-based approaches to discriminate microbial signatures associated with fermented fish products. The ongoing extension of the dataset will strengthen the assessment of matrix-, process- and location-associated microbial variation and support the future selection of beneficial strains for safer and more reproducible fermentations.

S1-P18: Physicochemical and Structural Characterization of Dextran-Type Exopolysaccharide (EPS) from *Weissella confusa* Isolated from Fermented Sprouted Rye

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Cereal-derived exopolysaccharides (EPS) are increasingly recognized as high-value functional ingredients and sustainable biopolymers within the food industry. These compounds serve as versatile hydrocolloids, thickeners, and stabilizers, playing a pivotal role in tailoring the rheological properties and overall texture of diverse food systems. Beyond these techno-functional attributes, EPS contributes to the development of "healthier" formulations by serving as natural prebiotics or fat mimetics. Within this framework, extracting EPS from cereal-based sources represents a circular bioeconomy model that valorizes agricultural raw materials through natural fermentation. This strategy not only improves the nutritional profile of cereal products but also meets the rising consumer demand for clean-label, eco-friendly ingredients.

In this study, we focused on isolating high-yield EPS-producing lactic acid bacteria from sprouted rye to investigate the physicochemical and structural traits of the resulting polymer. A high-density steeping method (1:2 w/w, rye : water) was conducted at 30°C and 150 rpm for 24 h, followed by a brief enrichment step on moistened sterile filter papers for 3–5 days. Post-sprouting, samples were milled, serially diluted, and cultured on sucrose-supplemented modified brain heart infusion (BHI) agar. The most promising isolate was identified as *Weissella confusa* via 16S rRNA sequencing. Following production in modified BHI broth, the EPS was recovered through double-step cold ethanol precipitation, purified using a 14 kDa cut-off dialysis membrane, and finally lyophilized.

The process yielded an overall EPS concentration of 4.5 g/L. Structural analyses using FT-IR and NMR confirmed that the polymer is a dextran-type EPS, primarily composed of α-(1→6) linkages with a low degree of α-(1→3) branching. XRD and AFM analyses provided evidence of its semi-crystalline nature and granular surface morphology, revealing a surprisingly uniform topography. Thermal profiling using TG/DTA and DSC demonstrated remarkable stability with a high degradation threshold. Such thermal resilience suggests that this dextran can withstand demanding food processing conditions, including pasteurization and extrusion. Taken together, the successful isolation and comprehensive characterization of this dextran underscore its potential as a robust, thermally stable functional biopolymer for sustainable food applications.

S1-P19: Moderate Pulsed Electric Fields as a Tool to Modulate Lactic Acid Bacteria Physiology: Linking Sub-Lethal Stress to Functional and Technological Traits

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Pulsed electric field (PEF) is emerging as a non-thermal technology capable of modulating microbial physiology beyond inactivation, offering new opportunities to enhance the functional and technological performance of beneficial microorganisms. This exploratory study evaluates moderate-intensity PEF (MI-PEF) as a tool to induce controlled sub-lethal stress and modulate the physiology of lactic acid bacteria (LAB). Selected autochthonous LAB strains were treated during the exponential and stationary growth phases under different MI-PEF conditions, defined by electric field strengths (1–2 kV/cm), pulse frequencies (150 or 250 Hz), and 300 or 600 pulses. Their responses were assessed with respect to growth behavior, stress tolerance, acidification, proteolytic activity, and cholesterol assimilation.

MI-PEF treatments induced strain-dependent sub-lethal injury, evidenced by reduced colony size and impaired growth under selective conditions, suggesting membrane damage and physiological stress. Notably, these stress conditions triggered adaptive responses that enhanced microbial performance. In some cases, treated cells exhibited greater tolerance to NaCl than untreated controls, suggesting improved environmental robustness. MI-PEF also modulated key metabolic functions. Selected treatments enhanced acidification kinetics, improving fermentation performance even in less favorable substrates such as plant-based matrices. In parallel, an increase in proteolytic activity and peptide release was observed. Furthermore, MI-PEF enhanced cholesterol assimilation capacity. These results suggest possible implications for both technological performance and functional properties of the final products.

Overall, the results of this study suggest that MI-PEF can serve as a tool for metabolic reprogramming of LAB through controlled sub-lethal stress. While the effects are strongly strain- and condition-dependent, the observed improvements in stress tolerance and metabolic activity highlight the potential of this approach for the development of tailored microbial cultures. This exploratory study lays the groundwork for future investigations to elucidate the mechanistic basis of PEF-induced physiological responses and to validate their impact on functional and technological properties in fermented food systems.

S1-P20: Bridging Phenotype and Genome to Reveal Functional Potential of Acetic Acid Bacteria for Food Applications

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Acetic acid bacteria (AAB) are essential microorganisms in fermented food systems; however, their functional potential beyond traditional fermentation remains underexplored. Integrating phenotypic characterization with genome-based analysis is essential for a comprehensive evaluation of their applicability and safety. This study aimed to evaluate an *Acetobacter pasteurianus* strain isolated from a traditional fermented food through a combined assessment of biosafety, functional properties, and genomic features. The isolate was evaluated for DNase, gelatinase and hemolytic activities, antibiotic susceptibility, and biofilm formation capacity. Antimicrobial activity against selected microorganisms was also assessed. Whole genome sequencing (WGS) was performed to investigate genetic determinants associated with safety and functional potential. The tested strain showed no DNase, gelatinase or hemolytic activity and exhibited susceptibility to most tested antibiotics, with only limited resistance observed, indicating the absence of a critical multidrug resistance profile. The strain exhibited a positive biofilm phenotype, with an OD_{570} value of 0.66, indicating measurable biofilm-forming capacity. Furthermore, antimicrobial activity was observed against selected indicator strains, with inhibition zones reaching approximately 14–15 mm against *Escherichia coli*. Whole-genome analysis revealed a draft genome of approximately 3.05 Mb with a GC content of 52.7%, consistent with the genomic features of *A. pasteurianus*. Importantly, no major virulence factors or acquired antibiotic resistance genes were detected, supporting its biosafety for food-related applications. Functional annotation indicated a metabolically versatile organism with a strong oxidative metabolism, particularly associated with organic acid production. In addition, genes related to stress response and redox balance suggest a high tolerance to environmental stresses and support a metabolite-driven antimicrobial mechanism. Overall, this study provides an integrated phenotype-genome framework for the evaluation of AAB, demonstrating their potential as safe and multifunctional candidates for food applications. These findings contribute to expanding the role of AAB beyond conventional fermentation toward targeted use in food safety and functional systems.

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S1-P21: Fermented Dairy Products and Vegetables as Sources of Protective and Functional Lactic Acid Bacteria

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Fermented foods, including dairy and plant-based products, are recognized for their safety, extended shelf life, and health benefits. These properties are largely attributed to lactic acid bacteria (LAB), which drive fermentation through the production of organic acids and antimicrobial metabolites, inhibiting pathogenic and spoilage microorganisms while also contributing to host health. Consequently, fermented foods represent an important source of microbial cultures with applications in food safety and functional food development. The aim of this study was to isolate and characterize indigenous LAB from fermented dairy and vegetable matrices, specifically goat cheese and spontaneously fermented tomato and carrot, and to evaluate their potential as protective cultures with health-promoting properties. Microbial analysis of goat cheese and spontaneously fermented tomato and carrot was performed using selective media, with de Man, Rogosa and Sharpe (MRS) agar for LAB enumeration and Rose Bengal Chloramphenicol (RBC) agar for the detection of yeasts and molds. In goat cheese, MRS agar plates were incubated at 30, 37, and 42°C to target mesophilic and thermophilic populations, whereas fermented vegetable samples were incubated at 30°C. Representative colonies from the media were isolated and preliminarily characterized using biochemical tests (Gram staining, catalase, and oxidase). Additionally, isolates were screened for antimicrobial activity using the microplate reader Tecan Spark Cyto, allowing real-time monitoring of growth inhibition. The analysis revealed that LAB populations in goat cheese reached approximately 6.7, 7.4, and 6.5 log CFU/g at 30, 37, and 42°C, respectively, indicating the presence of both mesophilic and thermophilic LAB. After 10 days of fermentation, tomato samples exhibited LAB counts of approximately 6.0 log CFU/g, while yeast populations reached approximately 5.0 log CFU/g. In contrast, carrot samples after 15 days of fermentation showed LAB counts of approximately 6.0 log CFU/g, whereas no detectable growth of yeasts and molds was observed. Molecular typing was then performed using M13-based Random Amplified Polymorphic DNA–Polymerase Chain Reaction (M13 RAPD-PCR), enabling strain-level discrimination based on strain-specific genetic fingerprints. The resulting banding patterns were analyzed to assess genetic diversity and cluster related isolates. Representative strains were selected and subjected to 16S rRNA gene amplification and sequencing for species-level identification. Overall, this study highlights fermented dairy and vegetable products as valuable sources of novel LAB strains with technological and functional potential. These isolates may be exploited as natural protective cultures in food systems and as contributors to the development of functional foods with added health benefits.

S1-P22: Microbial Dynamics and Colonization Efficacy of Two *Lactiplantibacillus* Strains During Kalamata Olive Fermentation

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The increasing consumer demand for functional foods, particularly those with probiotic properties, has stimulated interest in the incorporation of beneficial microorganisms into traditional fermented food matrices. The present study aimed to evaluate the colonization capacity and microbial dynamics of two lactic acid bacteria (LAB) strains, *Lactiplantibacillus pentosus* B281 with *in vitro* probiotic potential, and a commercial starter culture of *Lactiplantibacillus plantarum* (Vege-Start 60), during the fermentation of Kalamata black olives. Both strains were separately introduced as starter cultures under identical brining conditions (6% w/v NaCl), while a spontaneous fermentation served as the control. Changes in microbial counts were monitored for 145 days, whereas the survival of LAB strains on olive drupes was assessed using rep-PCR analysis at the early (day 1), middle (day 75) and final (day 145) stages of fermentation. Results showed that the population of LAB peaked at 6.9 and 7.0 log CFU/mL in spontaneous and inoculated fermentations after 22 and 12 days, respectively. Yeasts were maintained at ca. 2.0–2.5 log CFU/mL lower populations compared to LAB. Enterobacteria were undetectable in all fermentations after 22 days. The recovery rate of *L. pentosus* B281 from the surface of olives was 65%, 15% and 0% at the early, mid-fermentation and last stages, respectively, compared to 2.8%, 0% and 0% for the commercial starter culture *L. plantarum* (Vege-Start 60). SEM analysis revealed notable differences in microbial colonization. Specifically, olives inoculated with *L. pentosus* B281 presented large aggregates of microorganisms on the epidermis and in the mesocarp of olives, whereas smaller aggregates on the surface and absence of bacteria in the pulp were evident for the inoculated fermentation with the commercial starter *L. plantarum* (Vege-Start 60). In conclusion, the present study demonstrated that *L. pentosus* B281 exhibited better ability to colonize the surface of Kalamata olives compared to the commercial starter *L. plantarum* from Vege-Start 60, especially in the initial and middle stages of fermentation. However, the progressive decline of the strain during the course of fermentation indicates that further optimization of the processing conditions is required to improve long-term survival and maximize its probiotic delivery through the consumption of the final product.

S1-P23: Inoculated Fermentation of Kalamata Natural Black Olives in Reduced Salt Brines to Meet the "Reduced Salt" Nutrition Claim Under Regulation (EC) 1924/2006

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Table olives are among the most popular fermented foods in the Mediterranean diet and culture. Despite their economic significance, fermentation remains largely empirical, relying on the indigenous microbiota that increases spoilage risk and product variability. The use of starter cultures is therefore essential for process standardization, safety, and consistent quality. Although NaCl is technologically important for microbial stability and sensory properties, its high use conflicts with health-driven demand for low-sodium foods due to its association with cardiovascular disease. Therefore, although Kalamata olives align with modern consumer preferences for natural and minimally processed foods, the substantial use of NaCl (6–8%) may limit consumer acceptance.

The present study evaluated the microbiological, physicochemical, and sensory profile of cv. Kalamata natural black olives during inoculated fermentation with *Lactiplantibacillus plantarum* in three brines: (a) 7% (w/v) NaCl (control process), (b) 25% substitution of NaCl with KCl, and (c) 25% reduction of the initial NaCl concentration to meet the "reduced salt" nutrition claim under Regulation (EC) No 1924/2006. Microbiological (lactic acid bacteria [LAB], yeasts, and *Enterobacteriaceae*) and physicochemical (pH, titratable acidity) changes were monitored for 125 days. Starter survival was assessed by rep-PCR, and sensory evaluation was conducted at the end of fermentation according to the official International Olive Council method.

Results showed that LAB dominated all fermentations, followed by yeasts, whereas *Enterobacteriaceae* were undetectable in brines after 13 days. LAB populations were approximately 8.0 log CFU/mL at fermentation onset and gradually declined by ca. 2.0 log units, reaching 5.7–6.2 log CFU/mL at the end of fermentation. Yeast populations ranged from 3.7 to 4.5 log CFU/mL initially and remained relatively stable throughout the process. The pH decreased rapidly within the first 7 days and remained between 3.8 and 4.1 thereafter, while titratable acidity gradually increased to 6.4–7.7 g lactic acid/L brine. Sensory evaluation classified all samples as Extra, with no abnormal fermentations (zaparetia, putrid, or butyric) detected by the panel. Starter recovery from olives was 10% and 26% at early and mid-fermentation, respectively, in the control process, 6% and 25 % in the KCl-substituted brines, and 50% and 22% in the reduced-NaCl treatment. Overall, these findings support the development of reduced-salt Kalamata olives compliant with the "reduced-salt" claim of Regulation (EC) 1924/2006.

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S1-P24: Unveiling the Diversity of Commercial Kombuchas in Uruguay through an Integrated Physicochemical, Microbiological, and Bioactive Approach

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Kombucha is a tea-based fermented beverage whose popularity has increased considerably in recent years. In Uruguay, its production and commercialization are relatively recent and occur in a context where no specific regulations currently exist for this product. This situation has resulted in a highly heterogeneous and poorly characterized market, both in terms of physicochemical properties and microbial communities.

In this study, 24 commercial kombucha samples were analyzed, corresponding to 8 national brands, all evaluated in triplicate. Physicochemical and microbiological parameters were assessed in order to characterize the variability among products available in the Uruguayan market. The pH values ranged from 2.64 to 3.62, remaining within levels generally considered compatible with food safety. Titratable acidity ranged from 0.13 to 0.58% w/v, expressed as acetic acid, with only 64% of the samples falling within the reference range proposed by Kombucha Brewers International (KBI). Volatile acidity ranged from 13.40 to 55.41 mEq/L, and only half of the samples met the reference values established by the Argentine Food Code (CAA) and the Brazilian Food Code (CAB).

Sugar content also showed substantial variability, ranging from 5.37 to 49.70 g/L. Ethanol content averaged 1.12% v/v, exceeding the legal limit for non-alcoholic beverages in several national samples. Polyphenol content was also noteworthy, with a mean of 406.6 mg GAE/L, while antioxidant capacity reached maximum values of 22,720 µmol TE/L.

Microbiological characterization revealed a highly diverse and heterogeneous community of both bacteria and yeasts across samples. Among acetic acid bacteria, *Acetobacter* was the most frequently detected genus, followed by *Gluconobacter*, both of which are closely associated with sugar oxidation and organic acid production. In addition, lactic acid bacteria such as *Lactiplantibacillus* and *Liquorilactobacillus* were identified. Yeast diversity was also notable. *Zygotorulaspora* showed high frequency, while *Saccharomyces* was detected at intermediate frequency, indicating the presence of distinct fermentative profiles among brands. Other less frequent species were also recovered, highlighting the presence of uncommon microbial members with potential influence on aroma, flavor, and overall product differentiation.

Overall, the results demonstrated that commercial kombuchas marketed in Uruguay are not only physicochemically variable but also microbiologically diverse. This microbial heterogeneity is especially relevant, as it may underlie differences in acidification, ethanol production, sensory traits, and product stability. Therefore, these findings provide a valuable baseline for understanding the microbial ecology of kombucha in the national market and may contribute to future discussions on quality standards, product consistency, and regulatory development.

S1-P25: Resistome Profiling of Fermented Foods Using Shotgun Metagenomics

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Antimicrobial resistance is a critical global health issue that also affects the food production chain. Although microorganisms in fermented foods are generally regarded as safe, they may serve as reservoirs of antibiotic resistance genes (ARGs). While whole genome sequencing is routinely used for safety assessment of food-related strains, shotgun metagenomics provides a more comprehensive view by capturing the total resistome, including uncultivable microorganisms, and enabling the analysis of ARG diversity, abundance, and their association with mobile genetic elements (MGEs). Current knowledge of ARGs in fermented foods remains limited, particularly across diverse product types. To address this gap, this study investigates 120 artisanal fermented foods, including dairy, vegetable, meat, vinegar, and kombucha. We aimed to characterise their microbiota, functional potential, resistome, and mobilome, and to compare these features with those of corresponding raw materials. Samples were collected from farmers' markets and retail stores, diluted (1:10), homogenised, centrifuged, and pellets stored at −20°C. Metagenomic DNA was extracted using the Maxwell® 16 Tissue DNA Purification Kit (Promega) or NucleoSpin Food kit (MACHEREY-NAGEL GmbH & Co. KG) and used for shotgun sequencing. Bioinformatic analyses included de novo assembly, taxonomic profiling, and functional annotation using specialised databases for ARGs, MGEs, and virulence factors. The results revealed a diverse range of ARGs in fermented foods, including acquired genes such as *tetM*, *tetS*, and *mefE*, which are associated with MGEs that may facilitate their transfer to other bacteria. In addition, several virulence factors were identified, including *IlsD* and *plc2*. Overall, these findings emphasize that fermented foods can act as potential reservoirs of clinically relevant resistance and virulence determinants, warranting further surveillance across food systems.

S1-P26: Yeast-Driven Fermentation of Grape Pomace: Winery By-Product Valorisation through Pyranoanthocyanin Formation

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Agricultural side streams are increasingly recognised as valuable resources within circular bioeconomy strategies. Grape pomace (GP), the main solid residue of winemaking, is produced in large quantities worldwide and represents a rich source of polyphenols, particularly anthocyanins with known antioxidant properties. However, the limited stability of anthocyanins restricts their application. Pyranoanthocyanins, more stable anthocyanin-derived pigments formed during fermentation, offer a promising alternative. While widely studied in wines, their formation in winery by-products such as GP remains largely unexplored. Therefore, the targeted conversion of GP anthocyanins into pyranoanthocyanins represents a novel valorisation strategy aligned with circular economy principles.

This study investigates yeast-driven fermentation of GP skins from the red grapevine (*Vitis vinifera* L.) variety 'Refošk' and white variety 'Zelen' to promote pyranoanthocyanin formation. Initially, oven dried skins from 'Refošk' pressed GP were separated from the seeds, extracted to obtain an anthocyanin-rich skin extract. The lyophilized anthocyanin extract was reconstitued in a low pH (3.5) fermentation broth, supplemented with p-coumaric acid (500 mg/L) as a precursor of vinylphenolic pyranoanthocyanins, and inoculated with *Pichia guilliermondii* ZIM624, a high hydroxycinnamate decarboxylase activity yeast. In the second approach, a 50:50 (w/w) mixture of defrosted 'Refošk' and 'Zelen' GP skins was suspended in the same low pH fermentation broth using this matrix directly as a natural source of anthocyanins and hydroxycinnamic acids and inoculating with *P. guilliermondii* ZIM624. All fermentations were conducted in identical conditions, up to 10 days at 25°C, followed by cultures centrifugation and filtration, and supernatants' analysis employing spectrophotometer for colour evaluation and RP-HPLC on a C18 column coupled to UV–Vis detection for anthocyanins and pyranoanthocyanins composition and concentration determination. Moreover, fermentation supertantans were also evaluated using FRAP and ABTS assays, and their antioxidant and anti-inflammatory activity was assessed in human intestinal Caco-2 cells *in vitro*.

These findings demonstrate that targeted microbial fermentation of grape pomace can generate colour-stable pyranoanthocyanins while maintaining, and potentially enhancing, the bioactive properties of the resulting extracts, highlighting their potential as functional ingredients with health-promoting properties for human applications.

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S1-P27: Exploring the Impact of Native Yeasts on Cider Aroma and Composition: A Case Study from Hardanger

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This study investigates the role of native yeasts in shaping cider aroma and composition, focusing on strains isolated from the Hardanger region (Norway). A total of 40 *Saccharomyces* and non-*Saccharomyces* strains were selected from 372 identified isolates and evaluated in small-scale fermentations of Aroma apple juice at 15°C. Fermentations were comprehensively characterized using HPLC–UV/RI and HS-SPME-GC-MS, targeting sugars, organic acids, amino acids (AAs), biogenic amines (BAs), and volatile organic compounds (VOCs).

Multivariate analysis revealed pronounced, strain dependent metabolic diversity in both yeast groups. *Saccharomyces cerevisiae* and *Saccharomyces uvarum* strains exhibited distinct volatile profiles, with ester production emerging as the main driver of differentiation, while C6 alcohols and volatile phenols also contributed to strain discrimination, likely reflecting differences in yeast-mediated transformation of fruit-derived precursors during fermentation. Non-*Saccharomyces* strains formed four clusters corresponding to high ester- and volatile phenol-producing strains, acetate ester- and acetic acid-associated metabolism, mixed metabolic profiles, and low-activity phenotypes.

To further evaluate selected candidates, *S. uvarum* 2402 and *Pichia kluyveri* 2011 – representing clusters with high contents of ethyl esters, C6 alcohols and volatile phenols (*S. uvarum* 2402) or high ethyl and acetate ester production (*P. kluyveri* 2011) – were tested in lab-scale fermentations with Aroma apple juice, with a commercial *S. cerevisiae* strain as a control. Fermentations were carried out at 15°C without nitrogen addition and with diammonium phosphate supplementation to reach approximately 150 mg/L yeast-assimilable nitrogen, and ciders were analysed using the same HPLC–UV/RI and HS-SPME-GC-MS methods to quantify non-volatiles and volatiles. *S. uvarum* showed high fermentative efficiency and elevated VOC production with increased levels of key fruity esters (notably ethyl butyrate, ethyl octanoate, ethyl decanoate) and contributing floral compounds such as 2-phenylethanol. *P. kluyveri* enhanced the concentration of fruity ethyl and acetate esters in sequential fermentation with *S. uvarum*, and nitrogen supplementation further enhanced ester production. Additionally, sensory analysis was performed using quantitative descriptive profiling to evaluate the influence of yeast and nitrogen supplementation on cider aroma, with *S. uvarum* ciders perceived as more fruity and fresh apple, and *P. kluyveri*/*S. uvarum* sequential fermentations showing the most intense fruity and pear-like aromas, particularly under nitrogen supplementation.

Overall, native *Saccharomyces* and non-*Saccharomyces* yeasts from Hardanger exhibit distinct and complementary metabolic traits that can be exploited to modulate cider aroma, particularly in mixed or sequential fermentations combined with optimized nitrogen management.

S1-P28: Microbial Community Profiling of Greek-Style Black Table Olives of Cv. Conservolea and Cv. Kalamata Cultivars During Fermentation

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Fermentation of olives is essential for ensuring the quality and safety of the final product. Greek-style processing is one of the most widely used commercial methods, involving immersing olives in 6–10% (w/v) NaCl brine for 8–12 months, allowing natural fermentation driven by autochthonous microbiota. Despite its broad application, this process remains empirical. This study monitored physicochemical parameters and microbial dynamics during the Greek-style fermentation of two olive cultivars, cv. Conservolea and cv. Kalamata, to characterize microbial succession and its contribution to product quality.

Natural black Conservolea and Kalamata olives were fermented in plastic vessels containing 6% brine, in biological triplicates per cultivar. Physicochemical parameters (pH, salinity, titratable acidity) were monitored throughout fermentation, and salinity was adjusted to 6% when required. Microbial populations were enumerated by plate counting, targeting LAB, yeasts, moulds, *Enterobacteriaceae*, and *Staphylococcus aureus*. Microbial community composition in olives was assessed at the beginning, middle, and end of fermentation using 16S rRNA gene and ITS region amplicon sequencing.

The initial microbiota consisted mainly of *Enterobacteriaceae*, LAB, yeasts, and moulds. *Enterobacteriaceae* persisted during the first 15–20 days, while *S. aureus* was detected mainly in Conservolea during the first 4–10 days. In Conservolea samples, LAB dominated from day 5 until completion, whereas in Kalamata, LAB co-existed with yeasts and moulds throughout fermentation. Specifically, LAB initially ranged between 3–4 log CFU/g in olives for both cultivars, increased within the first 10 days, then declined to ~5 log CFU/g in Conservolea and ~3 log CFU/g in Kalamata. Yeasts and moulds ranged around ~5.5 log CFU/g in Conservolea and ~6 log CFU/g in Kalamata olives. Similar microbial population trends were observed in the brines. Taxonomic analysis in olives revealed that *Aspergillus* was the dominant fungal genus at all timepoints. Conservolea olives shifted from *Pantoea*, *Erwinia*, and *Leuconostoc* spp. to *Lactiplantibacillus* dominance, while Kalamata olives were characterized by *Leuconostoc* and *Lactiplantibacillus* co-dominance and a decline in *Lactococcus*. Both fermentations met International Olive Council standards (pH ≤ 4.3; acidity ≥ 0.3% lactic acid), with stronger acidification in Conservolea (pH 3.5; 0.7%) than in Kalamata (pH 4.2; 0.3%).

Both cultivars produced safe table olives but displayed distinct fermentation dynamics driven by variety-specific microbial communities. Conservolea supported a robust LAB-driven fermentation dominated by *Lactiplantibacillus* and greater acidification, whereas Kalamata maintained a more diverse ecosystem.

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S1-P29: Emerging *Hanseniaspora opuntiae* Populations in European Vineyards Show Hybrid Ancestry and Increased Stress tolerance

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Non-conventional yeasts of the genus *Hanseniaspora* are abundant in vineyards and grape must and one of the main contributors to the early stages of spontaneous fermentation and wine aroma development. *Hanseniaspora uvarum* has long been the predominant species isolated from European vineyards, but in recent decades *Hanseniaspora opuntiae*, originally found in natural environments such as cacti and other plants in warmer climates, has increasingly emerged in European vineyards, suggesting adaptation to human-influenced ecosystems and climate change.

To investigate the genomic and phenotypic basis of these microbiota changes, we analyzed a diverse collection of 200 *Hanseniaspora* isolates, including *H. uvarum* and *H. opuntiae* originating from different ecological niches. We combined high-throughput phenotyping under vineyard and fermentation relevant stress conditions with haplotype-resolved genome assemblies and population genomic analyses.

Our analyses showed that many European *H. opuntiae* isolates are hybrids with *Hanseniaspora pseudoguilliermondii*, suggesting that hybridization may have contributed to their successful adaptation and persistence in vineyard environments. In phenotypic assays, *H. opuntiae* and hybrid isolates showed an increased tolerance to multiple stress factors relevant to vineyard and fermentation environments, outperforming native *H. uvarum*. Genomic analyses further revealed extensive variation in ploidy, with many hybrids showing triploid genomes, indicating that changes in genomic architecture may contribute to increased fitness in these environments.

Together, these results suggest that hybridization and variation in genome structure and ploidy have played an important role in the recent emergence of *H. opuntiae* in European vineyards, with potential consequences for vineyard microbial ecology and fermentation performance.

S1-P30: Nitrogen Supplementation and Non-*Saccharomyces* Yeasts as Tools for Managing Challenging Alcoholic Fermentations

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Yeast-assimilable nitrogen (YAN) deficiency and the application of non-*Saccharomyces* starter cultures are among the most important variables influencing alcoholic fermentation performance and wine quality. This article integrates findings from four graduate research projects examining these two approaches across multiple grape varieties and fermentation conditions.

Nitrogen management was evaluated in musts of Malvasia, Welschriesling, and Žametna črnina (Black Velvet), representing challenging fermentation conditions due to either high sugar concentrations and/or low initial YAN levels. Three commercial nitrogen-enriched yeast nutrients — Go-Ferm Sterol Flash, Go-Ferm Protect Evolution, and Fermaid O — were applied at different stages of alcoholic fermentation (AF) and during yeast rehydration. Nitrogen supplementation consistently enhanced fermentation kinetics and positively affected the chemical composition of the resulting wines. The timing of nutrient addition proved decisive: supplementation at approximately one-third of AF duration yielded the most effective improvement in fermentation rate and nitrogen assimilation. The effectiveness of individual nutrient formulations was variety-dependent and closely linked to must composition, particularly initial YAN, free amino nitrogen (FAN), and ammoniacal nitrogen levels. Notably, extended yeast rehydration with Go-Ferm Sterol Flash consistently improved fermentation progression across all tested varieties, and wines (Malvasia and Welschriesling) produced using this approach were rated highest in sensory evaluation. Although nutrient supplementation did not always ensure complete fermentation in high-sugar musts, it reliably improved fermentation kinetics and wine chemistry in all tested varieties.

The contribution of non-*Saccharomyces* yeasts was assessed using commercial starter cultures — *Torulaspora delbrueckii* (Biodiva), *Lachancea thermotolerans* (Laktia), *Metschnikowia pulcherrima* (Flavia), and *Metschnikowia fructicola* (Gaia) — applied in sequential inoculation with *Saccharomyces cerevisiae* in Malvasia, Chardonnay, Merlot, and Blaufränkisch fermentations. Non-*Saccharomyces* strains alone were unable to complete AF, necessitating co-inoculation with *S. cerevisiae* to achieve fermentation completion and reduce residual sugars. Mixed-culture fermentations produced wines with greater aromatic complexity, higher glycerol content, and improved sensory scores. Optimal strain performance was variety-specific: *T. delbrueckii* and spontaneous AF yielded the most positively evaluated red wines, while outcomes for white varieties were strongly influenced by must composition.

Overall, these findings support an integrated fermentation management strategy, in which targeted nitrogen supplementation and tailored yeast selection are combined to ensure reliable fermentation completion and improved wine quality.

S1-P31: Untargeted Metabolomics for the Study of Sourdough Bread

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The present study investigates the impact of combining wheat and chickpea flours on the metabolite profile of previously stabilized sourdoughs, with a focus on microbial metabolic adaptation. The results demonstrate that introducing a novel food matrix creates a selective environment that modulates microbial metabolism and promotes alternative biochemical pathways. This metabolic shift leads to the production of compounds with potential nutraceutical value, which may positively influence the sensory attributes, shelf life, and nutritional quality of the final product.

These findings underscore the importance of controlled microbial adaptation as a strategy to enhance the functional properties of fermented foods. Specifically, the study demonstrates that adapting sourdough to legume-based substrates is a promising approach for developing innovative leavening agents with improved functional characteristics. Such methods can facilitate the production of next-generation fermented foods naturally enriched with bioactive compounds, aligning with contemporary consumer demand for health-focused and sustainable food products.

S1-P32: Mapping Microbial Dynamics and Functional Evolution During Spontaneous Fermentation of Bee Pollen from Different Origins

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The present study investigates the microbial dynamics and functional evolution during the spontaneous fermentation of bee pollen into bee bread. Using four fresh pollen samples from the Marche region (Italy) and a commercial reference, the research explores how botanical and geographical origins influence the microbial process over a 14-day period.

Microbiological analysis revealed a stable "fermentative core" dominated by the fructophilic lactic acid bacteria *Apilactobacillus kunkeei*, consistently present across all samples. A metabolic pathway analysis was performed to assess the microbial capacity for pollen wall degradation, highlighting functional traits potentially involved in the breakdown of complex pollen structures. This core microbiota was accompanied by a defined yeast succession, with the genus *Starmerella* prevailing in the early stages, while the osmotolerant yeast *Zygosaccharomyces rouxii* dominated the late-stage fermentation. Biochemical characterization of the fermented products showed a significantly enhanced nutritional profile, including increased protein content, reduced pH (3.5–4.0), and decreased total sugars. Microscopic observations confirmed that fermentation promotes the degradation of pollen grain structures, leading from intact morphology to cytoplasmic depletion, thereby improving nutrient bioavailability.

In addition, fermented bee bread exhibited enhanced antimicrobial activity against relevant foodborne pathogens such as *Staphylococcus aureus* and *Listeria monocytogenes*. These findings support spontaneous fermentation as a robust natural strategy for stabilizing bee products while enhancing their functional and therapeutic properties.

Further investigations were conducted on yeast isolates to deepen the functional characterization of key microbial players. *Starmerella* strains were analyzed for their ability to produce sophorolipids, while *Zygosaccharomyces* isolates were evaluated for ergosterol production. A comparative molecular analysis was also performed to identify genes potentially involved in these biosynthetic pathways, providing insights into the metabolic potential and industrial relevance of these yeasts. Overall, this study highlights the potential of controlled fermentation processes to standardize bee bread production, paving the way for its development as a stable, safe, and functional health product for consumers.

S1-P33: *Bacillus* spp. as Biopreservative Agents in Plant Food Systems

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The current research focuses on the microbiological safety of plant-based foods by drawing inspiration from Asian fermentation traditions, particularly natto. Natto is a Japanese superfood fermented by *Bacillus subtilis*; it is rich in vitamin K_2, poly-γ-glutamic acid, and nattokinase, and exhibits remarkable properties for inhibiting foodborne pathogens.

The goal of this work is to understand how *B. subtilis* prevents the growth of pathogenic bacteria in plant-based foods. By modeling microbial interactions, identifying key genes involved in biopreservation, and exploring the inhibitory capacities of various non-lactic bacterial species, this study aims to develop new food preservation systems.

These innovative biopreservation systems will meet the growing demand for safe, natural food products without the use of chemical preservatives. By leveraging the natural preservation mechanisms found in natto, this research aspires to enhance the safety of plant-based foods on an industrial scale, offering healthier alternatives to consumers.

S1-P34: From Preservation to Application: Study and Exploitation of Frozen and Freeze-Dried Microbiomes of Artisanal Natural Whey Starters and Sourdoughs.

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Microbial consortia (MC) play a fundamental role in many fermented foods. Unlike axenic cultures, the preservation of MC is still challenging, due to several constraints that may compromise viability and functionality.

In this study, we evaluated the effectiveness of cryopreservation (CrP), freeze-drying (FD), and different protective agents (e.g. skim milk, sucrose, glycerol, DMSO) on the survival (differential plate counting, fluorescence microscopy), composition (AT-HTS; V5-V6 region of 16S rRNA and ITS) and functionalities (acidification rate; EcoPlates® and OmniLog® Phenotype MicroArrays) of MCs from 5 natural whey starter (NWS) and 5 sourdoughs (SD) to identify the best conditions ensuring the maximum protection of all microbial fractions, after mid- (4 months) and long-term (8 months) preservation. Backslopping was applied on preserved MCs to verify the potential recovery of MC viability and functionality. The effectiveness of best-performing MCs (high survival and metabolic activity) was evaluated in model cheeses and sourdough breads. Microbiological profile (plate counting, AT-HTS) and volatilome (CG-MS) of final products were evaluated.

Microbial survival depended on the interaction between preservation technique and protective agents. High viability was found after 8 months in frozen MCs, while FD impaired microbial survival to a greater extent, in both NWS and SD samples. Thermophilic LAB in NWS and mesophilic ones in SD exhibited significant robustness to preservation processes, while yeasts were the most sensitive group. cFDA/PI staining was effective and rapid in assessing MC viability.

Metataxonomic and meta-phenomic analyses confirmed that composition and functionalities of preserved NWS and SD were correlated to the storage process and cryo-/lyo-protectants. Overall, CPr and FD did not significantly affect the macrostructure (dominant groups) of MCs, while more complex patterns were found for meta-phenomic profiles. FD and long-term storage significantly reduced the acidification rate (mainly for NWS), but backslopping procedures restored this functionality in both NWS and SD.

Model cheeses and SD breads produced with selected preserved MCs (after proper propagation cycles) had microbial and volatilome profiles similar to those of products obtained with control cultures.

The results confirm the effectiveness of CrP and FD in long-term storage of NWS and SD microbiomes. Some metabolic failures found in preserved cultures were reversed by proper backslopping procedures, leading to the production of products similar to those obtained with fresh starters, suggesting that our protocol may be used to overcome the constraints related to the microbial diversity and production variability of artisanal cultures.

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S1-P35: Site-Associated Yeast Communities in Blaufränkisch: Preliminary Insights from Slovenian Vineyards

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Microbial communities associated with grapes and fermentations have been proposed as one of several factors that may contribute to site-related differences in wine, but their role in shaping wine typicity remains insufficiently understood, particularly for Blaufränkisch wines in Slovenia. Within a multidisciplinary project that characterises vineyard sites through detailed geological, pedological and climatic analyses, we focus on yeasts as the most experimentally tractable part of the grape- and winery-associated microbiota and investigate *Saccharomyces* and non-*Saccharomyces* populations on Blaufränkisch grapes and in associated spontaneous fermentations from seven vineyards in the Dolenjska and Bela krajina wine-growing regions. Over two consecutive harvests, laboratory-scale spontaneous fermentations were performed and approximately 800 yeast isolates were obtained using a cultivation-based approach; these isolates are currently being molecularly identified and preserved in a dedicated culture collection.

Preliminary results indicate high yeast diversity and suggest site-dependent differences in species occurrence, while no clear grouping according to wine-growing region has been observed so far. As taxonomic resolution and statistical integration with geoclimatic datasets are still ongoing, these findings should be considered indicative and do not yet allow firm conclusions on microbiological contributions to vineyard or regional typicity. In the next phase, representative, well-characterised isolates will be used in controlled fermentations under standardised oenological conditions to evaluate the contribution of site-associated yeast consortia to wine chemistry and sensory attributes, providing an experimental basis to assess whether and to what extent microbial patterns are reflected in site-related wine style.

S1-P36: Starter Inoculation Shapes Microbial Succession, Metabolite Profiles and Intestinal Anti-Inflammatory Potential in Romanian Cucumber Fermentations

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Romanian fermented cucumbers (castraveți murați) are traditional ready-to-eat products produced at household level by spontaneous lactic acid fermentation in salt brine. Despite their widespread consumption across Eastern Europe, their microbiological safety and functional properties under real household conditions remain poorly characterised, and the impact of starter culture use has not been systematically evaluated.

Romanian-style cucumber fermentations were conducted over two weeks under spontaneous conditions or following inoculation with *Lactiplantibacillus plantarum* HF82. An integrated approach combined 16S rRNA amplicon sequencing, NMR-based metabolomics, antioxidant capacity assays, anti-inflammatory testing on HCT116-Dual intestinal epithelial cells, and digital PCR-based pathogen detection.

L. plantarum HF82 inoculation rapidly established *Lactiplantibacillus* dominance, driving faster acidification and more consistent microbial and metabolic trajectories compared with spontaneous fermentation. Inoculated fermentations accumulated lactate earlier and showed higher levels of pyridoxine and glutamine, while spontaneous fermentations were enriched in branched-chain amino acid catabolism products and formate. Both conditions reduced NF-κB activation in IL-1β-stimulated cells, but spontaneous fermentation was associated with higher IL-8 secretion. Neither *Listeria monocytogenes* nor *Salmonella* spp. were detected in any sample.

These results demonstrate that starter inoculation improves consistency, functional quality and safety predictability of artisanal Romanian cucumber fermentations, providing a scientific basis for the rational use of defined LAB cultures in traditional food production.

S1-P37: *Streptomyces rimosus* as a Non-GMO Platform for Natural Carotenoid Production

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Carotenoids are lipophilic pigments, typically yellow, orange, or red, and represent one of the most diverse and widespread classes of natural colorants in plants, algae, fungi, and microorganisms. Beyond their traditional use as food colorants, cosmetics ingredients, and feed additives, their importance for human health has only been fully appreciated in recent decades. Numerous studies and recent reviews highlight their antioxidant, anti-inflammatory, anti-tumor, cardioprotective and neuroprotective activities, as well as their role as provitamin A compounds and functional food ingredients. The chemical total synthesis of carotenoids is a well-established, cost-effective process that currently covers a major share of the global market, particularly for β-carotene and astaxanthin used as food and feed colorants. At the same time, there is growing interest in naturally derived carotenoids from microbial or plant biosynthesis, driven by consumer preference for natural ingredients, and by evidence that native isomeric mixtures and food matrices can modulate biological activity and health effects. At present, native β-carotene biosynthesis at industrially relevant levels is limited to a relatively small group of carotenoid-producing microorganisms such as *Blakeslea trispora*, *Rhodotorula glutinis* and *Dunaliella salina*, whose productivity and process costs can still be limiting for large-scale applications. Actinomycetes form another important group of natural carotenoid producers: several species harbour biosynthetic gene clusters for C_{40} and C_{50} carotenoids, for example isorenieratene and related aromatic carotenoids. In *Streptomyces rimosus* targeted deletion or modification of individual genes within these clusters can redirect the pathway towards intermediates such as lycopene or β-carotene, enabling the generation of non-GMO producer strains that accumulate different carotenoid profiles by exploiting native genomic plasticity rather than introducing foreign DNA. Such non GMO exploitation of actinomycetes opens up opportunities for the development of new, safety acceptable and industrially attractive sources of natural carotenoids for food and nutraceutical applications.

Keywords: carotenoids, β-carotene, actinomycetes, *Streptomyces rimosus*, non-GMO production, microbial biosynthesis

S1-P38: Legumes as a Sustainable Alternative to Animal Protein: Improving Sensory Properties of Pea and Faba Bean Concentrates by Lactic Acid Fermentation

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Legume-derived proteins are increasingly recognised as sustainable alternatives to animal protein due to their lower environmental footprint, favourable nutritional profile, and compatibility with plant-based food systems. Among these, pea and faba bean ingredients are particularly attractive for food applications. However, the broader use of legume protein concentrates and isolates is often limited by undesirable sensory characteristics, including bitterness, astringency, and green/beany off-flavours. In addition, these raw materials may contain antinutritional compounds such as phytates and saponins, which can negatively affect mineral bioavailability and consumer acceptance.

Fermentation with lactic acid bacteria (LAB) represents a promising strategy to improve both the sensory and functional properties of legume-based ingredients. Nevertheless, commercially available starter cultures specifically optimised for plant matrices remain limited. In this study, we evaluated the potential of LAB fermentation to enhance the quality of pea and faba bean concentrates using both commercial starter cultures and selected autochthonous strains isolated from plant-based environments.

Fermentation performance was monitored using isothermal microcalorimetry, which enabled real-time assessment of microbial metabolic activity and comparison of strain adaptation to legume substrates. Different flour concentrations, inoculation levels, and fermentation times were investigated to optimise process conditions. Volatile aroma compounds were subsequently analysed by gas chromatography–mass spectrometry (GC–MS) to identify changes associated with flavour improvement and reduction of undesirable notes.

Based on calorimetric and instrumental screening, the most promising fermentation candidates were selected for sensory evaluation. Human panel assessment was used to validate analytical findings and determine perceived improvements in aroma, taste, and overall acceptability.

Our results demonstrate that carefully selected LAB strains can significantly improve the sensory profile of pea and faba bean concentrates, while offering a natural processing solution for legume valorisation. This work supports the development of next-generation fermented legume ingredients with improved consumer acceptance and contributes to the transition toward more sustainable protein sources.

S1-P39: Evaluation of Lactic Acid Bacteria Isolated from Fish Sauce for Their Probiotic Potential and Antimicrobial Activity

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Lactic acid bacteria (LAB) isolated from traditional fermented foods have attracted significant attention due to their probiotic properties and potential applications as natural biopreservatives. This study aimed to isolate and characterize LAB strains from commercial fish sauce and evaluate their probiotic potential and antimicrobial activity against common foodborne pathogens. A total of fifteen LAB strains were successfully isolated and identified based on phenotypic characteristics and 16S rRNA gene sequencing, predominantly belonging to the genera *Lactobacillus* and *Pediococcus*. Among these, strain FS003 exhibited exceptional tolerance to simulated gastrointestinal conditions, maintaining a survival rate of over 85% after 3 h of exposure to gastric juice (pH 2.5) and 4 h of exposure to 0.3% bile salts. Furthermore, cell-free supernatants (CFS) from strain FS003 demonstrated robust antimicrobial activity against major foodborne pathogens, including *Staphylococcus aureus*, *Listeria monocytogenes*, and *Escherichia coli*. The antimicrobial effect was partially attributed to organic acid production, as acid-neutralized CFS retained significant inhibitory activity against *L. monocytogenes*, suggesting the presence of bacteriocin-like inhibitory substances. Additionally, strain FS003 displayed high cell-surface hydrophobicity (68%) and strong autoaggregation capability (72%), indicating a high potential for gastrointestinal colonization. These findings demonstrate that LAB strains derived from fish sauce, particularly strain FS003, possess excellent probiotic attributes and strong antimicrobial potential. Consequently, this strain represents a promising candidate for development as a functional probiotic culture or a natural bio-protective agent in the food industry to enhance microbial safety and extend product shelf life.

Keywords: lactic acid bacteria, fish sauce, probiotic potential, antimicrobial activity, foodborne pathogens

S1-P40: Kombucha as a Fermented Beverage: Bioactive Compounds and Antioxidant Capacity

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Kombucha is a traditional fermented beverage produced from sweetened tea, most commonly black or green tea, through the symbiotic activity of bacteria and yeasts known as a SCOBY (Symbiotic Culture of Bacteria and Yeast). During fermentation, sucrose is converted into organic acids, carbon dioxide, ethanol, and a variety of bioactive compounds, including polyphenols. These biochemical transformations significantly alter the chemical composition of the beverage, thereby influencing its antioxidant activity, sensory properties, and overall functional quality.

The aim of this study was to evaluate the effect of refrigerated storage on the stability of polyphenolic compounds and antioxidant capacity in five different kombucha formulations. Black tea was used as the conventional substrate, while alternative substrates included yerba mate, vinegar, a mixture of yerba mate and sencha, and cornelian cherry. These substrates were selected to assess how raw material composition affects the stability of bioactive compounds during storage. All samples were stored at 4°C and analyzed after 7, 14, and 21 days.

The analyzed parameters included total phenolic content, flavonoids, hydroxycinnamic acids, flavonols, monomeric anthocyanins, and proanthocyanidins. Antioxidant capacity was evaluated using ABTS and FRAP assays to provide a comprehensive assessment of antioxidant potential.

Results showed that both substrate type and storage duration significantly influenced the stability of bioactive compounds and antioxidant activity. The vinegar-based kombucha exhibited the highest levels of bioactive compounds and antioxidant capacity, indicating a strong substrate-dependent effect on product quality. During storage, total phenolic compounds and hydroxycinnamic acids generally increased in most samples, while flavonoid levels remained stable. Flavonols increased up to day 14 and then declined, whereas proanthocyanidins decreased over time. Antioxidant capacity changes varied depending on the assay and substrate used, reflecting the complexity of antioxidant behaviour in fermented systems.

Overall, the study demonstrates that both formulation and storage conditions are key factors determining the chemical stability and functional properties of kombucha beverages. Future research should focus on clarifying the mechanisms behind these changes during storage and on optimizing fermentation substrates and conditions to enhance the stability and bioactivity of Kombucha.

S1-P41: Effects of Antimicrobial Stress on the Fermentation Performance of an Antibiotic-Resistant *Lacticaseibacillus paracasei* Isolated from Fermented Dairy Products

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Lactic acid bacteria (LAB) represent a diverse group of Gram-positive microorganisms that have gained significant popularity as starter cultures in the fermentation of dairy products. This is due to their ability to enhance food safety, improve sensory properties, extend shelf life, and provide probiotic health benefits. Nevertheless, the appearance of antibiotic-resistant LAB has given rise to concerns, as these bacteria may function as reservoirs of antimicrobial resistance (AMR) genes. These genes could be transferred to pathogenic microorganisms via the food chain and lead to alterations in fermentation performance, as a consequence of changes in bacterial physiology or metabolism associated with the resistance mechanism. The objective of the present study was to isolate lactic acid bacteria (LAB) from dairy products sourced from small-scale Hungarian farmers, to test their antibiotic susceptibility, and, in the case of a selected resistant strain, to examine how its fermentation capacity changes in response to antibiotic stress.

LAB were isolated from raw milk, cheese, cottage cheese, yoghurt, and sour cream using M17 and MRS media. The isolates were identified by MALDI-TOF MS and 16S rDNA sequencing. The determination of antimicrobial susceptibility was conducted by employing the Kirby–Bauer disk diffusion method, utilising a total of twelve antibiotics. A ciprofloxacin-resistant LAB strain was then selected and used to ferment milk containing different concentrations of ciprofloxacin. The fermentation changes were monitored using conventional culturing methods, an electronic nose and an electronic tongue.

A total of thirteen LAB isolates were recovered from raw milk and fermented dairy products, and identified as *Lactococcus lactis*, *Lactobacillus curvatus*, and *Lacticaseibacillus paracasei*. It was observed that several isolates exhibited resistance, with no effect on growth in the presence of aztreonam, ciprofloxacin, kanamycin, and vancomycin. In LAB, resistance to aztreonam, kanamycin and vancomycin is generally regarded as intrinsic, whereas resistance to ciprofloxacin is more commonly associated with strain-specific or acquired resistance mechanisms. The presence of ciprofloxacin significantly altered the fermentation profile, as demonstrated by the clear discrimination between fermentations performed in the presence and absence of the antibiotic using electronic nose and electronic tongue analyses. These findings indicate that antibiotic-induced changes in milk fermentation can be effectively monitored by electronic nose and electronic tongue technologies, highlighting their potential as rapid analytical tools for detecting fermentation disturbances associated with antibiotic residues in milk.

S1-P42: Isolation and Characterization of Probiotic Bacteria from Eleusine Coracana (Finger Millet) -ML365 Fermented Flour

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The research investigated the isolation and identification of lactic acid bacteria (LAB) from fermented flour of finger millet (Eleusine coracana) flour to assess its potential for developing probiotic foods. The study involved two selective media (de Man Rogosa and Nutrient Agar) to isolate the most abundant bacteria from the millet flour. The isolates were tested for various traits such as gram staining, biochemical properties and morphology. Additionally, the probiotic potential of the isolates was assessed by evaluating their tolerance to acidic conditions, bile, NaCl, phenol and various antibiotics (e.g., tetracycline, ampicillin and ciprofloxacin). Based on these evaluations, five bacterial isolates were selected, and their 16S rDNA gene sequences were analyzed for identification. The bacterial isolates exhibited homology with Bacillus pumilus, Bacillus cereus, Bacillus subtilis, Bacillus amyloliquefaciens and Brevibacterium sp. The unique accession numbers were obtained following the submission of sequences to NCBI. The findings suggest that these isolates have potential as probiotic strains, particularly for populations with lactose tolerance. This opens the possibility for developing novel probiotic food products derived from finger millet, a nutrient-dense, under-utilized crop.

S1-P43: Screening of Yeast–Apple Cultivar Interactions and Their Impact on Chemical and Sensory Quality of Cider

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Cider quality is strongly influenced by both the apple raw material and the fermentation microbiota, yet systematic data on yeast–cultivar interactions under Nordic conditions remains limited. In this study, we conducted a screening experiment to assess how combinations of eight commercial *Saccharomyces cerevisiae* strains and five apple cultivars shape the chemical and sensory characteristics of cider.

Single-cultivar juices were fermented with each of the eight *S. cerevisiae* strains under standardized conditions to isolate the respective contributions of yeast strain and cultivar. Chemical composition was characterised by analyses of key fermentation parameters and volatile aroma compounds, while sensory quality was evaluated by a trained panel focusing on aroma and flavour profile.

Both yeast strain and apple cultivar exerted marked effects on cider composition and sensory expression, with clear yeast–cultivar interaction patterns. Certain strains enhanced cultivar-specific aroma characteristics, while others contributed more strongly to fermentation-derived notes and mouthfeel. Differences in volatile composition were closely associated with sensory outcomes.

This screening highlights the importance of tailoring yeast selection to the apple cultivar in order to optimize cider style and quality. The findings provide a basis for targeted strain choice and the development of differentiated cider products, particularly relevant for cool climate and emerging cider regions.

S1-P44: Effects of antimicrobial stress on the fermentation performance of an antibiotic-resistant Lacticaseibacillus paracasei isolated from fermented dairy products

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Lactic acid bacteria (LAB) represent a diverse group of Gram-positive microorganisms that have gained significant popularity as starter cultures in the fermentation of dairy products. This is due to their ability to enhance food safety, improve sensory properties, extend shelf life, and provide probiotic health benefits. Nevertheless, the appearance of antibiotic-resistant LAB has given rise to concerns, as these bacteria may function as reservoirs of antimicrobial resistance (AMR) genes. These genes could be transferred to pathogenic microorganisms via the food chain and lead to alterations in fermentation performance, as a consequence of changes in bacterial physiology or metabolism associated with the resistance mechanism. The objective of the present study was to isolate lactic acid bacteria (LAB) from dairy products sourced from small-scale Hungarian farmers, to test their antibiotic susceptibility, and, in the case of a selected resistant strain, to examine how its fermentation capacity changes in response to antibiotic stress.

LAB were isolated from raw milk, cheese, cottage cheese, yoghurt, and sour cream using M17 and MRS media. The isolates were identified by MALDI-TOF MS and16S rDNA sequencing. The determination of antimicrobial susceptibility was conducted by employing the Kirby–Bauer disk diffusion method, utilising a total of twelve antibiotics. A ciprofloxacin-resistant LAB strain was then selected and used to ferment milk containing different concentrations of ciprofloxacin. The fermentation changes were monitored using conventional culturing methods, an electronic nose and an electronic tongue.

A total of thirteen LAB isolates were recovered from raw milk and fermented dairy products, and identified as *Lactococcus lactis*, *Lactobacillus curvatus*, and *Lacticaseibacillus paracasei*. It was observed that several isolates exhibited resistance, with no effect on growth in the presence of aztreonam, ciprofloxacin, kanamycin, and vancomycin. In lactic acid bacteria, resistance to aztreonam, kanamycin and vancomycin is generally regarded as intrinsic, whereas resistance to ciprofloxacin is more commonly associated with strain-specific or acquired resistance mechanisms. The presence of ciprofloxacin significantly altered the fermentation profile, as demonstrated by the clear discrimination between fermentations performed in the presence and absence of the antibiotic using electronic nose and electronic tongue analyses. These findings indicate that antibiotic-induced changes in milk fermentation can be effectively monitored by electronic nose and electronic tongue technologies, highlighting their potential as rapid analytical tools for detecting fermentation disturbances associated with antibiotic residues in milk.

S2 Microbiomes in Sustainable Food Systems, Mitigation of Food Spoilage, Losses and Wastes

S2-P1: Modulation of Spoilage Microbiota During MAP Storage of Chicken Meat Treated with Fermentates: Case Studies from Norway and Portugal

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Microbial spoilage is a major constraint on the shelf life of fresh chicken meat, which varies widely depending on initial microbial load, packaging atmosphere, storage temperature, and product origin. Fermentates such as buffered vinegars are increasingly explored as natural preservation strategies. This study evaluated the effects of two commercial fermentates on the microbiota and spoilage progression of chicken breasts originating from Norway and Portugal, representing meat with variations in microbial status and shelf life characteristics.

Aligned experimental protocols were applied in both countries: Chicken breast portions of similar surface area (~50 g,) were immersed in 5% (w/w) aqueous solutions of the fermentates or sterile water (control), packaged under modified atmospheres (High CO_2: 50% CO_2/50% N_2 or High O_2: 70% O_2/30% CO_2), and stored chilled for 15 days in Portugal (4°C for 4 days, then 8°C) and 26 days in Norway (4°C for 7 days, then 8°C). The difference in duration of the storage experiments in Norway and Portugal was due to different shelf life of the chicken from these countries. Microbial growth and dynamics were monitored by enumeration on Plate Count Agar and MRS agar, and by 16S rRNA gene amplicon sequencing.

Marked differences were observed between the microbiological profiles of the Portuguese and Norwegian chicken. Bacterial loads increased during storage in all samples but remained lower in Norwegian meat (< 7 log CFU/cm²) compared with Portuguese samples (~8 log CFU/cm²). Fermentate treatments suppressed microbial growth during the early storage phase in products from both countries.

Control samples from Norway were dominated by *Lactobacillales* at the end of storage, while Portuguese controls showed a mixed community dominated by *Yersiniaceae* and *Carnobacterium* spp., with *Yersiniaceae* becoming predominant by day 15. Fermentate-treated samples exhibited microbiomes dominated by lactic acid bacteria in samples from both countries. Fermentate treatments reduced the relative abundance of spoilage-associated taxa such as *Hafnia* spp. and *Pseudomonas* spp. in Norwegian chicken meat samples and substantially suppressed (although still detected) *Yersiniaceae* in Portuguese samples. *Brochothrix* spp. were detected in Portuguese samples across all treatments and occurred at higher relative abundance in fermentate-treated samples.

Overall, fermentate application induced a restructuring of the chicken meat microbiome, selectively inhibiting common spoilage-associated bacteria while promoting lactic acid bacteria. However, *Brochothrix* spp. appeared to persist or potentially benefit from the altered microbial ecosystem. Further work will assess sensory impact and explore optimization of fermentate-based preservation strategies.

S2-P2: From Field to Plate: Unravelling the Interconnections Among Microbiota of Soil, Plant and Fermented Red Cabbage

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Fermented vegetables are widespread in different culinary traditions around the world and some of the most famous examples are kimchi, table olives and sauerkraut. Regarding the production processes, spontaneous fermentation is the most common way mainly because of tradition and the complex flavour profiles that it creates. In spontaneous fermentation the fermentation process is started by the microorganisms already present on the plant. The quality of the raw materials then is of great importance. The microbial community present on the raw material derives from the phyllosphere microbiota which is strictly connected to the soil microbiota. This means that great attention should be given also to the agricultural practices and to all the other factors influencing soil and phyllosphere microbiota moving the focus of research to the possible interconnections. In the last years, in fact, research mainly focused on the effects of fermented vegetables on human health and on the selection of starter cultures, rather than to the interconnections among microbiota of soil, plant and fermented products.

The aim of this project is to deepen the knowledge related to the link between the agroecological practices (such as the application of soil improvers, beneficial microorganisms and biotics to the soil) and the microbial composition involved in the fermentation of the vegetables.

To this end we studied the microbial communities present during the fermentation of red cabbage and the microbial communities present in the soil, rhizosphere and phyllosphere from the field where red cabbage was cultivated. To accomplish this, we employed culture-dependent methods such as the microbial plate count on selective media and culture-independent methods. Then to explore the interconnections between the field and the fermentation process we analysed the microbial communities through a metabarcoding approach.

By unravelling the interconnections between the field and the fermentation process we believe that this research contributes to the expansion of new research topics. These results, in fact, help to develop the optimal strategy to produce fermented vegetables of higher quality and optimize the spontaneous fermentation process.

S2-P3: Determinants of Spoilage Microbiota in Chilled Atlantic Salmon Fillets

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Predicting spoilage of chilled seafood remains challenging because microbial development reflects both variation introduced during processing and subsequent microbial selection during chilled storage. This study examines factors influencing spoilage microbiota in fillets of Atlantic salmon (*Salmo salar*) using an integrated design that combines monitoring of the processing environment with controlled shelf life experiments.

Sampling was conducted over two years at a commercial slaughter and filleting facility, covering 19 production days across different seasons. Microbial swab samples were collected from selected equipment surfaces and from salmon at key points along the production line. Fillets produced on the same days were wrapped in plastic film and stored on ice at 0°C in expanded polystyrene (EPS) boxes, or vacuum packaged and stored at both 0°C or 4°C. Microbial load and composition, and product quality were assessed during storage using culture-based methods, MALDI-TOF identification, and 16S rRNA gene amplicon sequencing, alongside sensory evaluation and instrumental measurements of firmness. In total, 616 fillets and 768 environmental samples were analysed.

The study design enabled direct comparison of microorganisms present on the product shortly after production with those dominating at later stages of storage, and assessment of how variation between production days and storage conditions influenced microbial succession. Despite generally low bacterial levels on fresh fillets, microbial development during storage showed substantial batch to batch variability. Initial product microbiota was typically dominated by *Pseudomonas*, whereas later during storage (14–19 days), the microbiota in most batches, both when stored in EPS boxes and in vacuum, shifted toward dominance by *Photobacterium*. Sensory differences between batches were modest and difficult to discern under proper chilled storage conditions. Final bacterial levels remained below levels typically associated with microbial spoilage (10^7 CFU/g). Contamination levels measured on salmon fillets sampled during production showed no significant association with bacterial loads in early product samples, indicating that simple hygiene indicators did not predict early product microbiota.

Rather than indicating a single controlling factor, these results show spoilage as the outcome of batch-specific microbial communities and selection during storage, with limited explanatory power from production hygiene indicators. Substantial batch to batch variation and modest sensory discrimination highlight the need for caution when interpreting spoilage patterns and shelf life expectations in chilled salmon.

S2-P4: Integrating Microbiology, 16S Metagenomics and Organoleptic Assessment to Investigate Fresh Pork Microbiota Dynamics During Shelf-Life

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This project aimed to establish a comprehensive overview of the microbiological composition of French pork at the cutting stage by combining culture-dependent methods, metagenomic analyses and sensory assessments. The objective was to build a knowledge base to better understand spoilage mechanisms and non-compliance issues, ultimately improving product quality control.

The study was conducted in five industrial cutting plants between 2023 and 2024. One sampling campaign was performed. Three types of pork cuts were analyzed at both the beginning and the end of shelf life: shoulder and belly stored under air, and pork loin chops stored under vacuum, air, and modified atmosphere packaging (70% CO_2/30% O_2). Analyses included the enumeration of several microbiological indicators (aerobic mesophilic count, lactic acid bacteria, *Enterobacteriaceae*, *Pseudomonas* spp., and sulfite-reducing anaerobes) by culture-dependent methods. These were complemented by bacterial populations analysis using metagenomic sequencing of a portion of the 16S rRNA gene, and a sensory evaluation of the products.

Microbiological results showed that initial contamination levels of fresh meat were globally similar between plants, whereas microbial concentration and diversity differed markedly at the end of shelf life. These variations were mainly associated with the initial composition of the product microbiota, probably shaped by the specific microbial populations of each processing environment (equipment, operators, surfaces…). In several cases, products exceeding hygiene criteria remained sensorially acceptable. This suggests that meat spoilage is more closely related to the composition of bacterial populations than to overall contamination levels. Metagenomic analysis provided deeper insight into the diversity and dynamics of bacterial populations. At day 0, microbial diversity was highly similar across the three pork cuts, with dominant genera including *Pseudomonas*, *Acinetobacter*, *Brochothrix*, *Achromobacter*, *Serratia*, and *Brevundimonas*. Some minor genera such as *Lactococcus*, *Dietzia* or *Massilia* were only identified in products from specific plants. This diversity decreased significantly at the end of shelf life, favoring bacterial genera adapted to storage conditions. Under aerobic packaging, *Pseudomonas* and *Brochothrix* became largely dominant while non psychrotrophic genera *Achromobacter* and *Brevundimonas* disappeared from the dominant microbiota. Under modified atmosphere packaging, lactic acid bacteria, including *Lactococcus*, *Leuconostoc*, and *Carnobacterium*, were favored in pork chops. In contrast, under vacuum packaging, the bacterial populations were dominated by *Latilactobacillus* and *Pseudomonas*, the latter being able to survive without oxygen.

A more detailed characterization of the species and their metabolic functions, particularly through shotgun metagenomics or metabolomics approaches, would help to precisely identify the microorganisms responsible for spoilage and the mechanisms involved.

S2-P5: Assessment of a Microbiome Engineering Approach Using *Lactilactobacillus sakei* as Bioprotective Culture to Extend the Shelf Life of Gilthead Seabream (*Sparus aurata*) Fillets

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The Mediterranean gilthead seabream (*Sparus aurata*) is a widely consumed seafood product, with global production reaching 354,920 tonnes in 2022, primarily from aquaculture (FAO, 2024). However, seabream is microbiologically sensitive and highly perishable, leading to significant food waste. Following slaughter, the seabream flesh is rapidly colonized by bacteria. Certain bacteria contribute to spoilage by producing metabolites responsible for quality degradation and sensory rejection.

Biopreservation is a promising soft preservation strategy that aims to modulate the microbiota of food product to slow spoilage or inhibit pathogen growth. It involves inoculating the product with microorganisms or metabolites that exhibit antimicrobial activity.

In the frame work of the European project Foodguard (https://www.foodguard-project.eu/), the biopreservative potential of five protective lactic acid bacteria (LAB) strains was accessed on seabream fillets stored under modified atmosphere packaging (MAP: 20% 0_2/30% CO_2/50% N_2) at 8°C during 11 days. To correlate microbiota composition dynamics, biochemical profiles and sensory attributes, multi-omics statistical analyses were performed. They highlight biologically relevant and robust molecular signatures and confirmed the protective culture effect. Among the tested strains, a *Latilactobacillus sakei* strain (belonging to the Qualified Presumption of Safety list), significantly reduced the growth of seabream spoilage-associated bacteria and preserve the fillet quality during storage. This strain was further evaluated under different packaging and storage conditions including different MAP conditions as well was low and abuse temperatures.

Finally, the effect of the lyophilized strain was validated in an industrial pilot-scale trial conducted in a seafood processing facility in Greece. During this shelf-life experiment performed, more than 350 fillets were stored at 4°, 8° or 12°C. The impact of the strain on the microbial community and fillet quality were evaluated using a combination of classical microbiological methods, 16S amplicons sequencing approach, biochemistry analysis and sensory evaluations conducted by an expert panel.

These results highlight the potential of *L. sakei* as an effective protective culture for improving the shelf life and quality of seabream fillet.

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S2-P6: Microbial Variation and Consumer-Anchored Shelf-Life of Chicken Breast Fillets

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A clear link between microbial spoilage, sensory change, and consumer rejection is needed to set a realistic shelf-life and reduce avoidable food waste. This study combined a year-long shelf-life investigation of chicken breast fillets from a Norwegian producer using high-CO_2 MAP and a Portuguese producer using high-O_2 MAP with complementary consumer tests on selected products. Fillets were stored at constant 4°C or subjected to a temperature shift from 4 to 8°C simulating domestic temperature abuse.

Spoilage and rejection were characterised using total viable counts (TVC), 16S rRNA gene amplicon sequencing, MALDI-TOF MS, descriptive sensory profiling, and consumer odour-acceptance testing. Initial TVC were lower for Norwegian than Portuguese products (mean 2.4 vs. 4.3 $\log_{10}$ CFU/g), with ≈2-log variation across batches. Variation in both initial TVC and microbiota composition was greater between production days than between batches produced on the same day.

Late-stage spoilage communities remained producer-specific, with *Lactobacillales* and *Hafnia* spp. dominating Norwegian products and *Brochothrix* spp. and *Yersiniaceae* dominating Portuguese products. Portuguese products reached the commonly applied 7 $\log_{10}$ CFU/g spoilage threshold earlier than the Norwegian products. For the Norwegian products, days to 7 $\log_{10}$ CFU/g ranged from 16–29 days at 4°C (mean 21) and 12–20 days under temperature shift (mean 14). For the Portuguese products, the corresponding ranges were 8–17 days at 4°C (mean 11) and 6–10 days under temperature shift (mean 7). Days to 7 $\log_{10}$ CFU/g correlated strongly with the estimated lag time, but only weakly with initial TVC. Sensory attributes changed largely in parallel during storage and closely tracked bacterial load, while temperature shift mainly accelerated these changes.

In the consumer study (one batch from each producer), 25% rejection at 4°C occurred after 25 days in Norway and after 12 days in Portugal, corresponding to TVCs of 7.4 and 7.3 $\log_{10}$ CFU/g, respectively. These rejection levels were slightly above the conventional 7 $\log_{10}$ CFU/g benchmark and broadly consistent with the producer-specific growth modelling.

Overall, shelf-life was associated primarily with growth dynamics, particularly the lag phase, whereas initial TVC had limited predictive value within producers. An additional early TVC measurement to refine batch-specific lag may provide a practical basis for adaptive, data-driven shelf-life setting. Days to 7 $\log_{10}$ CFU/g can be a useful shelf-life indicator when the spoilage microbiota is stable, provided that the threshold has been anchored to consumer rejection for the relevant product.

S2-P7: Microbiome Dynamics in Chilled Pikeperch and Development of a Sustainable Printed Spoilage Indicator for Reducing Food Waste

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Food spoilage in fish is driven by shifts in microbial communities that generate characteristic metabolites during storage. In this study, we applied both metabarcoding-based microbiome profiling and culture-based enumeration to characterize microbial succession in chilled pikeperch and to provide microbiological knowledge essential for optimizing a low-cost, colour-changing printed indicator designed to detect spoilage-associated volatiles.

A small-scale trial storage experiment was conducted using pikeperch packaged under modified atmosphere (60% CO_2, 40% N_2) and stored at 4°C and 6°C for six days. Microbial quality was assessed on days 0, 1, 3, and 6. Bacterial and fungal communities were analyzed from DNA extracted from fish using a multi-marker metabarcoding method, while detailed bacterial composition was also quantified by plating onto selective agar media. Performance of printed spoilage indicators – tag based on specific chromogenic reaction responding to the presence of TVB-N (Type a) and to the presence of aldehydes and ketones (Type b) in the package headspace was studied. In addition, volatile compounds were analyzed with GC-MS.

Culture-based enumeration showed that at the beginning of the trial, the total viable count was 5 log CFU/g, with H_2S-producing bacteria and lactic acid bacteria (LAB) dominating. *Enterobacteriaceae* were initially present as a minor group. By the end of storage (6 days), the total viable count and H_2S-producing bacteria exceeded 7 log CFU/g, surpassing national microbiological guideline values (ETL, 2022), and spoilage indicators clearly confirmed product spoilage after 6 days. LAB dominated the bacterial community, closely followed by H_2S producers. *Brochothrix* and *Enterobacteriaceae* exhibited substantial microbial growth during chilled storage, confirming significant proliferation.

Metabarcoding revealed clear temperature-dependent bacterial succession patterns. During the first 1–3 days, psychrotrophic spoilers such as *Pseudomonas* and *Photobacterium* increased rapidly, indicating early aerobic spoilage. By day 6, storage at 6°C favoured continued aerobic growth, including *Shewanella*, whereas 4°C sufficiently suppressed aerobes and allowed *Carnobacterium* to become more prominent, consistent with LAB dominance observed in culture-based results. Fungal succession followed a parallel pattern, shifting from early filamentous fungi (*Neurospora*, *Lecanicillium*) to late-stage cold-tolerant yeasts (*Mrakia*, *Rhodotorula*), with faster progression at 6°C.

Together, the combined spoilage-associated microbiome and volatiles provide a robust microbiological baseline for validating and refining the sensitivity and operational performance of the printed spoilage indicator.

References: Finnish Food and Drink Industries' Federation (ETL), 2022. Microbiological guideline values for food products. Updated version 25 May 2023. Available at: https://www.etl.fi/wp-content/uploads/2023/08/etl_microbiological_guideline-values-oct-2022-english-final-25.5.2023.pdf

S2-P8: Control of *Sporolactobacillus* spp. Growth in Diced Fruit Pulp-Containing Beverages Using Lactic Acid as an Alternative Acidulant

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Sporolactobacillus spp. (SPLB) are recognized as spoilage organisms in acidic beverages, particularly those containing fruit juice. Owing to their heat resistance, SPLB can survive thermal pasteurization processes and subsequently proliferate at pH values below 4.0, posing a microbiological risk in acidic beverages. However, the growth behavior of SPLB in beverage systems containing diced fruit pulp pieces has not been sufficiently investigated. Therefore, this study aimed to assess the growth potential of SPLB in such beverages and to explore effective control strategies. Microbial tests were conducted using ready-to-drink (RTD) beverages containing diced yellow peach pulp pieces, supplemented with bacteriostatic emulsifiers. SPLB was inoculated at 10 CFU/mL and stored at 25°C for three months. By the third month, spoilage caused by SPLB was observed, characterized by an increase in pH and turbidity, despite the presence of emulsifiers. These results suggest that the presence of diced fruit pulp pieces interferes with the effectiveness of bacteriostatic emulsifiers. To investigate the mechanism, organic acid profiles in the spoiled beverages were analyzed, revealing a substantial decrease in citric acid accompanied by increases in lactic acid and acetic acid. These findings indicate that SPLB proliferation led to citric acid consumption, resulting in pH elevation. Based on these results, the impact of acidulant composition on SPLB growth was further investigated. In a subsequent product challenge test, substituting citric acid with lactic acid resulted in no spoilage during three months of storage. This demonstrates the effective control of SPLB by lactic acid in diced fruit pulp–containing RTD beverages, where bacteriostatic emulsifiers alone were insufficient. The potential of lactic acid for SPLB control was further evaluated under beverage-relevant conditions using non-alcoholic, non-carbonated model beverages containing lactic acid ranging from 0 to 2200 ppm. At an inoculum of 10 CFU/mL, lactic acid concentrations of 1500 ppm or higher exhibited a clear bacteriostatic effect under anaerobic conditions at 25°C for 7 days. Overall, acidulant selection was identified as a critical determinant of microbial stability in diced fruit pulp–containing beverages. Lactic acid serves as an effective alternative to emulsifiers for suppressing SPLB growth and was shown to be further applicable beyond RTD beverages. As this strategy does not rely on synthetic preservatives or emulsifiers, it avoids additive-related regulatory restrictions and consumer perception concerns, making it a practical approach for industry application.

S2-P9: Are Standard Microbiological Controls Suitable for Edible Insect Matrices? A Case Study on *Bacillus cereus* and *Clostridium perfringens* in *Hermetia illucens*

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Edible insects are increasingly promoted as sustainable protein sources, yet their microbiological safety assessment still largely relies on conventional cultural methods developed for other animal-derived foods. Their suitability for insect-derived matrices, however, remains poorly documented. This study aimed to evaluate the performance of standard methods for the detection of *Bacillus cereus* and *Clostridium perfringens* in *Hermetia illucens* matrices and to assess the added value of molecular and genomic tools for pathogen confirmation and hazard characterization.

Fifteen samples covering the production continuum, including substrate, frass, larvae and insect powder, were selected from previous investigations because standard analyses had indicated presumptive *C. perfringens* and/or *B. cereus* group contamination. These samples were then re-investigated using selective culture protocols, biochemical confirmation tests, DNA extraction, targeted PCR assays and whole genome sequencing (WGS) to recover presumptive isolates and determine whether conventional identification reflected the presence of the pathogens. DNA was extracted from presumptive isolates prior to PCR screening targeting *cpa* gene for *C. perfringens*, *cesB* and *panC* genes for the *B. cereus* group. Selected isolates were further characterized by WGS using Illumina sequencing. Genome annotation and comparative genomic analyses were then performed with the MicroScope (MaGe/Genoscope) platform to refine taxonomic assignment and investigate virulence-associated determinants, antimicrobial resistance markers and metabolic potential.

Standard methods yielded 31 presumptive *C. perfringens* isolates from 8 samples and 26 presumptive *B. cereus* isolates from 7 samples. Biochemical confirmation reduced these numbers to 10 and 21 isolates, respectively. However, PCR screening showed a discrepancy with culture-based identification. Only 6 isolates were positive for the expected pathogen targets, and all originated from wheat-based substrate samples, whereas none of the presumptive *B. cereus* isolates were molecularly confirmed. These results indicate that many isolates classified as positive by selective media and biochemical assays were not supported by molecular evidence. WGS further demonstrated taxonomic misassignments among conventionally confirmed isolates. Two powder isolates initially identified as *B. cereus* and *C. perfringens* were reassigned to *Bacillus tropicus* and *Clostridium tepidium*, respectively. Comparative genome analysis also highlighted the value of genomics for exploring traits that cannot be resolved by food control methods alone, including resistance, virulence-related features and functional potential.

Overall, this study shows that microbiological controls transposed from conventional food systems to edible insect matrices lack specificity and may lead to false-positive hazard identification. More robust confirmatory approaches, including targeted qPCR and genome-based analyses, should therefore be integrated into food safety assessment frameworks for edible insect products.

S2-P10: Safety Meets Quality: *Carnobacterium* Cultures as a Dual-Action Preservative for Cold-Smoked Salmon

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The cold-smoked salmon (CSS) industry, a major sector in Europe, faces persistent microbial challenges, particularly contamination by *Listeria monocytogenes* and early sensory degradation. The protective potential of five commercial or experimental food cultures (FC) or FC cocktails, including *Carnobacterium divergens*, *Carnobacterium maltaromaticum*, *Latilactobacillus curvatus*, and *Lactococcus piscium*, were compared on products with distinct autochthonous microbiota, provided by two processing plants (A and B). CSS slices were sprayed with a cocktail of *L. monocytogenes* (10^2 CFU/g) and FC (5×10^6 CFU/g) when appropriated. Sample were stored under vacuum packaging at 4°C for 10 days followed by 8°C for 20 days. Every 10 days, three independent biological samples were analyzed to assess bacterial load (total psychrothrophic count, total lactic acid bacteria, *Listeria*, *Enterobacteriaceae*, *Photobacterium* and *Brochothrix*), microbial biodiversity via metabarcoding, pH, biogenic amines, and organoleptic characteristics (evaluated by trained panel of 12 judges). Without FC addition, batches from the processing Plant A, initially colonized by *Photobacterium*, *Brochothrix*, *Serratia*, *Yersinia* and lactic acid bacteria strongly spoiled at Day 30. Batches from the processing Plant B, mainly colonized by *Vibrio*, *Salinivibrio*, *Pseudomonas*, *Psychrobacter* and *Serratia* did not spoil till the end of shelf-life.

Microbiological and metabarcoding analyses of biopreserved CSS confirmed the successful colonization of all *Carnobacterium* strains in the CSS matrix, while *L. curvatus* and *L. piscium* showed limited persistence. All FCs, except *L. curvatus*, significantly limited the growth potential of *L. monocytogenes* by 3 to 4 Log (CFU/g) regardless of the smokehouse.

An integrative multi-omics analysis revealed that CSS quality was primarily influenced by the processing plant and storage time, although some few differences between FCs efficacy against spoilage and biogenic amines were noticed. Addition of *L. piscium* to *C. maltaromaticum* did not enhance the protective effects of *C. maltaromaticum* in single culture.

Overall, *Carnobacterium*-based cultures, notably a non-tyramine-producing *C. divergens* chemical mutant, demonstrated the most robust anti-listerial and anti-spoilage activity without production of any biogenic amines, and a minimal sensory impact in unaltered product, offering a promising natural preservation strategy for CSS.

S2-P11: Selective Antimicrobial Behaviour of PVA Nanocellulose–Polyphenol Biocomposite Films Against Gram-Positive and Gram-Negative Foodborne Bacteria

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Microbial contamination of food-contact materials represents a critical route for the transmission of foodborne pathogens. Surface-associated bacterial cells can persist and initiate colonisation even at low initial loads, highlighting the need for antimicrobial packaging materials capable of reducing viable bacterial counts upon contact.

In this study, poly(vinyl alcohol) (PVA)-based biocomposite films functionalised with nanocellulose and natural polyphenolic compounds were evaluated as antimicrobial surfaces. Native cellulose nanofibrils (CNF), lignin-containing nanofibrils (LCNF), and TEMPO-oxidised nanofibrils (TCNF) were incorporated into the PVA matrix in combination with selected polyphenols, including tannic acid (TA) and wood-derived extract (EX).

Antibacterial activity was assessed according to ISO 22196 using a quantitative surface contact assay against the Gram-positive foodborne pathogen *Staphylococcus aureus* ATCC 6538 and the Gram-negative bacterium *Escherichia coli* ATCC 8739. The films achieved significant reductions in viable cell counts, depending on formulation.

A strain-dependent response was observed. Films containing wood extract (EX) exhibited higher antibacterial efficacy against *S. aureus*, whereas tannic acid (TA)-functionalised films demonstrated superior activity against *E. coli*. These differences are consistent with selective interactions between specific polyphenolic compounds and structural features of the bacterial cell envelope.

The results demonstrate that antimicrobial performance can be systematically tuned through controlled material design. Bio-based PVA nanocomposite films therefore represent promising active food-contact materials for improving microbiological safety.

S2-P12: Cold-Smoked Salmon Synthetic Microbiomes to Optimize the Biopreservation

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The cold-smoked salmon (CSS) is a widely consumed seafood but highly perishable. The CSS microbiome is composed of bacteria that may contribute to spoilage or, sometimes, include human pathogens like *Listeria monocytogenes*. This CSS microbiota originates from the fish itself but also from the processing plant environment. Recent studies have shown the existence of distinct bacterial signatures for each processing plant.

Biopreservation is a microbiome engineering strategy used to limit the development of pathogenic and spoilage bacteria. This method consists in inoculating microorganisms, or their metabolites with antimicrobial activities, on a food matrix without altering its organoleptic properties. Biosolutions based on living lactic acid bacterial strains such as *Carnobacterium* spp. have successfully enabled inhibition of *Listeria* in CSS. However, sometimes variation in the inhibition effect as well as organoleptic side effects of the strain have been observed and seem to be link with the product endogenous microbiota, and thus with processing plant.

Here, we aim to understand the impact of those CSS plant-signatures microbiota on the efficiency of the biopreservation. Based on metabarcoding data of different French processing plant samples, a large experimental setup using 16 synthetic microbiotas (SynComs) was built to mimic their bacterial signatures. Between 2 to 13 strains from different species were combined together. Each combination was also inoculated with *Listeria innocua*, safe surrogate for sensory analysis. The 16 SynComs were either biopreserved or not with *Carnobacterium divergens* V41. All 32 SynComs were incubated in sterile CSS juice at 8°C, an abusive refrigeration temperature, and monitored over 28 days, the maximum storage limit of CSS in France, for maximal bacterial growth. Biochemical and sensory spoilage indicators were statistically correlated to the microbial dynamics thanks to newly developed specific qPCR. *Listeria* was monitored by plate counts.

Contrasted spoilage responses and *Listeria* inhibitions were observed. *Listeria* was systematically inhibited by *C. divergens* V41 in early storage but its effect varies during time. Certain LAB strains specially *Latilactobacillus* included in SynComs extended the anti-*Listeria* effect and could be used as biosolution cocktails. Concerning spoilage, results are currently being analyzed. High levels of biogenic amines and off-odors in the SynComs were correlated to the presence of specific spoilers such as *Brochothrix thermosphacta* and *Photobacterium phosphoreum*. These results highlighted for the first time the role of the endogenous microbiota in the variation of biopreservation responses. This study will allow a better understanding of bacterial interactions and explore the possibility of optimized biosolutions.

S2-P13: Development of High-Throughput qPCR to Monitor Eighteen Main Bacterial Genera of Seafood Microbiome

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Seafood products and their storage conditions provide a suitable environment for the development of microorganisms originating from fish and seawater or resulting from contamination during the food chain processing. They are thus highly susceptible to spoilage due to biochemical and microbial degradations and are considered as potential vectors of human pathogens (e.g. *Listeria monocytogenes*). During the product storage, the microbiome diversity and its dynamics evolve though the influence of abiotic parameter such as packaging, processing and temperature. In order to establish structural and functional relationships of seafood microbiome (i.e. presence of spoilers and organoleptic changes), we developed quantitative qPCR methods to complete metabarcoding and cultural approaches. These qPCR methods specifically targeted the main bacterial genera, including seafood spoilers (*Brochothrix*, *Hafnia*, *Photobacterium*, *Serratia*), lactic acid bacteria (*Carnobacterium*, *Latilactobacillus*, *Lactococcus*, *Leuconostoc*), the pathogen *Listeria* and endogenous bacteria frequently found in microbiotas (*Buttiauxella*, *Pseudomonas*, *Psychrobacter* and *Vibrio*). The specificity, sensitivity and efficiency of each 18 qPCR methods were first validated in cold-smoked salmon juice, and then validated in naturally contaminated samples of cold-smoked salmon and seabream. Finally, the use of a high-throughput qPCR method using the microfluidic-based Biomark HD system to quantify simultaneously those key genera in seafood samples was assessed.

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S2-P14: Structural Shifts in the Rhizosphere Microbiome of a Pea-Camelina Intercropping System Assessed by Quantitative Microbiome Profiling

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Plant-microbe interactions are foundational to sustainable agriculture and global food security. Intercropping co-cultivation systems are increasingly adopted to enhance land-use efficiency, however, their impact on rhizosphere microbial communities remains poorly understood due to the compositional constraints of standard metabarcoding sequencing, which cannot resolve absolute microbial abundances.

We aimed to quantify absolute changes in rhizosphere bacterial communities, with emphasis on potential nitrogen-fixing taxa, under intercropping versus monoculture conditions using quantitative microbiome profiling.

An intercropping system including pea (*Pisum sativum*) and camelina (*Camelina sativa*), characterized by nearly synchronous growing cycles, was sown at a 50:50 seeding ratio for two consecutive years (2022–2024) at the experimental organic farm of Bologna University, and compared against their respective full-density monocultures. 16S rRNA amplicon sequencing data were corrected for 16S copy number variation and integrated with total bacterial loads quantified by qPCR to derive absolute cell abundances per gram of rhizosphere soil.

Using standard relative quantification, the overall potential diazotrophic guild remained stable across both cropping systems. However, differential abundance analysis revealed host-specific shifts: in the pea rhizosphere, intercropping competitively excluded specialized plant-associated bacteria like *Devosia*, while significantly enriching stress-tolerant opportunists such as *Pseudomonas*. Conversely, the camelina rhizosphere microbiome remained compositionally stable. When evaluating absolute abundances via qPCR, the total diazotrophic cell count was nearly two-fold higher in the intercropping regime, although high inter-replicate variability masked statistical significance. Despite this variance, the quantitative enrichment was distinctly driven by associative diazotrophs, namely *Paenibacillus*, *Variovorax*, and the *Burkholderia-Caballeronia-Paraburkholderia* complex. Finally, topological spatial analysis based on evenness index revealed a systemic structural reorganization. Intercropping induced significant niche expansion, leading to a broader and shared spatial distribution of non-constitutive symbionts, such as *Paenibacillus*, *Sphingomonas*, and *Variovorax*, across the entire intercropping system.

Intercropping restructures the rhizosphere microbiome by favouring generalist diazotrophs and plant growth-promoting bacteria over specialized symbionts, such as *Rhizobium* or *Mesorhizobium*, subjected to more stochastic dynamics. While monoculture maintains a stable rhizosphere assembly, intercropping expands microbial niche breadth and increases community evenness. The enrichment of associative diazotrophs suggests an improved nitrogen cycling capacity, although high spatial heterogeneity indicates reduced determinism in microbial assembly. These findings highlight the importance of absolute quantification approaches for understanding microbiome dynamics in sustainable agriculture systems.

S2-P15: Unseen Architects of Cider: Phage Diversity Across Fermentation Microbiomes

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Bacteriophages (phages) are viruses that specifically infect bacteria. Despite their ecological importance, phage communities (phageomes) remain poorly understood, particularly in fermented plant-based ecosystems compared to dairy systems. In fermented beverages, phages are thought to influence microbial population dynamics and community structure, potentially in a temperature-dependent manner. Better characterization could improve our understanding of fermentation processes and, ultimately, their control. This study aimed to explore the diversity of lytic phages in cider, a complex fermented matrix that remains largely unexplored from a viral perspective.

Cider samples were collected in the Normandy region (France) from 12 producers in 2024 and 5 producers in 2025, resulting in 29 samples taken at early stages of fermentation (one day to one month). Microbial enumeration and isolation were performed using nine different culture media, and bacterial isolates were identified by MALDI-TOF. To improve phage detection, the pH of each sample was adjusted to 7 prior to centrifugation and filtration (0.45 µm). Culture-dependent screening in double layer was conducted by spot assays using filtrates against bacterial isolates from the same ciders. Isolated phages were subsequently characterized based on morphology using transmission electron microscopy, host range and genomic content.

Screening of 505 bacterial isolates led to the isolation of 21 phages from five cider samples. Most phages were associated with acetic acid bacteria, including sixteen myophages infecting *Gluconobacter* spp. (*G. cerinus*, *G. thailandicus* and *G. frateurii*) and *Acetobacter lambici* detected from early fermentation stages (1 day to 1 month), and one siphophage targeting the enterobacterium *Rahnella aquatilis*. In addition, a myophage infecting *Komagataeibacter intermedius* isolated from cider vinegar was identified. Notably, several phages infected *G. cerinus*, a species for which only one phage has previously been described in wine (GC1 phage with *Tectiviridae* morphology). Furthermore, a siphophage infecting the lactic acid bacterium *Oenococcus oeni* was isolated from a cider sample after at least one month of fermentation, representing a unique observation outside wine environments.

These results suggest a temporal structuring of phage-host interactions during cider fermentation, with acetic acid bacteria phages dominating early stages and lactic acid bacteria phages emerging later. Altogether, this study provides new insights into phage diversity in cider and supports the hypothesis that phages may contribute to microbial succession in fermented beverages. Ongoing genomic analyses and the development of a model microbial community will further elucidate phage–bacteria interactions in this ecosystem.

S2-P16: From Bubbles to Biology: Microbial Drivers of Late Blowing in Beetroot Kimchi

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Fermented vegetables are increasingly consumed for their nutritional value and the presence of live lactic acid bacteria (LAB). Unpasteurized, spontaneously fermented products appeal due to their sensory complexity and low-input, sustainable production, but they are vulnerable to quality deviations in the absence of preservation steps. A critical defect is excessive gas production that leads to late blowing and "second fermentation", jeopardizing product stability, safety, and consumer acceptance. This work investigates the microbial and metabolic drivers underlying this phenomenon in beetroot kimchi. Pre-fermented beetroot matrices were inoculated with selected homofermentative and heterofermentative LAB and yeast strains previously isolated from spontaneous fermentations exhibiting late blowing. Fermentations were performed under process-relevant conditions. Product performance was evaluated by visual assessments (color change, gas formation, microbial growth), microbial enumeration on PCA, MRS, and YGC media, with species-level identification by MALDI-TOF MS, and physicochemical measurements of pH, sugars (glucose, fructose), and organic acids (lactic, acetic). Preliminary results showed that overall microbial counts increased during the early fermentation phase (day 0–11) and declined during prolonged fermentation (day 11–32). Samples inoculated with heterofermentative LAB exhibited lower pH values than the controls, likely reflecting enhanced organic acid production, whereas yeast-inoculated samples maintained comparatively higher pH values. A pronounced reduction in microbial counts was observed in samples inoculated with *Lentilactobacillus buchneri*. In fermentations inoculated with *Kazachstania exigua*, yeast counts decreased significantly during extended fermentation, suggesting competitive suppression of yeasts by LAB over time. In addition, heterofermentative LAB were associated with visible gas bubble formation, whereas yeast and homofermentative LAB inoculation alone did not produce comparable effects under the tested conditions. The association of heterofermentative LAB with both acidification and gas production points to their potential role as key drivers of late blowing in beetroot kimchi. Overall, this work contributes to a better understanding of the mechanisms underlying late blowing in fermented vegetables and may support the development of microbial and physicochemical markers, as well as practical process strategies, to improve the stability of spontaneously fermented products without compromising their sensory quality or sustainability advantages.

S2-P17: Soil Microbiome Responses to Water Limitation in Wheat Cropping Systems Across Spain and Italy

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Crop-associated microbial communities play a central role in soil fertility and plant adaptation to environmental stress under climate change. Within the TRIBIOME project, we investigated the diversity and composition of wheat-associated microbiomes, focusing on the effects of water availability on bacterial and fungal communities. Field trials were conducted during the 2022/2023 growing season in Spain and Italy using soft and durum wheat. Geo-referenced soil and plant samples were collected at phenological stages including flowering, maturity and post-harvest, and processed for DNA extraction followed by amplicon sequencing.

A total of 1052 rhizosphere and bulk soil samples were analysed through 16S rRNA and ITS metabarcoding to characterize microbiome dynamics under contrasting irrigation regimes. Across wheat species, differences were observed between rhizosphere and bulk microbial assemblages, confirming the influence of the root environment on community structure. Water regime was identified as a determinant of microbial diversity and composition. Irrigated plots showed higher alpha diversity, whereas arid conditions reduced diversity and shifted the relative abundance of specific taxa. Under drought conditions, bacterial genera such as *Sphingomonas*, *Paenibacillus*, *Microvirga*, *Gemmata*, *Variovorax*, *Microbacterium* and *Bradyrhizobium* were enriched in rhizosphere and bulk soil. Likewise, fungal communities in arid plots showed higher abundance of *Talaromyces*, *Periconia*, *Mortierella*, *Curvularia* and *Rhizopus*.

Comparable trends were detected in both wheat species, suggesting that responses to aridity were largely independent of host type, although intrinsic soil properties may further modulate these patterns. These findings improve our understanding of microbiome responses to climate-related stress and support microbiome-based strategies for sustainable wheat production systems.

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S2-P18: Pig Slurry Fertilization Increases the Number of Antimicrobial-Resistant Bacteria in Agricultural Fields

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The widespread use of antimicrobials has contributed to the emergence and dissemination of antimicrobial resistance (AMR), which is currently considered one of the major threats to public health under the One Health framework. In animal production, the use of antimicrobials can alter the animal microbiota and the presence of resistance genes. Pig slurry, commonly applied as agricultural fertiliser, may act as a reservoir of AMR microorganisms and facilitate their spread into the environment. The aim of this study was to evaluate the impact of pig slurry fertilisation on the transmission of AMR bacteria from slurry to soil and plant products.

During 2024–2025, slurry samples were collected from five pig farming companies. Each slurry sample was also applied as fertiliser to a different agricultural field. Soil samples were collected before and after fertilisation, along with rhizoplane samples from harvested crops. Bacterial isolation (*n* = 2034) was performed using selective media (bile esculin azide agar) to isolate *Enterococcus* spp., followed by phenotypic antimicrobial susceptibility testing. The taxonomic identification of resistant isolates was confirmed using MALDI-TOF mass spectrometry.

Prior to fertilisation, soil samples showed very low resistance levels, ranging from 0 to 0.71% for tetracycline and 0 to 7.14% for erythromycin depending on the field.

In slurry samples, resistance to both antibiotics showed high variability among the five farms, ranging from 41.1 to 96.6% for tetracycline and 17.8 to 85.4% for erythromycin. Co-resistance to both antibiotics ranged from 14.4 to 83.1% of isolates, reflecting variability between slurry samples. Most resistant isolates were identified as *Enterococcus* spp., predominantly *Enterococcus durans*, while *Mammaliicoccus* spp. represented a minor fraction (~1.8%). Following slurry application, resistance to antibiotics increased markedly in three of the fields, reaching up to 35.4% for tetracycline and 9.1% for erythromycin, with co-resistance also rising to 9.1% of isolates. No resistant isolates were detected in one field, while in another field only erythromycin resistance increased. In harvested products, resistant isolates were detected only for erythromycin and at very low levels (~1%), suggesting a limited establishment of resistant enterococci in the plant rhizoplane.

Overall, these findings identify slurry as a major reservoir AMR bacteria and demonstrate that its application can significantly increase resistance levels in agricultural soils, although this effect varies depending on application conditions. This study highlights the need to need to better understand and control the role of organic fertilisation practices in the dissemination of AMR.

S2-P19: Comparative Microbial assessment of Pea-Based Burgers Formulated with Distinct Biosolutions

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Biosolutions, including food cultures and fermentates, are increasingly recognised as effective alternatives to conventional preservation strategies. In line with current industry and consumer-driven trends, this study aimed to develop an innovative pea-based burger through four distinct formulations, each incorporating a different biosolution. These formulations were assessed for their capacity to modulate the product's microbial ecology and inhibit the growth of spoilage-associated microorganisms.

Five batches were produced: V1 (control, no preservatives); V2 (fermentation extract, 0.5%); V3 (*Lactiplantibacillus plantarum* + *Lactococcus lactis*); V4 (pea fermented with *Latilactobacillus curvatus*); and V5 (pea fermented with *Leuconostoc carnosum*). Burgers were stored at 7°C for 28 days. Analyses were conducted at days 0, 7, 14, 21 and 28, including pH, total viable counts (TVC), lactic acid bacteria (LAB), yeasts, moulds, and spores of aerobic (AE-Bac) and anaerobic bacilli (ANAE-Bac). Water activity was measured at days 0 and 28.

Despite marked differences at day 0, TVC and LAB counts converged across all formulations, reaching approximately 9.0 log CFU/g after day 14, indicating a rapid and similar microbial evolution irrespective of formulation. Spores of AE-Bac and ANAE-Bac were generally below the enumeration limit (< 2 log CFU/g) but were found sporadically at levels between 2 and 5 log CFU/g. No moulds were enumerated. Yeasts, however, were present in multiple samples. Formulations V3, V4 and V5 reached 2.7, 2.3 and 3.8 log CFU/g, respectively, after 14 days, whereas V2 showed a higher level (5.2 log CFU/g) at day 21. The control, V1, reached 2.7 log CFU/g after 28 days. These findings suggest that the tested biosolutions were not effective in controlling yeast proliferation and might have contributed to their introduction.

Water activity remained stable throughout shelf life (0.96 to 0.94), while pH decreased in all formulations, from approximately 6.0 to 4.8 (for V1, V2 and V3) or 5.2 (V4 and V5), remaining within acceptable limits for based burgers.

Overall, yeast contamination emerged as the primary limitation to shelf-life extension. Identifying its origin and implementing mitigation strategies will be critical in future work, alongside sensory evaluation to assess consumer acceptance.

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S2-P20: Structural Reorganization and Antiviral Activity of Heat-Denatured Lysozyme: Implications for Food Applications

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Human norovirus is a leading cause of foodborne illness worldwide, and the development of effective antiviral strategies applicable to food systems remains a major challenge. Conventional disinfectants such as sodium hypochlorite are limited by reduced efficacy in the presence of organic matter and restrictions in food applications. We previously reported that heat-denatured lysozyme, a food-grade protein derived from egg white, exhibits strong antiviral activity against murine norovirus (MNV), an experimental model for human norovirus, achieving up to approximately 4.5 log reduction in infectivity. However, the structural basis underlying this antiviral activity has not been elucidated.

In this study, we investigated the relationship between structural changes and antiviral activity of lysozyme using Fourier-transform infrared spectroscopy (FT-IR). Heat-denatured lysozyme (80°C and 100°C for 40 min, pH 7 during heating) and chemically reduced lysozyme (TCEP-treated) were prepared, and secondary structural changes were analyzed based on the amide I region.

Heat treatment induced pronounced structural reorganization characterized by a decrease in random coil content and an increase in β-sheet structures. In contrast, reduced lysozyme also showed an increase in β-sheet content, but the extent of structural change was limited, indicating a distinct mode of structural alteration. Deconvolution of FT-IR spectra and secondary structure assignment supported these differences.

These structural differences were consistent with antiviral activity. Heat-denatured lysozyme reduced MNV infectivity by approximately 2.7–3.0 log PFU/mL, whereas reduced lysozyme showed only limited reduction (~1.0 log PFU/mL). Notably, increased β-sheet content alone did not correlate with antiviral efficacy, suggesting that antiviral activity depends on a specific mode of structural reorganization rather than secondary structure composition alone.

We further examined the effect of pH during heat treatment. Under acidic conditions (pH 3), structural changes were minimal and antiviral activity was limited (~1.1 log reduction), whereas pronounced structural reorganization and higher antiviral activity were observed under neutral conditions (pH 7). These results suggest that structural reorganization during heat denaturation is influenced by pH and associated with antiviral activity.

This study provides structural insight into the antiviral mechanism of denatured lysozyme and demonstrates a link between processing conditions, protein structure, and antiviral function. These findings highlight the potential of heat-denatured lysozyme as a safe and tunable antiviral agent for food applications. Ongoing work focuses on systematic evaluation of pH-dependent structural changes and their quantitative correlation with antiviral activity.

S2-P21: Assessment of Bioaerosols of a Commercial Craft Brewery

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Bioaerosols are small airborne microbiological particles and other components that may lead to potential spoilage or contamination of products in the production environment, and pose occupational health risks for food handlers. Craft beer is more prone to spoilage than beer from large-scale breweries since it is usually neither pasteurized nor filtered. The study aimed to assess microbial diversity of bioaerosols in the craft beer production environment using culture-dependent and -independent approaches. Active sampling was performed with a SKC Biosampler. Samples were collected in PBS from 6 areas: milling, brewhouse, yeast propagation, fermenters, kegging and canning. Culturable diversity was assessed using 15 selective media, while targeted metagenomics (16S rRNA and ITS) was performed with PacBio SMRT. Bioaerosol concentrations in all 6 areas exceeded the thresholds set by the American Industrial Hygiene Association for total bacteria (< 500 CFU/m^3) and fungi (< 250 CFU/m^3). Cultured microbes were categorized into beer spoilers and potential risks to human health. Beer spoilage microbes detected included *Bacillus* spp., *Kocuria* spp., acetic acid bacteria (AAB), and *Zymomonas* spp.. Microbes that could impact human health included *Penicillium* spp., *Bacillus* spp., *Micrococcus* spp., and *Staphylococcus* spp.. Uncultured bacterial diversity was dominated by potential beer spoilage lactic acid bacteria (40%) in all areas of the brewery. Genera included *Enterococcus*, *Lactococcus*, *Leuconostoc*, *Streptococcus* and *Weissella*. Brewhouse, yeast propagation and kegging areas showed consistently high relative abundance of *Lactococcus garvieae*, *Lactococcus lactis*, *Leuconostoc citreum*, *Leuconostoc pseudomesenteroides*, *Weissella confusa*, *Weissella paramesenteroides* and *Weissella soli*. Opportunistic pathogens *Kocuria rosea*, *Micrococcus lateus*, *Staphylococcus epidermis*, *Raoultella ornithinolytica*, *Pantoea agglomerans*, *Enterobacter cloacae* and *Escherichia coli* were also detected. Relative abundance of yeast and mould in most areas was low (1.01–5.92%) with the exception of *Pichia mandshurica* (23%) in the milling area. It is often associated with AAB and known biofilm forming wine spoiler. Pathogenic yeast *Candida tropicalis* was detected in all areas except the milling area. *Penicillium bialowiezense* (blue mould) and *Penicillium javanicum* present in the milling and kegging areas may rarely cause allergic respiratory responses. The milling area also contained *Naganishia diffluens* considered an emerging human pathogen. *Aspergillus penicillioides* and *Aspergillus ruber* were detected in the kegging area; they are considered to have pathogenic potential and are agents of allergy. No recognized or relevant spoiler or wild yeast were detected. This research emphasizes the need for air quality monitoring in craft breweries, highlights risks to product quality and consistency and advocates for the health of workers.

S2-P22: *Pseudomonas* Isolates from Dairy Plants: Genomic Insights and Implications for Dairy Product Spoilage and Biopreservation

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Pseudomonas spp. constitute one of the main groups of psychrotrophic bacteria responsible for spoilage and economic losses in dairy production. This is mainly due to their ability to produce pigments and extracellular enzymes, form biofilms, and resist various antimicrobial compounds. Biopreservation through the addition of lactic acid bacteria (LAB) is one of the strategies used to control *Pseudomonas* spp., for example, when applied during milk refrigeration to inhibit microbial growth.

In this study, a phenotypic and genomic characterization of six *Pseudomonas* strains isolated from dairy plants was carried out to elucidate the genetic basis of their spoilage potential and environmental persistence. The effect of four commercial bioprotective cultures (L1–L4) on a cocktail of *Pseudomonas* spp. inoculated into pasteurized goat and cow milk during storage at 8°C for 72–96 h was investigated at the molecular level and by assessing growth kinetics. Phenotypic assays evaluated the ability of the strains to produce pigments, acidify milk, and form biofilms. Genome sequencing was performed using Oxford Nanopore Technology, followed by assembly and annotation.

Genome sizes of the studied *Pseudomonas* spp. strains ranged from 5.59 to 6.85 Mb. The strains were assigned to the species *Pseudomonas putida*, *Pseudomonas paracarnis*, *Pseudomonas haemolytica*, and *Pseudomonas shahriarae*. Genomic analysis identified genes involved in dairy product spoilage, including those encoding extracellular proteases, as well as biosynthetic clusters for exopolysaccharide and pigment production. Resistome analysis revealed the presence of genes associated with resistance to fluoroquinolones, tetracyclines, and beta-lactams, among others. Additionally, putative resistance to quaternary ammonium compounds and other disinfectants was identified, suggesting possible tolerance to biocides used in the dairy industry. The addition of LAB cultures L3 (*Lacticaseibacillus paracasei* + *Lactiplantibacillus plantarum*) and L5 (*L. plantarum* + *Lacticaseibacillus rhamnosus*) was experimentally shown to have the greatest protective effect against *Pseudomonas* spp. in refrigerated milk.

This study demonstrates the relationship between genomic features and dairy spoilage phenotypes in *Pseudomonas* spp., highlighting their persistence and the challenges they pose for product quality and hygiene in the food industry. Additionally, it evaluates the efficacy of biopreservation methods as potential strategies to mitigate microbial spoilage, which can negatively impact cheese production and overall dairy operations.

S2-P23: High-Throughput Screening of Lactic Acid Bacteria-Derived Antimicrobials and Nano-Emulsified Essential Oils Against *Pseudomonas* spp.

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Spoilage-associated bacteria such as *Pseudomonas* spp. pose a significant challenge in food systems due to their metabolic adaptability and resistance to traditional preservation methods. Lactic acid bacteria (LAB) derived antimicrobials, including bacteriocins and antimicrobial peptides, present a promising natural alternative, providing targeted bactericidal activity while remaining safe for food use. Moreover, nano-emulsified essential oils represent a promising strategy for food preservation, improving the delivery and antimicrobial performance of natural compounds against spoilage-associated bacteria.

This study presents a high-throughput screening platform to evaluate the antimicrobial potential of LAB-derived compounds against *Pseudomonas* spp.. The LAB strains under investigation include *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Pediococcus* spp., and *Carnobacterium* spp.. Combinations of different LAB are also assessed to explore complementary inhibitory effects. In addition, nano-emulsified essential oils are tested as a complementary antimicrobial strategy. Oils such as *Origanum vulgare* subsp. *hirtum*, *Coridothymus capitatus*, and *Thymus vulgaris* have been studied and have demonstrated activity against relevant foodborne bacteria. Nano-emulsification enhances dispersion, stability, and bioavailability of these hydrophobic compounds, improving their interaction with bacterial cells. The potential inhibitory effects of nano-emulsified essential oils on lactic acid bacteria are also evaluated to assess their compatibility and ensure the effectiveness of combined antimicrobial strategies. A 96-well microplate-based assay is optimized to allow rapid, parallel testing of multiple formulations and concentrations. Assay preparation and liquid handling are performed using an Opentrons OT-2 platform to ensure precision, reproducibility, and scalability. Bacterial growth and metabolic activity are monitored using tetrazolium chloride as a colorimetric indicator. This design enables standardized conditions and systematic evaluation of antimicrobial combinations, including potential synergistic effects. This approach is expected to provide a robust methodology for identifying and optimizing LAB-derived antimicrobials against *Pseudomonas* spp., while also evaluating the complementary efficacy of nano-emulsified essential oils. The combined strategy supports the development of natural preservation solutions to improve food quality and extend shelf life.

S2-P25: Microbial Risk Profiling in Plant-Based Dairy Alternatives: A Comparative Bioinformatic Study

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Plant-based fermented dairy alternatives are becoming increasingly popular and mainstream as consumers seek non-animal-origin, sustainable, nutritious, and lactose-free options. However, such matrices may carry broad and variable microbial risks, which can significantly impact the safety and quality of the final product. Identifying the microorganisms associated with these products is important for fermentation optimization, quality control, and food safety risk assessment. The aim of the present study was to compare the microbiota of commercial plant-based cheese and kefir alternatives with microorganisms reported in NCBI repositories for plant-based foods.

Plant-based cheese and kefir alternatives, previously analyzed by metabarcoding targeting the bacterial 16S rRNA gene and the fungal ITS region, were compared with a set of NCBI data (including FASTA sequences) explicitly linked to plant-based foods and fermented plant-based products. According to NCBI data, plant-based foods and meat analogues were associated with *Salmonella enterica*, *Enterobacter*, *Klebsiella*, *Citrobacter*, *Cronobacter*, *Siccibacter*, *Lelliottia*, *Serratia*, and *Escherichia*. Quinoa and chickpea-based products were linked with *S. enterica*, fava bean products with *Listeria monocytogenes*, and lentils with *Bacillus* spp.. Soy-based matrices (including soy drinks, soy sauce mash, natto, traditional soy paste, and fermented soybean condiments) were associated with *Bacillus* spp., including *B. subtilis*, *B. licheniformis*, *B. velezensis*, *B. amyloliquefaciens*, *B. cereus*, and *B. pumilus*. In contrast, our products were mainly characterized by fermentation-associated microbiota, and more specifically, plant-based cheeses were associated with *Lacticaseibacillus*, *Leuconostoc*, *Lactobacillus* and *Enterococcus* (with occasional *Raoultella*), while kefir with *Lactococcus* and *Streptococcus*. Notably, no reads in our data were assigned to the key pathogen-associated taxa highlighted by the NCBI records (e.g., *S. enterica* and *L. monocytogenes*).

Overall, comparing product-specific metabarcoding with NCBI records provides essential context for interpreting the microbiota of plant-based fermented foods. These findings can support food safety risk assessment and highlight the importance of monitoring microbial hazards along the plant-based fermented foods value chain.

S2-P26: Occurrence of Mycotoxin-Producing Microorganisms in Dried Fruits by HPLC-UV and Flow Cytometry Analysis

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Dried fruits are commonly consumed due to their extended shelf life, ease of use, and high nutritional value. Despite these advantages, inadequate processing and storage conditions may promote the persistence and proliferation of microorganisms, particularly moulds and yeasts, which can compromise product quality and safety. Some of these fungi are capable of producing hazardous secondary metabolites, including mycotoxins such as ochratoxin A (OTA), aflatoxins (AFs), and patulin (PAT). Therefore, the present study aimed to assess the microbiological safety of dried fruits available on the Slovenian market, with emphasis on the identification of fungal and yeast contaminants and their potential to produce mycotoxins.

Samples were collected from three producers of raisins, two producers of dried apricots, and one producer of table grapes. Microorganisms were isolated by culturing fruit samples on malt extract agar (MEA), followed by subcultivation on WL (Wallerstein Laboratory) nutrient agar and potato dextrose agar (PDA). Identification of fungal and yeast isolates was performed using morphological characteristics in combination with molecular techniques.

To evaluate mycotoxin production, fungal biomass cultivated on agar media was extracted using methanol–water (80:20, v/v) and ethyl acetate for OTA and PAT, respectively. For PAT detection extracts were purified Affinimip SPE columns (Affinisep, France) and analysed by HPLC using C18 column (3 µm, 150 × 4.6 mm) mantained at 35°C, UV detection at 276 nm. In addition, rapid screening for aflatoxins and ochratoxin in dried fruit extracts was performed using the SAFIA Mycotoxin kit, a flow cytometry-based suspension array fluorescence immunoassay (SAFIA Technologies GmbH, Germany).

The results showed that species belonging to *Aspergillus* section Nigri predominated in certain raisin samples, while *Penicillium* spp. were mainly associated with table grapes. Typical spoilage yeasts, including *Zygosaccharomyces rouxii* and *Schizosaccharomyces pombe*, were detected in dried apricot samples. These findings indicate that dried fruits provide favorable conditions for the growth of fungi capable of producing mycotoxins.

HPLC-UV analysis of fungal biomass extracts confirmed the presence of PAT in several samples (8.6–93 µg/kg), with the highest concentrations observed in raisins, whereas OTA was below the limit of detection. SAFIA revealed the presence of AF and OTA in some of selected dried fruit samples, although their concentrations did not exceed the maximum limits established by EU legislation. Overall, the results highlight the importance of continuous microbiological surveillance and targeted mycotoxin analysis to ensure the safety and quality of dried fruit products.

S2-P27: Evaluating the Inhibitory Effects of Chemical and Natural Antimicrobials on Food Spoilage Bacteria

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Food-spoilage microorganisms, notably *Pseudomonas* species and *Hafnia alvei*, are common contaminants in food-processing environments. Their metabolic activities and biofilm-forming capabilities significantly contribute to product degradation and pose hygiene challenges. As interest increases in integrating conventional disinfectants with natural antimicrobial compounds, it is crucial to understand the inhibitory effects of these agents on key spoilage organisms to enhance sanitation strategies.

The objective of this study was to assess the antimicrobial responses of selected food-related isolates, including *Pseudomonas aeruginosa*, *Pseudomonas lundensis*, *Pseudomonas fluorescens*, and *H. alvei*, to a chlorine-based disinfectant and carvacrol, a natural phenolic compound. Antimicrobial susceptibility was evaluated using disk diffusion assays and broth microdilution, with growth monitored through Multiskan Ascent Microplate photometric measurements. In some cases, both total and partial inhibition zones were recorded to better characterize subtle antimicrobial interactions.

Preliminary observations showed that the disinfectant exhibited consistent inhibition across a wide concentration range, whereas carvacrol showed strong activity at higher concentrations, with a distinct transition zone at lower levels. In several cases, no growth was detected even at the most diluted concentrations, indicating high susceptibility and guiding the selection of concentrations for subsequent assays.

The findings indicate that both chemical and natural antimicrobials have the potential to inhibit the growth of spoilage-associated microorganisms on food-contact surfaces. Current research efforts involve expanding the range of antimicrobials and examining biofilms on various contact surfaces to gain a deeper understanding of early attachment and metabolic activity. Collectively, these results contribute to the enhancement of hygiene management strategies within food-processing environments.

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S2-P28: Shotgun Metagenomics of Castelvetrano Olives

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Table olives processed in the Castelvetrano style are rapidly debittered with sodium hydroxide, then treated with sea salt and stored in cold rooms to preserve sensory properties and the proliferation of pathogenic microorganisms. Before commercialisation, olives undergo multiple rinses to remove residual soda, immersion in acidified brine, and, in some cases, pasteurisation, followed by packaging. At the industrial level, even when stored at low temperatures, olives sometimes spoil and become unsuitable for sale. To identify the microbial communities involved in the spoilage of table olives during storage prior to commercialisation, a shotgun metagenomic investigation of both suitable and unsuitable olives was conducted.

DNA extracted from olives and brine was quantified and submitted for library preparation following Illumina DNA Prep instructions. The prepared libraries were sequenced using P3 reagents (2 × 150 cycles) on the Illumina Nextseq 2000 platform. Raw sequencing data were analysed using open-access bioinformatics tools, including quality control (Falco, version 1.2.4), trimming (FastP, version 1.0.1), depletion of olive DNA (Bowtie 2, version 2.5.3), taxonomic classification (Kraken 2, version 2.1.3), and core-metagenome visualisation (Krona pie chart, version 2.7.1). Alpha- and beta-diversity were calculated using Shannon and Bray-Curtis indices, respectively.

The main results demonstrated that, on an industrial scale, in preserved olives suitable for further processing according to the Castelvetrano style, *Leuconostoc mesenteroides*, *Loigolactobacillus coryniformis*, and *Paucilactobacillus nenjiangensis* are the most represented species. In olives, these microorganisms primarily participate in oleuropein hydrolysis and secondary debittering. In particular, *L. coryniformis* and *P. nenjiangensis* help reduce softening by regulating pectinolytic activity and contribute to the sensory profile. Conversely, in olives unsuitable for Castelvetrano-style processing, the most abundant species is *Pichia membranifaciens*, followed by *Kluyveromyces lactis* and *Debaryomyces hansenii*. *P. membranifaciens* is a yeast with high osmotic tolerance, which gives it a competitive advantage over lactic acid bacteria. It can produce compounds that contribute to off-flavours and biotin, which can promote the growth of undesirable and sometimes pathogenic microorganisms.

S2-P29: Liquid vs Solid: Matrix-Dependent Heat Stability of Spoilage *Pseudomonas* Proteases

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Bacteria of the genus *Pseudomonas* are important spoilage microorganisms in refrigerated poultry meat due to their production of extracellular proteases, which degrade muscle proteins and negatively impact product quality. The thermal stability of these enzymes is a critical factor in determining their spoilage potential following thermal processing of food. In this study, we investigated the heat stability of proteases produced by *Pseudomonas* isolates in chicken meat. We compared natural *Pseudomonas* isolates with the presence or absence of *aprX* gene for their protease activity and its thermostability at temperatures between 50 and 90°C. Isolates were cultivated under different conditions, including two liquid matrices – tryptic soy broth (TSB) and chicken meat homogenate – and solid matrix – chicken meat. Isolates were cultivated at 5 and 15°C for 3 and 7 days. Following incubation, samples were subjected to control heat treatments, and residual proteolytic activity was measured using azocasein assay. Final samples were evaluated also with zymography. Protease activity of the *Pseudomonas* strains was significantly higher in chicken meat homogenate compared to TSB, indicating a strong nutrient-dependent effect on protease production. Across incubation time, proteolytic activity increased from day 3 to day 7, demonstrating a clear time-dependent enhancement of extracellular enzyme expression. In addition, temperature had a pronounced effect on protease production, with substantially higher activity observed at 15°C compared to 5°C. Despite substantial differences in the intensity of protease activity among the individual isolates, their thermal stability was found to be remarkably comparable. Following 30 min of exposure to elevated temperatures, all isolates showed similar patterns of protease inactivation, regardless of differences in initial protease activity or genetic background. In contrast, a clear matrix-dependent effect was observed on thermal resistance. Residual protease activity was higher in liquid media compared to meat, where protease activity was diminished under identical thermal conditions. In addition, zymographic analysis revealed that proteases produced in all experimental conditions made major bands of identical molecular size, indicating comparable protease type and activity in both liquid and solid matrices, and implying that the variability in heat stability is likely driven by structural or post-translational differences within the proteases or differences in their microenvironmental context, which could influence their susceptibility to thermal denaturation, which needs to be further studied. These results indicate that liquid homogenate food systems tend to overestimate the apparent thermal stability of *Pseudomonas* proteases relative to real food matrices.

S2-P30: Evaluating Yeasts as Biocontrol Agents Against Pathogenic and Spoilage Microorganisms in Agri-Food: Action of Volatile Organic Compounds (VOCs)

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Yeasts are widely recognized for their industrial and fermentative relevance, but their potential as source of antibacterial compounds remains significantly underexplored compared to bacteria or filamentous fungi. In this study, a wide taxonomically characterized population of yeast strains for their antimicrobial activity against enteropathogenic bacteria, plant pathogens and spoilage molds was evaluated.

Metschnikowia pulcherrima exhibited a broad inhibitory spectrum against both molds and bacteria, driven by non-volatile, multifactorial mechanisms such as iron chelation and antimicrobial metabolites. In contrast, *Hanseniaspora* and *Wickerhamomyces* strains displayed more versatile behavior, combining strong antifungal properties with VOC-mediated antibacterial activity. *Brettanomyces bruxellensis* showed a wide antimicrobial action among the population producing bioactive VOCs. The identification and evaluation of the antimicrobial action of VOCs compounds produced by *Brettanomyces* yeasts are under evaluation.

S2-P31: Combining Culture-Dependent and Culture-Independent Methods to Unravel Grape Berry Microbiota Across European Wine-Growing Countries

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Grape microbial communities have an important role in shaping grape quality and wine characteristics. Growing evidence shows that grape microbiota exhibits consistent regional patterns with wine composition. That makes microbial biogeography another relevant aspect of terroir. However, characterising these communities remains challenging, as different methodologies capture distinct fractions of microbial diversity. Integrating multiple analytical approaches is therefore essential for a more comprehensive assessment of grape microbiota.

To capture complex structure of grape berry microbiota, the study combines culture-dependent and culture-independent workflows across nine vineyards sampled at technological maturity in five relevant wine-growing countries (Spain, Italy, France, and Georgia). Grape juice pH and sugar content (°Brix) were measured, and vineyard-specific, environmental and climatic metadata were collected. For culture-dependent analysis grape juice samples were plated on selective media targeting yeasts, moulds, lactic acid bacteria, and acetic acid bacteria. Representative morphotypes were isolated and identified by Sanger sequencing. In parallel, culture-independent metagenomic analysis targeting fungal ITS and partial 26S rDNA, and bacterial 16S rDNA markers was performed using total DNA extracted directly from grape pellets. Samples were then sequenced on a MinION platform.

Culturomics analysis suggested higher abundance of yeast colonies compared to moulds, while metagenomic analysis showed a predominance of filamentous fungi. This discrepancy reflects methodological differences, as culture-dependent approaches favour the yeast growth, while metagenomic analyses capture a broader spectrum of biodiversity, including low abundant and non-culturable taxa. Despite the differences, overall trends were partially consistent. For example, in samples where yeast colonies were not observed on plates, metagenomic data did not detect yeast-associated taxa among dominant species.

The results demonstrate complementary roles of the two approaches. Culture-independent sequencing allows identification of total community structure, enabling detection of rare and non-culturable taxa, whereas culture-dependent methods enable isolation of viable strains and functional validation of traits relevant to oenology.

S2-P32: Development and Evaluation of the Antifungal Activity of Cerulenin and Biosurfactant-Based Biofungicidal Formulations for Postharvest Control of *Penicillium digitatum*

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The development of bioactive coatings to control the significant *Penicillium digitatum* disease in citrus fruits (green mold) offers a sustainable alternative to synthetic fungicides. This study aimed to develop various coating formulations based on sodium alginate (SA) and carboxymethylcellulose (CMC), incorporating cerulenin (CERU) and the biosurfactant monorhamnolipid (mono-RML) against *P. digitatum*. Solutions of SA (1.0% w/w) and CMC (0.5% w/w) were prepared under agitation at 70°C with glycerol added as a plasticizer. Mono-RML was incorporated at a concentration of 4,650 mg/L, along with two concentrations of CERU, forming solutions S5 [SA/CMC + mono-RML + CERU 2.5 mg/L] and S6 [SA/CMC + mono-RML + CERU 7.5 mg/L]. Control solutions without bioactive compounds and with the compounds incorporated separately were also used: S1 [SA/CMC]; S2 [SA/CMC + mono-RML]; S3 [SA/CMC + CERU 2.5 mg/L]; and S4 [SA/CMC + CERU 7.5 mg/L]. The antifungal activity of the solutions was evaluated using a mycelial growth inhibition assay (ICM%). Five hundred microliters of each formulation were spread on PDA medium, and 10 µL of *P. digitatum* suspension (10^5 spores/mL) was inoculated onto a sterile paper disc (6 mm) placed in the center of the plate. Colony diameter was measured after 8 days in a BOD at 25°C. Results show that mono-RML and CERU, when used individually, inhibited *P. digitatum* development at concentrations of 4,650 mg/L (S2) and 7.5 mg/L (S4), respectively. However, S4 was more effective, promoting 97.2% inhibition of fungal growth, while S2 showed approximately 25% less inhibition than S4. Formulation S6 achieved total inhibition (100%) of fungal growth, not differing statistically from S4 ($p > 0.05$). Solution S5 also inhibited mycelial growth by more than 70%, as did S2. Solution S3 inhibited fungal growth by 18.7%, and S1 showed no inhibition of *P. digitatum*. These results indicate that CERU at 7.5 mg/L was sufficient to control *P. digitatum in vitro* without the need for combination with the biosurfactant to enhance the antifungal effect. In contrast, 2.5 mg/L of CERU was not effective in controlling *P. digitatum* growth, even when combined with mono-RML, with no synergistic effect observed. Therefore, further studies evaluating intermediate concentrations of CERU (between 2.5 and 7.5 mg/L) in combination with mono-RML (4,650 mg/L) may help identify possible synergistic interactions, aiming for more efficient and economically viable formulations.

S2-P33: Plasma-Activated Aerosol for the Decontamination of Fresh Strawberries: Effects on Natural Microbiota and Quality Attributes

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Plasma-activated aerosol (PAA) is an emerging non-thermal technology for the surface decontamination and shelf-life extension of fresh produce. Unlike conventional plasma-activated water treatments, the PAA system generates a plasma-activated aerosol by exposing liquid microdroplets to a gas plasma discharge, enabling the in-situ formation and delivery of reactive oxygen and nitrogen species (RONS) and potentially improving the transfer of short-lived reactive species to the product surface. Aim of the study was to investigate the effects of in situ generated PAA on microbial inactivation and quality attributes of fresh strawberries during refrigerated storage.

Fresh strawberries were treated in a lab-scale PAA system operating at 10 kV and 4 Hz for four 5-min cycles. Three sample groups were produced and tested: non-treated control (NT), water aerosol without plasma activation (C-PAA), and plasma-activated aerosol (PAA). C-PAA and PAA samples were treated for 20 min and stored at 10°C for 5 days (t5). Specifically, natural microbiota (mesophilic and psychrophilic bacteria, lactic acid bacteria, *Pseudomonadaceae*, *Enterobacteriaceae*, yeasts, and molds) quality parameters (moisture content, firmness, total soluble solids, pH and CIE Lab* colour), enzymatic activity (phenylalanine ammonia-lyase and polyphenol oxidase activities, superoxide dismutase) and antioxidant capacity (TPC and ABTS assay) were evaluated after treatments (t0) and at the storage end point (t5).

Microbiological analyses revealed significant ($p < 0.05$) inhibition of endogenous microbial growth, although reductions were moderate and not uniform across microbial groups. PAA application was particularly effective in the reduction of psychrophilic count, lactic acid bacteria, yeasts and molds, suggesting a positive impact on strawberries shelf-life.

Enzymatic analyses showed no effects on phenylalanine ammonia-lyase and polyphenol oxidase activities, while superoxide dismutase was activated immediately after PAA treatment; however, after storage, no significant differences were observed among PAA and the control samples (NT and C-PAA). No significant changes were detected in antioxidant-related indices.

In conclusion, in situ generated PAA contributed to preserving strawberry quality by limiting microbial growth and colour changes during refrigerated storage; however, process optimisation is required to enhance its efficacy on fresh fruit matrices.

S2-P34: Electrically-Assisted Supersonic Solution Blow-Spun Porous Nonwovens for Rapid Monitoring of Bacterial Growth, Volatile Amines, and Fish Spoilage

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Rapid and non-destructive tools capable of detecting microbial spoilage are essential for improving food safety and reducing food waste. In this study, highly porous polycaprolactone (PCL) nonwoven mats were developed through the combination of non-solvent-induced phase separation and electrically-assisted supersonic solution blow spinning (EASBS) to produce intelligent colorimetric indicators for bacterial growth, volatile amines, and food freshness monitoring. Bromophenol blue, alizarin, and curcumin were incorporated as pH-sensitive indicators, and the performance of porous EASBS materials was compared with non-porous EASBS fibers. The ultrafine porous nonwovens exhibited substantially smaller fiber diameters and higher specific surface area than the reference materials, resulting in enhanced interaction with volatile spoilage compounds. Among all formulations, bromophenol blue-loaded porous nonwovens demonstrated the highest analytical performance, showing the lowest detection and quantification limits for ammonia and volatile amines. The developed materials also exhibited a clear colorimetric response after 24 h of direct exposure to *Escherichia coli*, demonstrating their ability to detect bacterial metabolic activity without inhibiting bacterial growth. During storage of fish fillets at 25°C, the indicators exhibited a progressive color transition from yellow to green, closely following increases in pH, total volatile basic nitrogen, and total viable counts over 72 h. These findings demonstrate that integrating NIPS with EASBS enables the fabrication of ultrathin, highly porous smart nonwovens with superior sensitivity toward bacterial metabolism and volatile spoilage compounds. The developed materials represent a promising platform for intelligent food packaging that can rapidly detect bacterial activity and monitor freshness in real time, contributing to safer food and reduced food waste.

S2-P35: Effect of an Olive Oil, Lemon Juice, and Essential Oil–Based Marinade on the Survival of Salmonella spp. and Listeria monocytogenes in Vacuum-Packaged Sea Bass Fillets (Dicentrarchus labrax)

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In recent years, the demand for convenient ready-to-cook products has increased significantly due to changing consumer lifestyles and preferences. However, the high perishability of fish, combined with the risk of contamination by pathogenic microorganisms, poses a serious challenge for ensuring the safety and quality of such products. The main objective of the present study was to evaluate the antimicrobial activity of a marinade composed of olive oil and lemon juice, with or without the addition of oregano essential oil, aiming to assess the effect of the marinade on the survival of Salmonella spp. and Listeria monocytogenes.

During the experimental procedure, two independent replicates of two different batches were performed. Fish fillet samples were inoculated with a three-strain cocktail of Salmonella spp. or Listeria monocytogenes at ca. 10^4 CFU/g. The fillet treatments included the following: a control group (non-marinated), an olive oil-lemon juice-based marinade, and an olive oil-lemon juice-essential oil-based marinade. The samples were stored under vacuum packaging at two different temperatures (4 and 12 ^{o}C). Microbiological analysis, pH measurements, and sensory evaluation (odor, color, and texture) upon package opening were performed. In addition, spectral data were collected at the same time intervals using two Multispectral imaging devices (Benchtop and Portable MSI). The spectral data were further processed, and predictive models were developed to estimate microbiological counts and predict the presence or absence of pathogenic microorganisms.

Marination, with or without the addition of oregano essential oil, reduced the levels of Salmonella spp. and Listeria monocytogenes compared to non-marinated (control) samples by 0.93 and 0.70 log CFU/g, respectively. According to sensory evaluation, the shelf life of the fillets was extended by up to 2 days for samples without essential oil and up to 4 days for samples with essential oil, in both Salmonella spp. and Listeria monocytogenes cases. Regarding the spectral data, the examined models showed promising results for rapid microbiological assessment and performed effectively in predicting the presence of pathogenic microorganisms in the product, reaching accuracy levels of up to 93–95%.

Overall, the results indicate that olive oil–lemon juice–based marinades can reduce Salmonella spp. and Listeria monocytogenes levels, while multispectral imaging combined with predictive modeling demonstrated great potential as a rapid, non-invasive method for microbiological assessment, achieving high accuracy in detecting pathogenic microorganisms.

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S2-P36: Evaluation of quality and safety of gutted gilthead sea bream (Sparus aurata) using classic and rapid detection methods with tag sensors and multispectral imaging (MSI)

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The gilthead sea bream (Sparus aurata) is a highly perishable species in which microbial spoilage and post-mortem biochemical changes rapidly compromise food safety, organoleptic quality, and shelf life. Therefore, real-time and non-invasive monitoring of spoilage is essential to reduce waste and improve quality control. In this context, rapid and non-destructive methods for assessing fish freshness are increasingly needed, with sensor tags and multispectral imaging (MSI) emerging as promising tools for monitoring spoilage during storage.

Farmed whole gutted sea bream were packed under modified atmosphere packaging (45% CO2 / 35% N2 / 20% O2) with tag sensors embedded inside the packaging and stored at 0,4,8, and 12°C. The designed tag sensors changed color from yellow to blue in response to volatile compounds produced during spoilage. During storage, microbiological counts and pH levels were measured, and sensory evaluations were performed. Additionally, multispectral imaging (MSI) analysis of the sensor tags was carried out at each sampling point.

At all temperatures, microbial populations increased progressively, reaching 5.3–7.8 log CFU/g at the end of storage with Pseudomonas spp. and H_2S-producing bacteria as the dominant spoilage organisms. Based on sensory rejection, the shelf life of the samples was 18, 12, 6, and 5 days at 0, 4, 8, and 12°C, respectively. It should be noted that the fish used in this study were of high initial freshness (initial TVC 3.1 log CFU/g) and originated from batches, which likely resulted in lower initial microbial loads and may partly explain the prolonged organoleptic acceptance, particularly at 12°C. The sensor tags exhibited a gradual color transition from yellow to blue during storage, corresponding to the progression of spoilage. This visual change was consistently monitored alongside MSI analysis. MSI measurements demonstrated a gradual reduction in the reflectance spectra of the sensor tags over time, with the most pronounced decrease observed in the 600–700 nm region. pH values remained relatively stable during storage, ranging from 5.8 to 6.3 across all storage temperatures.

Overall, the results of the present study are promising and highlight the potential of tag sensors combined with multispectral imaging (MSI) as a rapid and non-destructive tool for monitoring fish spoilage and assessing microbial quality.

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S3 Novel and Sustainable Bioprocesses in Circular Economy

S3-P1: Fresh-Cut Lettuce – Industrial Application of Plasma-Treated Water for Food Safety and Food Quality

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Bacteria, including human pathogens and zoonoses, are known for (persistent) biofilm growth. Especially in fresh food production this can be challenging, as high humidity and nutrient availability provide a perfect fundament for biofilm growth. Equipment with hygienic design, antimicrobial properties of the process water and CIP/SIP-processes offer avoidance of biofilm growth. Here, plasma treated water (PTW) was combined with hygienic design within a commercial lettuce washing line to investigate its industrial applicability, antimicrobial effectiveness and preservation of food quality. PTW was applied within a lettuce washing line to investigate total viable count (TVC), microbial viability, and vitality of the fresh cut lettuce itself and the process water with/without storage respecting the food quality and toxicology. In dependency of the PTW concentration TVC reductions of 2 lg-steps on the lettuce and 3 lg-steps in the process water were detected. Viability and vitality of the microorganisms were decreased, too. Food quality like color was not affected and no toxicological effects have been seen. The implementation of PTW at different washing steps was realized at industrial conditions. The promising results of this project, the strong industrial need and the realistic implementation possibilities offer new non-thermal plasma-based tools for the challenging defence against biofilm growth in food industry.

S3-P2: Beneficial Properties of Lactic Acid Bacteria with Emphasis on γ - Aminobutyric Acid and Bacteriocins Production

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Production of metabolites beneficial to health, such as bacteriocins and γ-aminobutyric acid (GABA) are some of the metabolites of interested in beneficial microbes. Our objective was to identify new safe strains and to evaluate their multitasking beneficial properties, including bacteriocin and GABA production. Role of environmental factors on expression of bacteriocins and GABA.

More than 146 isolates were obtained from fermented dairy products and evaluated for bacteriocin and GABA production. From them 17 were identified as bacteriocin producers (versus *Listeria monocytogenes* and *Staphylococcus aureus*) and 59 as producers of GABA based on colorimetric test. Crossing the safety assets, bacteriocin production, and GABA production, 7 strains were selected for further investigations, including strains: *Pediococcus pentosaceus* ST0401KOC, *Latilactobacillus curvatus* ST0403KOC, *Pediococcus acidilactici* ST0412KOC, *Lactiplantibacillus plantarum* ST0414KOC, *Loigolactobacillus coryniformis* ST0105KOC, *Lacticaseibacillus rhamnosus* ST0154KOC and *Lacticaseibacillus paracasei* ST0110KOC.

Studies cultures were evaluated for GABA biosynthesis, including production capacity was explored under different conditions of cellular density (105–108 CFU/mL), monosodium glutamate (30 mM–120 mM), pH (4.0–8.0), temperatures (30°C–50°C) and incubation time (24h–120h), varying one-method-at-time, maintaining other standard conditions of 106 CFU/mL, 60 mM, pH 6.0, 37°C and 24 h and following to additional experimental set-ups.

As example, for *P. pentosaceus* ST0401KOC was recorded superior levels for GABA production, when the strain was cultured with low concentration of monosodium glutamate of 30 mM (37.17 mg/mL) and 24 h of incubation (18.34 mg/mL), respectively. Intermediate temperatures of 37°C and 40°C presented better amounts of GABA production (10.55 mg/mL and 15.00 mg/mL, respectively), demonstrating that standard culture temperatures can be considered as optimal for GABA production by *P. pentosaceus* ST0401KOC. Variations in pH and cell density were not influencing the production of GABA, maintaining close values between their respective variations.

Whole genomic sequence for *P. pentosaceus* ST0401KOC was performed and analyzed by bioinformatic tools regarding safety and beneficial metabolites production ability. Ten genes were identified (by VFanalyzer) and classified into the categories of antiphagocytosis (*cpsA*/*uppS*, *cpsI*), toxin (hemolysin), immune evasion (*cps4I*, *epsE*, *manA*), protease (*htrA*/*degP*, *tig*/*ropA*), and secretion system (T6SS II). Regarding mobile genetic elements, two mobilizable plasmids were found in strain ST0401KOC, with a predicted host range restricted to *Lactobacillaceae*.

Performed biochemical and physiological tests, and results from whole genome sequence and following bioinformatic analysis, hemolytic, gelatinase, proteolytic and lipolytic activity, biogenic amines production and antibiotic resistance, including different van genes, suggested that *P. pentosaceus* ST0401KOC can be considered as a safe strain, where GABA production can be influenced by environmental conditions.

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S3-P3: Growth of *Lacticaseibacillus rhamnosus* GG in Plant-Based Beverages

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The growing demand for plant-based milk alternatives highlights the need to evaluate their properties and suitability as carriers of probiotic cultures. This study aimed to characterise the growth of *Lacticaseibacillus rhamnosus* GG (LGG) in UHT-treated oat, rice, soya, and almond beverages, and to compare the obtained parameters with published data for milk. Growth data were analysed using a two-step modelling approach comprising the primary Baranyi–Roberts (BR) model and secondary temperature models (SQRT and cardinal models), enabling quantification of the effects of temperature on μ_{max} and lag phase across the biokinetic range. The cardinal temperatures of LGG were similar in all plant-based beverages (T_{min} ≈ 5°C, T_{opt} ≈ 40°C, T_{max} ≈ 46°C). In the rice beverage, for example, μ_{max} ranged from 0.043 to 0.601 h^{-1} between 8°C and 44°C, reaching a maximum of 0.753 h^{-1} at 40°C (R^2 = 0.996). Recently introduced correction factors derived from the SQRT and CTM parameters, b and μ_{opt}, respectively, confirmed that the nutritive potential of plant matrices is lower than that of milk. These findings demonstrate that predictive modelling is an effective tool for evaluating and ranking the suitability of plant-based beverage substrates for probiotic strains, providing a basis for their application in developing new fermented dairy-free probiotic products.

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S3-P4: D-Glucose Oxidation in Acetic Acid Bacteria: An *in silico* Study of *Acetobacter*

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Microorganisms with unique metabolisms are of particular industrial interest. These include acetic acid bacteria (AAB), which possess numerous membrane-bound dehydrogenases for the incomplete oxidation of diverse substrates. Although AAB were named for their ability to oxidize ethanol to acetic acid, certain strains are also used in the food industry for the oxidation of D-glucose to D-gluconic acid (GA) and various keto-D-gluconic acids (KGA).

To date, this metabolic pathway has been largely characterized only in the genus *Gluconobacter*, which inhabits sugar-rich environments, while information on its presence in other AAB genera remains scarce. A similar situation applies to the largest AAB genus, *Acetobacter*, in which oxidized forms of D-glucose have been detected after fermentation. However, a comprehensive bioinformatic reconstruction of the metabolic pathway linking these compounds to the corresponding enzymes remains lacking.

Our objective was to evaluate the capacity for D-glucose oxidation in the genus *Acetobacter* in silico using genomes from 25 strains representing 14 species. Strains of the genus *Gluconobacter* were used to define target proteins in the pathway and to provide reference amino acid sequences. These *Gluconobacter* reference sequences were compared with amino acid sequences of predicted gene products derived from the annotated *Acetobacter* genomes using protein–protein BLAST. BLAST output was filtered by identity (≥ 50%), similarity (≥ 60%), and E-value (≤ 10^{-50}), with hit validity assessed using a length ratio coefficient. Finally, keyword-based screening, reconstruction of multi-subunit proteins from annotation numbers, and verification of hit identity using the Conserved Domain Database were used to complement the analysis.

Compared with *Gluconobacter*, the reconstructed metabolic pathway in *Acetobacter* is shorter, indicating a reduced capacity for D-glucose oxidation. All strains appear capable of the initial step, the oxidation of D-glucose to GA, whereas ketogenic dehydrogenases – common in *Gluconobacter* – occur in only half of the tested species. Although 2KGA and 5KGA production are similarly represented in *Gluconobacter*, with many strains producing both structural isomers, the enzyme responsible for 2KGA production predominates in our set of *Acetobacter* strains.

Our results show that the ability to oxidize D-glucose is not restricted to the model genus *Gluconobacter*, with some *Acetobacter* strains exhibiting comparable industrial potential. Expanding bioinformatic analyses to other AAB genera and corroborating them with laboratory experiments would be valuable future directions.

S3-P5: Advancing Sustainable Bioactive Solutions Through the Microbial Assessment of Valorized Olive Processing Residues

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The olive oil industry, one of the largest in the Mediterranean region, generates vast quantities of bioactive-rich residues that represent both an environmental challenge and a significant opportunity for valorization. While these by-products are known for their high phenolic content, their potential to serve as natural, selective microbial growth regulators remains quite unexplored. The aim of the present study was to evaluate the impact of olive residue extracts on a diverse range of food - related microorganisms, focusing on their ability to inhibit undesirable microorganisms while potentially supporting beneficial technological strains. By assessing these complex interactions, this research aims to advance the development of sustainable and multifunctional bioactive solutions for the food and pharmaceutical sectors. Extracts from olive by-products, namely olive pomace, olive leaves and olive kernels, were prepared with ultrasound-assisted extraction under optimized conditions using natural deep eutectic solvents (NADES) as green solvents. Aqueous extracts were obtained from olive pruning residues through controlled magnetic stirring. Simultaneously, olive mill wastewater was subjected to liquid-liquid extraction using ethyl acetate to isolate a concentrated phenolic-rich fraction. Ethyl acetate was subsequently removed with rotary evaporation, and the resulting extract was re-dissolved in a water-ethanol solution. The antimicrobial activity of the extracts was tested against a wide range of food related microorganisms using the agar well diffusion method. Specifically, *Pseudomonas*, *Lactiplantibacillus*, *Levilactobacillus*, *Listeria*, *Escherichia*, *Candida* and *Wickerhamomyces* strains were included in this study. After incubation for 48 h, the diameters (in mm) of growth inhibition zones were measured. The respective solvents of each extract were also tested as control samples. The extracts exhibiting initial antimicrobial activity were further evaluated using microplate growth assays to monitor microbial inhibition and growth kinetics over a 48-h period. Results demonstrated that each olive residue extract affected microbial growth in a slightly different way. The present study is promising and highlights the potential future application of bioactive-rich olive by-products extracts as additives in functional food products.

S3-P7: Antibacterial and Physicochemical Properties of Vinegars Produced from Beer and Sweet Potato

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Converting spoiled alcoholic drinks or surplus crops into vinegar is a promising approach to reducing biomass waste and generating economic value. This study sought to characterize vinegar produced from beer and sweet potato using the Orleans method, to highlight their potential as sources of specific nutrients, and to unravel some of their interesting biological activities. Physicochemical parameters (pH, total acidity, °Brix, total phenolic content [TPC], flavonoid content [TFC], total antioxidant capacity [TAC], and mineral composition) were assessed. Antimicrobial activity against bacteria and yeasts was also evaluated. TAC values were 0.40 ± 0.001 and 0.134 ± 0.035 DPPH, µg Trolox/mL, for beer and sweet potato vinegars, respectively. Sweet potato vinegar showed the highest TFC (102.318 ± 5.785 mg catechin/L), while beer vinegar had the highest TPC (0.62 ± 0.06 mg gallic acid/mL). Potassium, calcium, and sodium were the major minerals detected, with sweet potato vinegar showing high levels of potassium (366.33 ± 29.03 mg/mL), calcium (3.27 ± 0.17 mg/mL), sodium (68.67 ± 2.12 mg/mL), and zinc (0.98 ± 0.00 mg/mL). The enterobacteria *Cronobacter sakazakii* and *Salmonella* Typhimurium were the most tolerant (10.00 ± 0.00–25.75 ± 0.96 mm), while *Pseudomonas aeruginosa* was the most sensitive (30.00 ± 5.77–30.00 ± 1.29 mm), along with *Staphylococcus aureus* and *Listeria innocua* (26.09 ± 0.58–29.33 ± 0.58 mm). The acetification yielded vinegars with acetic acid levels above 5% in accordance with legal standards, and the final products are valuable sources of phenolic compounds, antibacterial agents, and minerals. These processes provide an effective way to add value to materials that would otherwise be discarded, offering a profitable strategy for managing and valorizing losses and waste.

S3-P8: Hazard Analysis and Risk Assessment of Animal-Free Casein Production by Precision Fermentation

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Precision fermentation (PF) is a process that utilizes genetically modified microorganisms (GMMs) to produce specific ingredients or compounds through fermentation. PF is considered a sustainable alternative for, amongst others, the production of animal free dairy proteins. While the PF process itself is not new, the envisioned scale at which proteins are foreseen to be produced for sustainable food production is unprecedented. PF processes can vary greatly depending on the choice of product, microorganism, fermentation feedstock, production scale, and Down Stream Processing (DSP) strategy, which influence not only the final product but also the hazards associated with the process.

The objective of this research is firstly to identify potential hazards and critical control points (CCPs) in two generic precision fermentation processes for recombinant casein production and, secondly, to conduct a microbial risk assessment for the precision fermentation processes.

Two generic, representative industrial scale processes for recombinant casein production were evaluated, including upstream processing, fermentation and DSP. One process uses the yeast *Komagataella phaffii* and the other the bacterium *Escherichia coli*, resulting in two very different processes to produce the same protein. To identify the most significant hazards in these processes, information was collected through literature research, expert consultations, and interviews with recombinant protein producers. For each of the processing steps, hazards were identified and evaluated using the FAO HACCP decision tree to determine whether the step constituted a CCP. It was concluded that individual hazards can be identified for both processes, with overlapping key hazards in these processes, mostly related to microbial contamination and subsequent toxin production by pathogens, like *Bacillus cereus*. To determine whether these hazards pose a realistic risk in PF-derived products, a quantitative microbial risk assessment was performed using microbial growth and inactivation models. The results of this risk assessment pointed towards sensitivities in the PF process, both during fermentation and DSP. It can be concluded that pathogen growth and toxin production can be a risk in PF processes, which should be properly addressed. Generic HACCP guidelines can be established for PF processes, however, further optimization and process-specific refinements are necessary prior to their implementation in alternative production systems for recombinant dairy proteins intended for food applications.

S3-P9: Exploring Acetic Acid Bacteria Diversity Across Natural and Fermentative Environments

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Acetic acid bacteria (AAB) exhibit a wide range of beneficial properties, most notably their role in food and beverage fermentation and their ability to produce extracellular polysaccharides. Consequently, the isolation and characterization of novel strains are essential for expanding culture collections of industrially relevant microorganisms. In this study, a total of 104 AAB isolates were obtained from diverse natural and fermentative environments, including flowers, fruit and vegetable surfaces, vinegars, naturally pressed juices, and pomace. The results revealed a broad distribution of AAB, with members of the genus Acetobacter present across most sample types. Vinegar samples exhibited the highest species diversity, highlighting them as a particularly rich reservoir of AAB and a source of novel bacterial species. Isolation from flowers was relatively inefficient, with successful isolation in only 34.8% of samples. Despite the large number of samples, only 4 AAB species were identified in flowers, with each sample typically containing a single species, suggesting dominance by the most abundant strain. Notably, 91.3% of flower isolates belonged to the genus *Acetobacter*, either to *Acetobacter pasteurianus* or *Acetobacter senegalensis*, with single representatives of *Bombella apis* and *Gluconacetobacter cerinus*. From fruits and vegetables, 32 isolates representing 17 species across five genera (Acetobacter, *Gluconobacter*, *Gluconacetobacter*, *Komagataeibacter*, *Novacetimonas*, and one potentially novel genus) were obtained. Similar species compositions were observed in the juice and pomace samples, as expected given their origin. Initial screening of the 16S-23S rRNA intergenic spacer region analysis indicated the presence of multiple potentially novel taxa. Subsequent phylogenetic and phenotypic analyses confirmed several previously undescribed species, particularly among vinegar isolates. Two novel species, *Komagataeibacter viniaceti* sp. nov. and *Novacetimonas teuberi* sp. nov., are proposed in this study, both isolated from vinegar samples and characterized using a polyphasic taxonomic approach. The strains are Gram-negative, aerobic (tolerant of reduced oxygen conditions), motile bacteria capable of oxidizing ethanol to acetic acid, which is then further oxidized to CO_2 and H_2O. Notably, both species produce bacterial cellulose, highlighting their potential for industrial applications. This study represents one of the first comprehensive large-scale investigations of AAB diversity across diverse habitats within a European environment. The results provide new insights into the ecological distribution of AAB and identify vinegar as a promising source of novel taxa. These findings contribute to the development of microbial resources with potential applications in biotechnology and industrial processes.

S3-P10: Eco-Friendly and Sustainable Chitosan-Based Edible Coatings for Fish Fillets Shelf-Life Extension

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Nowadays, people are becoming increasingly concerned about climate change, environmental pollution, and food waste, tending to adopt more environmentally friendly habits. Domestic and industrial food waste creates a significant problem that the food industry is facing and requires changes in processing techniques in order to achieve more sustainable production practices. Use of natural preservatives and materials obtained from side-streams, such as shrimp shells, can contribute to improved sustainability. Capsaicin is the active compound derived from chili peppers, with important properties such as anti-inflammatory, anticancer, antioxidant, and thermoregulatory effects. On the other hand, chitosan is a natural, biocompatible, and biodegradable polysaccharide derived from chitin (found in shellfish), with remarkable antibacterial properties.

The present study focuses on the use of biodegradable packaging materials in combination with natural antimicrobials for the preservation and shelf-life extension of fish, with minimal environmental impact. Commercial chitosan (co) powder and laboratory-made chitosan (lab) (Atatürk University) were used, with or without the addition of capsaicin, as edible coatings on sea bream fillets. Samples were stored at 4°C for up to 19 days and evaluated for sensory characteristics (odor, appearance, and firmness), physicochemical (color, pH, texture) and microbiological changes, including Total Viable Counts (TVC), yeasts and molds, lactic acid bacteria (LAB), *Enterobacteriaceae*, *Pseudomonas* spp., and H_2S-producing bacteria, in to determine shelf-life.

Chitosan (co), chitosan (co) with 0.5 mg/L capsaicin, chitosan (lab), and chitosan (lab) with 0.5 mg/L capsaicin samples showed no significant odor changes compared to the control samples. The use of capsaicin had no effect on extending the shelf-life of the fillets. Chitosan (co) and chitosan (lab), with or without capsaicin, reduced the population levels of both *Enterobacteriaceae* and H_2S-producing bacteria below the detection limit, while LAB populations increased after 5th day, reaching 3 log CFU/g. *Pseudomonas* spp., as well as yeasts and molds, increased gradually after the 5th day of storage. However, TVC on 9th day for samples chitosan (lab) and chitosan (lab) with capsaicin remained approximately 2 log levels lower than those of chitosan (co) with or without capsaicin. Overall, laboratory-produced chitosan demonstrated better performance compared to commercial chitosan.

In conclusion, the use of side-stream, edible, and environmentally friendly materials can effectively extend the shelf-life of sea bream fillets. The development of alternative bio-based packaging solutions for seafood preservation contributes to greener processing technologies by reducing both plastic usage and food waste, thereby minimizing environmental impact.

S3-P11: Upcycling Banana Peel into a Sustainable Functional Ingredient Supporting Probiotic Growth and Enhancing Postbiotic Antioxidant Activity

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The valorization of agro-industrial by-products represents a promising strategy for developing sustainable and functional food systems. Banana peel, a widely generated waste material, is a rich source of bioactive compounds with potential applications as a functional ingredient. This study aimed to produce a stable banana peel powder and to evaluate its physicochemical properties, antioxidant and antimicrobial activities, as well as its ability to support probiotic growth and enhance postbiotic functionality. Banana peels were washed, size reduced, freeze-dried, and milled into a stable powder. In addition, ultrasonic-assisted extraction was applied to obtain bioactive-rich extracts. The powder was characterized in terms of moisture content (6.9%), ash content (11.49%), pH (5.57), and titratable acidity (0.51 g/100 g). Antioxidant analysis revealed high radical scavenging activity (DPPH: 79.8%) along with considerable total phenolic content (39.22 mg GAE/g extract). Supplementation with banana peel powder (2%, w/v) enhanced *Pediococcus acidilactici* growth, resulting in an increase of 1.98 log colony forming units (CFU)/mL (~95-fold) compared to the control. Additionally, fermentation led to marked physicochemical changes, including decreased pH and increased titratable acidity, indicating enhanced metabolic activity. Postbiotic obtained from cultures (cell free supernatant) supplemented with banana peel powder maintained high antioxidant activity, with DPPH values reaching approximately 90.60%, demonstrating that substrate composition can influence postbiotic functionality. Furthermore, banana peel extracts exhibited selective antimicrobial activity, particularly against Gram-positive bacteria such as *Bacillus cereus*, *Staphylococcus aureus*, and *Listeria monocytogenes*, while no inhibition was observed against Gram-negative strains such as *Escherichia coli* O157:H7, *Salmonella* Typhimurium. Overall, these findings demonstrate that banana peel can be transformed into a multifunctional and sustainable ingredient that supports probiotic growth, enhances metabolic activity, and preserves bioactivity. This approach highlights its potential as a low-cost substrate for the development of next-generation functional food systems aligned with circular economy principles.

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S3-P12: Oleaginous Yeast Biomass for Application in Meat Analogues

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Oleaginous yeasts have emerged as sustainable platforms for producing lipids and biomass with promising applications in the food industry, particularly in meat analogue development. Within a circular economy perspective, the conversion of agro-industrial residues into high-value ingredients represents a key strategy to reduce environmental impacts and enhance resource efficiency. In this context, this study evaluated oleaginous yeasts cultivated on low-cost substrates, with emphasis on crude glycerol, a biodiesel by-product, as alternative sources of lipids and proteins. Cultivation conditions were optimized, and the fatty acid profiles of the resulting biomasses were compared with those of bovine meat to assess their technological and nutritional relevance.

The strains *Yarrowia lipolytica* CCMA 0357 and *Cystofilobasidium ferigula* CCMA 1623 were cultivated under optimized conditions and characterized in terms of biomass yield, lipid accumulation, and total protein content. Lipid contents reached 51.79% for *Y. lipolytica* and 63.12% for *C. ferigula*, confirming their high oleaginous potential. Additionally, both biomasses showed relevant protein levels, with 16.4 ± 0.1% (w/w) and 12.1 ± 0.1% (w/w), respectively, reinforcing their dual functionality as lipid and protein sources.

Fatty acid profile analysis, performed through heatmap visualization, indicated that the predominant fatty acids in both yeast-derived lipids and bovine meat were palmitic (C16:0), stearic (C18:0), and oleic (C18:1) acids, which are essential for sensory properties and structural behavior in meat analogues. Minor fatty acids included myristic (C14:0) and linoleic (C18:2) acids; notably, myristic acid was more abundant in meat, whereas linoleic acid was more prevalent in yeast biomasses, suggesting potential advantages in terms of nutritional profile.

Overall, the findings demonstrate the feasibility of integrating microbial bioprocesses into circular bioeconomy systems by valorizing low-value residues such as crude glycerol into high-value food ingredients. This approach aligns with sustainability goals by potentially reducing waste generation, lowering reliance on conventional livestock systems, and contributing to decreased greenhouse gas emissions, as could be further quantified through life cycle assessment (LCA) and carbon footprint analyses. Oleaginous yeasts thus represent promising and scalable platforms for next-generation meat analogues. Future work should focus on lipid oxidative stability, techno-functional performance in complex food matrices, and comprehensive environmental assessments to support industrial implementation.

S3-P13: Cultivation Strategy-Driven Lipid Accumulation and Fatty Acid Profiling of Yeast Biomass for Application in Meat Analogues

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Two cultivation trials were conducted within a circular economy framework, aiming to valorize low-cost agro-industrial residues – namely molasses and waste oil – as substrates to produce value-added microbial biomass. The study evaluated biomass yield, lipid accumulation, and the physicochemical properties and fatty acid profile of the resulting biomass. Fermentations (35–50 L) were carried out under different conditions: a stainless-steel airlift bioreactor and a polyethylene bioreactor with mechanical agitation. In the first trial, performed as a two-stage fed-batch process, molasses was initially used as the carbon source. After sugar depletion or when °Brix dropped below 5, feeding with waste oil was initiated. This condition resulted in a biomass yield of 1%, with an average biomass volume of approximately 500 mL, but low lipid accumulation (8% total lipids). Despite the use of residual substrates, the delayed introduction of the hydrophobic carbon source likely limited lipid biosynthesis. In the second trial, conducted in a single stage with lower molasses concentrations and the addition of waste oil from the beginning, biomass yield increased to 1.43%, with a similar biomass volume (~500 mL), and a substantially higher lipid accumulation (51.58%). This approach enhanced substrate co-utilization and improved the conversion of waste streams into lipid-rich biomass, reinforcing the efficiency of circular bioprocessing strategies. The high-lipid biomass showed higher moisture content (75.01%), whereas the low-lipid biomass presented higher protein (34.43%) and ash (3.98%) contents. Fiber levels were low in both samples. These findings indicate that cultivation conditions strongly influence biomass composition, particularly the balance among lipids, proteins, and minerals, and can be tailored depending on the intended food application. Fatty acid profiling showed a predominance of long-chain fatty acids, mainly unsaturated. In the low-lipid biomass, linoleic acid (C18:2) was the major component (44.81%), followed by oleic (C18:1) (19.05%) and palmitic acids (C16:0) (16.83%). In the high-lipid biomass, linoleic (33.48%) and oleic acids (28.23%) remained predominant, followed by stearic (C18:0) (14.07%) and palmitic acids (8.84%). Comparison with different beef cuts (chuck, flank, shoulder, round, and burger) showed a similar predominance of long-chain fatty acids. Overall, the results demonstrate that cultivation strategy and hydrophobic substrate availability directly affect lipid accumulation and biomass composition. Importantly, they highlight how circular economy approaches - through the upcycling of industrial residues - can support the sustainable production of yeast-derived lipids and proteins as functional ingredients, serving as partial substitutes for animal fats and as alternative protein sources in meat analogues.

S3-P14: Ensuring Safe Reuse of Aquaculture Side Streams: Heat Hygienisation and Documentation Using Seven Microbial Indicators for Feed and Food Safety

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Sustainable utilisation of side streams from aquaculture is increasingly important as global food systems face shortages of feed and fertiliser. Current regulations restrict the use of fish sludge and by products due to concerns about zoonotic transmission, cannibalism, and recycling of pathogens—rules largely developed before circular economy principles and resource scarcity became pressing. To support safe valorisation of marine side streams, this study evaluated whether hygienisation through heat treatment can be reliably documented and used to ensure microbiological safety in feed and fertiliser production.

The work comprised three components: (1) selecting indicator organisms suitable for hygienisation tests in cold water marine by-products, (2) investigating survival of both legislated indicator microbes and relevant aquaculture-associated pathogens in minced whole fish during heat treatment, and (3) proposing practical approaches for documenting effective hygienisation under full-scale industrial conditions. Seven test organisms were examined: *Escherichia coli*, *Salmonella* Senftenberg, *Clostridium perfringens*, *Bacillus subtilis*, *Listeria monocytogenes*, *Moritella viscosa*, and *Carnobacterium maltaromaticum*. Minced fish (≤ 15 mm particle size) was heated statically in a heating block at 65°C and 80°C to simulate conditions typical of biogas and feed-preprocessing systems.

Five of the organisms showed similar survival patterns: initial stability for approximately 5 min, followed by a rapid 3–4 log reduction after 10–15 min, and then unexpected persistence the next hour until the end of the experment. Only *M. viscosa* showed complete inactivation within 5 min at 85°C and 25 min at 65°C. The incomplete inactivation of the other microbes was likely caused by uneven heat penetration, including poor heating at the top of the test tubes and formation of a fat layer that insulated upper regions of the material.

These findings demonstrate that uneven heat distribution—not the inherent heat resistance of the microbes—poses the main risk for survival. Consequently, documentation of hygienisation must focus on identifying cold spots within processing equipment. Approaches include using temperature mapping, introducing non-pathogenic biological indicators with known heat tolerance, or analysing microbiomes before and after treatment to confirm loss of heat-sensitive species. Methods detecting dead cells should be avoided.

In conclusion, heat treatment is effective for hygienisation of marine by products, but only when temperature distribution is uniform. Reliable documentation must therefore include assessment of areas with potentially reduced heat transfer. This study proposes three practical microbiological approaches to verify effective hygienisation and support safe circular use of aquaculture side streams.

S3-P15: TRANSConTAFOOD Project – Assessment of Risks Associated with Cross-Contamination (Microbial, Chemical, Allergenic) During the Reuse of Food Packaging

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The reuse of food packaging is a key environmental strategy to reduce waste generated by consumption while preserving the resources required for packaging production. As part of the circular economy and supported by the AGEC law, this approach represents a sustainable alternative to single-use packaging. However, it raises major sanitary concerns, particularly regarding cross-contamination between food and reused packaging.

The safety of reusable packaging depends on its integrity (tightness, cleanliness, absence of wear), as well as on the effectiveness of cleaning and disinfection procedures. These protocols must ensure the removal of microorganisms and allergens while limiting the presence of biocide residues. This requires a better understanding of the mechanisms governing the transfer of these contaminants between reused packaging surfaces and food.

The TRANSCONTAFOOD project (2025–2028), funded by the French National Research Agency (ANR), aims to assess the risks of cross-contamination (microbiological, chemical, and allergenic) associated with the reuse of food packaging. Regarding the microbiological component, the study will focus on several foodborne pathogens of interest (*Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Salmonella* spp., *Bacillus cereus*, *Bacillus subtilis*), as well as a spoilage-associated bacterium *Pseudomonas* spp..

The work will investigate the ability of these microorganisms to persist on different materials (plastics and glass) subjected to repeated washing cycles and material aging due to use. In particular, real-use conditions (thermal treatment, cold or ambient storage) will be simulated to assess biofilm formation, resistance to disinfectants, and the risk of transfer to food products. The evolution of bacterial communities will be analyzed using both conventional approaches (culture, PCR, microscopy) and innovative techniques (Raman microspectroscopy, predictive modeling).

The generated data will feed into a global risk modeling framework integrating microbial, chemical, and allergenic contaminants, enabling a comprehensive assessment of the safety of reusable food packaging.

Acknowledgements: This poster presents the different research components of the TRANSCONTAFOOD project, with a focus on the microbiological aspects.

S3-P16: Fingerprinting Fermentation: Evaluating Fourier Transform Infrared Spectroscopy for Rapid Strain-Level Differentiation of Starter Cultures

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Reliable strain-level identification of starter cultures is essential for maintaining product quality and consistency in fermentation. *Lactococcus lactis*, one of the most widely used dairy starter species, exhibits substantial phenotypic and genomic diversity, and its two major groups, *L. lactis* subsp. *lactis* and *L. lactis* subsp. *lactis* biovar (bv.) *diacetylactis*, cannot be consistently distinguished using routine tools such as MALDI-TOF MS. This study evaluated the discriminatory performance of Fourier transform infrared (FTIR) spectroscopy using the IR Biotyper® system for species-, biovar-, and strain-level characterization of starter culture isolates. FTIR reliably differentiated six representative starter culture species and produced highly reproducible spectra using an optimized sample-preparation protocol. Within *L. lactis*, FTIR achieved clear biovar separation that closely reflected whole-genome sequence (WGS)-based phylogeny and correctly identified historically misclassified strains. Strain-level clustering was stable across biological replicates, growth media, and analytical pipelines, and strongly concordant with WGS-derived relationships. Building on these findings, we developed an artificial neural network (ANN) classifier trained on curated FTIR spectra from 15 *L. lactis* strains. Validation with 21 additional strains demonstrated high sensitivity, specificity, and predictive values, while confidence scoring confirmed robust exclusion of non-*L. lactis* species. Overall, this study establishes FTIR spectroscopy as a rapid, high-throughput, and cost-effective tool for routine monitoring of *L. lactis* strain identity, with strong potential for early detection of misclassification, culture drift, or contamination in fermentation workflows.

S3-P17: Starch-Dependent Changes in Bioactive Compounds and Microbiological Stability of 3D-Printed Jostaberry Products

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3D printed food represents an emerging and rapidly developing approach in food science that enables the design and production of personalized and functionally tailored food products with precisely controlled structure, composition, and nutritional value. One of the key determinants of the quality of the final printed product is the selection of appropriate raw materials, as ingredient composition strongly influences printability, rheological behaviour, structural integrity, and the stability of the printed matrix. In addition to achieving desirable technological and sensory properties, a major challenge in 3D food printing is ensuring the stability and safety of the product during storage. Changes occurring post-processing, such as moisture redistribution, structural relaxation, and degradation of bioactive compounds, can significantly affect both the nutritional quality and microbiological safety of the final product. Therefore, stability assessment during storage is essential for the successful development of functional 3D-printed foods.

In this study, the suitability of 3D printing technology was investigated using jostaberry (*Ribes* × *nidigrolaria*) as a functional fruit ingredient, with a particular focus on the influence of starch type (corn or wheat) and refrigerated storage (4°C for 12 days) on the physicochemical properties, bioactive profile, and microbiological stability of printed snacks. Both starch systems enabled successful printing, however, they differently affected product quality. Wheat starch increased total soluble solids and improved anthocyanin retention, whereas corn starch contributed to higher levels of other polyphenolic subclasses, indicating substrate-dependent modulation of bioactive compound stability.

Storage time had the most pronounced effect on product stability. Colour changes became more evident during storage, with a marked increase in ΔE values after day 5, mainly associated with moisture loss and starch retrogradation processes. The degradation of polyphenols was compound-specific: condensed tannins and anthocyanins decreased by 27.6% and 37.3%, respectively, while total phenolic content remained relatively stable throughout storage.

Microbiological analysis confirmed the absence of *Salmonella*, *Enterobacteriaceae*, yeasts, and moulds after 12 days of storage, demonstrating that the printed products maintained microbiological safety under refrigerated conditions. Overall, the results indicate that jostaberry is a suitable ingredient for the development of functional 3D-printed snacks with acceptable structural, bioactive, and microbiological stability during short-term refrigerated storage.

Keywords: 3D printed food, jostaberry (*Ribes* × *nidigrolaria*), bioactive compounds, microbiological stability, refrigerated storage

S3-P18: *In vitro* Antimicrobial Assessment of Lactic Acid Bacteria Isolates and Their Postbiotics for Integration into Edible Membranes

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The rising demand for safe, natural food preservation methods has driven significant interest in active food packaging, particularly edible membranes. Probiotics and their cell-free metabolic products (i.e. postbiotics) offer a highly promising, environmentally friendly alternative to synthetic antimicrobials. This study aimed to screen the antimicrobial efficacy of various lactic acid bacteria (LAB) strains and their corresponding postbiotics against common foodborne pathogens and fish related microorganisms.

A broad spectrum of LAB strains, including *Lactiplantibacillus plantarum*, *Lactococcus lactis*, *Leuconostoc lactis*, and *Lacticaseibacillus* species, was utilized. These strains were isolated from varied geographical and dietary origins, including traditional Greek products (Staka from Crete, Feta cheese from Lesvos, and Kerasoelia olive fermentations from Agio Oros), as well as reconstituted cow milk powder from the Fiji Islands. The antagonistic activity of the live probiotic cells was evaluated using the soft agar overlay method. Furthermore, postbiotics were produced via centrifugation and 0.22 µm filtration, and their antimicrobial capacity was assessed using the well diffusion method. The target pathogens included *Staphylococcus aureus*, *Salmonella enterica*, *Listeria monocytogenes*, and *Escherichia coli*. To broadly evaluate the antimicrobial efficacy, the study also targeted species of *Pseudomonas*, *Aeromonas*, *Vibrio*, and *Brochothrix*, which are widely recognized for their roles in fish spoilage and pathogenesis.

The results indicated that the living probiotic strains possess potent, broad-spectrum antimicrobial activity. In the soft agar overlay assay, several strains demonstrated pronounced inhibitory zones across all tested pathogens, with maximum inhibition reaching up to 24.7 mm against *L. monocytogenes*. The postbiotics also exhibited notable, although milder, antimicrobial activity in the well diffusion assay, yielding inhibition zones generally ranging from 3.0 to 10.5 mm depending on the strain and target pathogen. Isolates, such as *Leuconostoc lactis* and specific *L. plantarum* strains, maintained competitive inhibition profiles in both their viable and postbiotic states.

In conclusion, the significant antagonistic properties of these LAB isolates and their metabolic byproducts underscore their strong potential as natural biocontrol agents. The complementary efficacy of both the living cells and their postbiotics provides a viable foundation for their application as active antimicrobial ingredients in edible membranes, aiming to enhance food safety and extend product shelf life.

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S3-P19: Solid-State Fermentation of Faba Bean for Protein-Rich Ingredients

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Plant-based protein ingredients are needed to support the transition towards more sustainable and healthier food systems. The use of faba bean is limited by antinutrients, off-flavours and suboptimal functionality. This study investigated solid-state fermentation (SSF) as a sustainable processing approach to improve the nutritional and techno-functional quality of protein-rich ingredients based on faba bean. Pre-treatment of faba beans was optimized to increase moisture content, soften the seed structure and reduce microbial load prior fermentation. SSF was carried out using various filamentous fungi, including *Neurospora tetrasperma*, *Aspergillus oryzae* and *Rhizopus microsporus* var. *oligosporus*, as single cultures and in coculture with lactic (LAB) or propionic acid bacteria (PAB). Fermented materials were dried, milled and analysed for key compositional and functional properties, raffinose-family oligosaccharides, phytic acid, degree of protein hydrolysis and free amino acids and for particle size. All studied strains and cocultures grew in pre-treated faba beans. Growth rates and effects of fermentation on flavour, texture and pH varied among them. SSF clearly decreased antinutritional factors. *A. oryzae* reduced raffinose-family oligosaccharides by more than 70%, while the highest phytic acid degradation obtained with *R. microsporus* reached 39%. Cocultivation of *R. microsporus* with *A. oryzae* or LAB, especially *Lactiplantibacillus plantarum*, or *Propionibacterium freudenreichii* further enhanced phytic acid reduction. Fermentation also increased free amino acid content ca. 4–5-fold in the dried ingredients, with *R. microsporus*-based fermentations producing higher levels than *A. oryzae* alone. The highest accumulation was observed in cocultures. Amino acid profiles were modified and for instance enrichment of umami amino acids or GABA was detected depending on the used strains. In addition, fermented samples showed improved dry matter and nitrogen extractability compared with pre-treated unfermented beans. Overall, SSF using cocultures is a promising strategy for producing dried, protein-rich faba bean ingredients with lower antinutrient levels, enhanced protein hydrolysis and improved extractability, demonstrating potential for sustainable plant-based food applications.

S3-P20: Development of Fungal–Bacterial Consortia for the Bioconversion of Plastic in Agri-Food Waste Streams

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The escalating global production of plastic polymers for food packaging and agricultural applications, alongside the accumulation of recalcitrant waste, necessitates innovative bioprocessing strategies to close the loop in the circular economy. While microbial degradation offers a sustainable alternative to traditional waste management, the enzymatic complexity required to break down high-density polymers such as polypropylene (PP), polyethylene (PE), and polyamide (PA) often exceeds the capabilities of individual organisms. Fungi, with their potent enzymatic machinery and hyphal growth, are particularly effective at colonizing and depolymerizing complex substrates. Species originating from extreme or oligotrophic environments may provide resilient enzymes capable of driving sustainable bioprocesses under harsh conditions.

This study investigates the development of specialized fungal–bacterial consortia through selective enrichment as a novel platform for plastic valorisation. A complex inoculum was created by combining plastic-contaminated environmental samples with fungal isolates from extreme habitats known for their plastic-degrading potential. These mixed communities were subjected to successive enrichment cycles in defined media, where microplastics served as the primary carbon source. To characterize the resulting diversity and community shifts, high-throughput sequencing of bacterial and archaeal 16S rDNA, fungal ITS, and eukaryotic 18S markers was performed. Amplicon sequence variants (ASVs) were analysed to assess the impact of selective pressure on community architecture.

Our findings highlight the dominant role of fungi in maintaining consortium stability during this bioprocess. The introduced fungal strains remained influential throughout the enrichment cycles and successfully integrated into the developing consortia without being outcompeted. The presence of microplastics significantly reshaped community structures, with fungal persistence supporting broader microbial diversity. This potentially provides the physical scaffolding and initial enzymatic attack required for subsequent bacterial co-degradation. These results suggest microbial consortia may represent a scalable approach for the bioconversion and upcycling of mixed microplastic waste, offering a promising new tool of circular economy in the food sector.

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S3-P21: Incorporation of a Fucoidan-Rich Extract into Citric Acid Crosslinked Alginate Films as Active Food Packaging Systems: Physicochemical and Functional Characterization

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The growing demand for sustainable materials has driven the development of biopolymer-based films as alternatives to plastic packaging. These materials can reduce environmental impact while extending food shelf life by limiting moisture loss, oxidative deterioration and microbial spoilage. Among them, alginates derived from brown algae are widely used due to their eco-friendly properties, while fucoidans, also derived from brown algae, are sulphated polysaccharides rich in fucose, exhibiting notable antioxidant activity. This study aimed to develop and characterize citric acid (CA) crosslinked alginate-based films containing a fucoidan rich extract for active food packaging applications. Alginate (1% w/w) was used as the film forming polymer, and fucoidan rich extract (Fuc. Ex.) was incorporated at ratios of 1:0 (control), 1:0.25, 1:0.5, 1:1 and 1:2 (% w/w, alginate/Fuc. Ex. in the film forming solutions). The solutions were cast into films and after drying crosslinking was elaborated on the surface of each film with citric acid (CA, 1.0 M). Crosslinked films were either rinsed with distilled water or left unrinsed prior to further characterization. The produced films were characterized in terms of mechanical properties: tensile strength (TS, MPa), elongation at break (EAB, %) and Young's modulus (YM, MPa), water vapor permeability (WVP, g mm/m^2 day kPa), water solubility (WS, %) and swelling degree (SD, %). FTIR analysis and morphological characterization (SEM, AFM) were performed, while also the release of phenolics compounds (mg catechin/g film), antioxidant potential (ABTS assay) and antimicrobial properties (zone inhibition assay) of produced films against *Escherichia coli* and *Listeria innocua* were also determined. The incorporation of Fuc. Ex. was significantly reduced WVP in CA alginate films leading to notably low WVP values (~0.14 g mm/m^2 day kPa), while the WS, SD, release of phenolic compounds and antioxidant activity were substantially increased. Unrinsed CA-crosslinked films exhibited inherent antimicrobial activity against *E. coli* and *L. innocua*, which was reduced after rinsing, indicating the key role of residual CA, while the incorporation of Fuc. Ex, led to a slight enhancement of antimicrobial activity. Regarding mechanical properties, no clear trend was observed with Fuc. Ex. incorporation. However, unrinsed CA-crosslinked films exhibited higher EAB and lower TS and YM compared to rinsed films, indicating a plasticizing effect of residual CA. Overall, the developed films demonstrate promising potential as active food packaging materials, combining improved barrier and antioxidant properties due to Fuc. Ex. incorporation, while the antimicrobial activity is mainly attributed to residual CA.

S4 Foodborne Pathogens and Their Resistance

S4-P1: Development of Resazurin-Based Assay for Rapid Evaluation of Sodium Hypochlorite Tolerance in *Salmonella*

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Sodium hypochlorite (NaClO) is frequently utilized in food processing. Exposure to NaClO disinfection may lead to the emergence of bacterial tolerance to chlorine, which is frequently associated with antibiotic cross-resistance. The broth microdilution approach with turbidity measurement is appropriate for comparing chlorine tolerance among isolates. However, it is a laborious and time-consuming method. At present, few studies investigated on the resazurin-based assay for rapid evaluation of sodium hypochlorite tolerance in *Salmonella*.

This study developed a method based on resazurin assay for evaluating NaClO tolerance in *Salmonella*, utilizing the changes of colorimetric and fluorescence intensity as the indicator of NaClO tolerance.

Chlorine tolerance assays were performed in 96-well microtiter plates. Each well contained 100 µL of NaClO solution at final concentrations of 8, 16, 32, 64, 128, 256, and 512 mg/L, followed by the addition of 100 µL of bacterial suspension. After 30 min of exposure at room temperature, the reaction was quenched by adding 20 µL of 2.5% $Na_2S_2O_3$ and incubating for an additional 10 min at room temperature. A total of 20 µL of PrestoBlue resazurin solution was added to each well.

The results shows that the NaClO tolerance is readily observable in colorimetric during incubating 7 h, while the NaClO tolerance was not detectable in the broth microdilution method by monitoring the turbidity. For the evaluate NaClO tolerance using fluorescence intensity, a significant rise in fluorescence was noted with the addition of the resazurin reagent and a 1-h incubation period when the initial inoculum concentration at 10^7 CFU/mL. Our research indicated that the *Salmonella* concentration identifiable varied from 10^4 to 10^7 CFU/mL by our resazurin-based approach, employing fluorescence intensity as an indicator of NaClO tolerance. Furthermore, the broth microdilution method revealed NaClO tolerance (MIC > 256 mg/L) in 1.6% (1/64) of the *Salmonella* isolates. The modified resazurin assay, by contrast, detected tolerance in 6.3% (4/64) of isolates. The findings suggest that the resazurin-based assay offers a more sensitive identification of NaClO-tolerant bacteria. When the strains exhibiting an MIC value of 256 mg/L, the fluorescence intensity varied from around 1.2×10^5 to 4×10^5 RFU, reflecting inactivation effects at the same MIC values.

Evaluation method of sodium hypochlorite tolerance in *Salmonella* using resazurin was developed. The results of NaClO tolerance can be finalized within one hour. This methodology is recognized as an effective, accurate, and rapid approach for assessing bacterial disinfectant resistance.

S4-P2: Tracking *Salmonella* from Hatchery to Slaughter: The First Integrated Surveillance of Free-Range Poultry Production in South Africa

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This study investigated the prevalence of *Salmonella*, its antimicrobial resistance and genomic characteristics across a South African free-range poultry plant (hatchery, broiler farm and slaughterhouse). Sampling took place over two trials (December 2024–April 2025) at each facility. Samples (*n* = 891) (eggshells, hatchery fluff, environmental, stool, specimens etc.) were collected (hatchery *n* = 83, broiler farm *n* = 528, slaughterhouse *n* = 200), of which 115 were confirmed to be *Salmonella* with the VITEK® 2 Compact system. The hatchery had a zero-detection rate for *Salmonella* compared to a higher prevalence observed at the broiler farm and slaughterhouse ($p \leq 0.01$), however the detection rate at the broiler farm (15%) and the slaughterhouse (17%) were similar ($p = 1$). A cohort of 80 isolates were selected for antimicrobial susceptibility testing (AST), which were analyzed using the VITEK® 2 Compact system, (AST-GN97 card) that included a range of veterinary relevant antibiotics. Testing revealed resistance to cefalotin, amikacin and gentamicin for all isolates. One isolate was additionally resistant to ampicillin. Of these, 20 isolates were selected for AST testing using the clinical AST-N255 card, which confirmed consistent resistance to aminoglycoside and cefalotin with additional resistance to cefoxitin, cefuroxime, cefuroxime axetil and intermediate resistance to nitrofurantoin. Representative isolates (*n* = 13) were selected for whole genome sequencing (WGS). Genotypic resistance profiles for AST matched with phenotypic profiles. In silico serotyping showed that there were two main serotypes: *Salmonella enterica* subspecies *enterica* serovar Ohio and serovar Hadar. One isolate was detected as a rare *Salmonella* serovar Baildon, which originated from a stool sample collected from the truck transporting live birds from the broiler farm to the slaughterhouse. *S.* Ohio was found in six out of seven broiler farm isolates across the various zones, whilst *S.* Hadar was found in five out of six isolates at the slaughterhouse across production stages. One stool isolate from the broiler farm (zone 4 – chicks near slaughter age) was also identified as *S.* Hadar, reflecting potential cross contamination from the broiler farm to the slaughterhouse. Using a ≤ 10 SNP cutoff, phylogenetic analysis revealed tight clustering (0–3 SNPs) among slaughterhouse *S.* Hadar isolates from multiple processing stages. The broiler farm *S.* Hadar isolate showed 0 SNP differences, indicating cross-facility dissemination beyond the critical control point (CCP). *S.* Ohio exhibited farm-specific clustering (1–23 SNPs), suggesting persistence. This represents the first integrated free-range poultry chain surveillance in South Africa. Enhanced biosecurity, CCP validation, and routine WGS integration are recommended.

S4-P3: Enhancing Foodborne Pathogen Surveillance: Harmonisation of Genomic Typing Methods for Staphylococcal Food Poisoning Outbreak Investigation

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Staphylococcal food poisoning outbreaks (SFPOs) remain a significant cause of foodborne illness in Europe. In 2023, 221 SFPOs were reported across EU Member States, representing 23% of bacterial toxin-related outbreaks and ranking staphylococcal enterotoxins (SEs) as the second leading cause. The French National Agency for Food, Environmental, and Occupational Health and Safety (ANSES) acts as the European Union Reference Laboratory (EURL) for coagulase-positive staphylococci (CPS), providing guidance on typing methodologies to a network of 33 National Reference Laboratories (NRLs). Harmonising typing approaches is essential to ensure data comparability, support coordinated food safety actions, and enhance cross-border outbreak investigations. To determine the most suitable tools for SFPO investigation within a food safety surveillance framework, we compared conventional typing methods—pulsed-field gel electrophoresis (PFGE) and 7-loci multi-locus sequence typing (MLST)—with whole genome sequencing (WGS)-based approaches, namely core genome MLST (cgMLST) and core single nucleotide polymorphism (cSNP) analysis. Method performance was statistically evaluated in terms of discriminatory power, reproducibility, and concordance, building on previous assessments. A retrospective analysis of 31 outbreaks demonstrated that all methods showed broadly comparable ability to discriminate epidemiologically related strains. While conventional methods such as MLST and PFGE remain useful for outbreak investigation, their limited resolution and reduced inter-laboratory comparability reduce their effectiveness for integrated, large-scale foodborne pathogen surveillance. In contrast, WGS-based approaches provide substantially higher resolution, allowing more accurate strain discrimination, improved traceability, and strengthened epidemiological linkage of cases. Despite these advantages, routine implementation of WGS in food safety laboratories remains constrained by the lack of standardized laboratory workflows and harmonised bioinformatics pipelines. Current EURL initiatives aim to validate WGS methodologies, refine genetic distance thresholds for cgMLST and cSNP analyses, and coordinate inter-laboratory comparison studies. By promoting harmonised genomic typing strategies, these efforts contribute directly to strengthening European foodborne pathogen surveillance systems, improving outbreak detection and response, and supporting evidence-based food safety risk management.

S4-P4: Genotypic and Phenotypic Characterization of *Bacillus cereus* Isolates from Human Clinical Cases in Slovenia

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In 2022, European Union member states reported 127 foodborne outbreaks attributed to *Bacillus cereus* toxins. Although foodborne illness is typically associated with *B. cereus* sensu stricto (s.s.) and biovar Emeticus, the recovery of biovar Thuringiensis from clinical cases raises concerns. The goal of this project was to characterize the genotypic and phenotypic diversity of 47 *B. cereus* group isolates recovered from human clinical cases by the Slovenian National Laboratory of Health, Environment, and Food between April 2024 and July 2025. Genomic DNA was extracted using the QIAGEN DNA Blood Mini Kit. Libraries were prepared with the Rapid Barcoding Kit and sequenced on Nanopore MinION R9 flow cells. Genome assembly, polishing, and quality assessment were conducted with Flye (v2.9.5), Medaka (v1.3.2), and QUAST (v5.2.0), respectively. Taxonomic classification and virulence gene detection were performed using BTyper3 (v3.4.0), and core genome SNPs were detected using kSNP4 (v4.1.0). Maximum-likelihood phylogenetic trees were constructed with RAxML (Galaxy v8.2.12) and visualized in iTOL (v6). Cytotoxicity was assessed using Caco-2 cells exposed to 15% (v/v) culture supernatant, followed by WST-1 metabolic activity measurement. Isolates with good quality of assemblies (*n* = 43) were classified as *B. cereus* s.s. (*n* = 25, including 9 biovar Thuringiensis), *Bacillus mosaicus* (*n* = 12, including one biovar Emeticus), *Bacillus mosaicus*/*luti* (*n* = 2), and *Bacillus toyonensis* (*n* = 4). BTyper3 detected *nhe* genes in 86% of 43 isolates, *hbl* in 65%, *cytK-2* in 60%, and *sph* in 86%, while *cesABCD* was present in only one isolate (2%). Isolates from group IV (*B. cereus* s.s.) had the highest average cytotoxicity (0.57 ± 0.32). In contrast, group II showed the lowest cytotoxicity (0.119 ± 0.02), with no significant differences between *panC* groups ($p = 0.0716$). *B. cereus* s.s. predominated among tested isolates and included nine biovar Thuringiensis strains, six of which were closely related (i.e., > 99% average nucleotide identity) to commercial biopesticide strains. The high genetic relatedness of several biovar Thuringiensis isolates to commercial biopesticide strains underscores the need for exposure assessment frameworks that integrate both genomic and phenotypic data to more accurately predict food safety risk associated with these strains.

S4-P5: Slaughterhouses as Reservoirs of Antimicrobial-Resistant Bacteria: Effects of Disinfection Treatments on Microbial Communities

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Slaughterhouses are important hotspots for the accumulation and dissemination of antibiotic-resistant bacteria (ARB) and antimicrobial resistance genes (ARGs), largely due to antimicrobial use in livestock production. While disinfection practices help control microbial contamination, some biocides may contribute to the spread of resistance genes and promote cross-resistance to antibiotics, enhancing the persistence of antimicrobial resistance in the food production chain and the environment.

In this context, the present study characterized microorganisms isolated from various slaughterhouse areas (cold storage, cutting room and slaughter room). Each facility was subjected to different disinfection protocols to evaluate the impact on the microbial communities. The treatments included an HLE disinfectant solution, ultraviolet radiation (UV-C), a routine disinfection strategy used by the slaughterhouse and a combination thereof. The isolated microorganisms were analyzed using molecular biology techniques to determine their taxonomic identity and antibiotic resistance profiles. The phenotypic and genotypic resistance profiles of the isolates showed resistance to multiple antibiotics (more than 3 antibiotics belonging to different classes) such as beta-lactams (ampicillin, amoxicillin-clavulanic acid, imipenem and cefuroxime), gentamicin, ciprofloxacin, tetracycline, rifampicin, erythromycin, nitrofurantoin, chloramphenicol and sulfamethoxazole.

Among the multidrug-resistant genera identified by molecular tools (REP-PCR and 16S rRNA sequencing), *Pseudomonas* sp. constituted the majority of the isolates, while *Escherichia coli* and *Staphylococcus* sp. were also detected. These genera are of particular importance because of their recognized role in foodborne contamination and their potential impact on public health. Analysis of these multidrug-resistant (MDR) pathogens revealed their dissemination throughout the slaughterhouse areas, from the slaughter room to the cold storage room. Notably, this spread was significantly influenced by specific disinfection protocols.

In conclusion, these findings demonstrate the critical need to implement rigorous and targeted hygiene, control, and disinfection protocols in industrial settings. Mitigating the environmental prevalence and dissemination of pathogenic multidrug resistant bacteria and their resistance genes in these environments is essential not only to limit localized spread but also to preserve the therapeutic efficacy of antibiotics.

S4-P6: Genetic Characterization of Antibiotic Resistance and Virulence Genes in *Acinetobacter* spp. from Raw Milk

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The genus *Acinetobacter* comprises a diverse group of Gram-negative bacteria widely distributed in environmental and food-associated habitats, including raw milk. While species such as *A. baumannii* are well-known opportunistic pathogens with increasing antibiotic resistance, the role of non-clinical *Acinetobacter* spp. from food matrices as reservoirs of virulence and resistance determinants remains less well understood.

In this study, 39 *Acinetobacter* spp. isolates obtained from raw milk across Germany underwent phenotypic and genomic characterization. Whole-genome sequencing was performed using a hybrid approach combining Illumina NextSeq2000 and Oxford Nanopore PromethION technology, followed by assembly with Unicycler (v0.5.0) and subsequent bioinformatic analyses. Species assignment based on average nucleotide identity (ANI) and in silico DNA–DNA hybridization (dDDH) revealed several distinct taxa. The majority of isolates were identified as *Acinetobacter* (*A.*) *guillouiae*, while *A. baumannii*, *A. ursingii*, *A. gerneri*, and *A. entericus* were detected less frequently. A subset of genomes did not meet the species delineation thresholds, suggesting potentially novel species within the genus.

Antibiotic resistance was assessed using the disk diffusion method, while resistance genes were determined using the ResFinder pipeline. All isolates displayed susceptibility to erythromycin, tobramycin, ciprofloxacin, meropenem, and tetracycline. Resistance was most common against ampicillin (84.6%), followed by cefoxitin (59%), chloramphenicol (17.9%), cefotaxime (12.8%), and streptomycin (10.3%). Genotypic analyses supported these findings, with multiple genomes harboring β-lactamase genes, predominantly bla_{OXA}, as well as aminoglycoside resistance variants. Furthermore, the presences of virulence genes involved in adhesion, biofilm formation, and type VI secretion system functions were detected.

These findings indicate that raw milk serves as a reservoir for diverse *Acinetobacter* species, some of which can harbor resistance and virulence-associated genes. While their clinical relevance remains to be fully determined, these findings emphasize the importance of continued investigation into *Acinetobacter* spp. and their potential contribution to pathogenic development.

S4-P7: *Debaryomyces hansenii* and Rosemary Reduce Ochratoxin A Contamination in Dry-Cured Fermented Sausages

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Ochratoxin A (OTA) in dry-cured meat products has attracted attention in recent years, particularly following a Scientific Opinion of the European Food Safety Authority (EFSA), which identified them as one of the main contributors to chronic dietary exposure to this mycotoxin. In dry-cured fermented sausages, microorganisms and plants, either individually or in combination, have been proposed as the most promising approach for alleviating this hazard. However, these biological strategies have been tested against ochratoxigenic moulds inoculated individually into dry-cured fermented sausages and not under conditions of coexistence, which is what actually occurs in curing facilities. The aim of this study was to evaluate the antiochratoxigenic activity of *Debaryomyces hansenii* (Dh) and rosemary-based approaches to control the OTA accumulation due to *Penicillium nordicum* and *Aspergillus westerdijkiae*, the most common ochratoxigenic moulds in dry-cured fermented sausages. Rosemary leaves (RL), rosemary essential oil (REO) and casings macerated with RL (CMR) were used. Two different kinds of these foodstuffs, "salchichón" and "chorizo", were selected for assessing the influence of their distinct ingredients. Their production was performed at the pilot plant of the Faculty of Veterinary Sciences (University of Extremadura, Cáceres, Spain). RL and Dh were applied into the meat dough before stuffing. REO was sprayed onto the pieces after stuffing. The treatments were applied alone and in combination against a mix of *P. nordicum* and *A. westerdijkiae*. The ripening of sausages was performed in a climate-controlled environment for 25 days. The surface mould growth, fungi counts and OTA levels were finally analysed. All "salchichón" and "chorizo" batches displayed fungi levels from 7 to 8 log CFU/g. The highest score of mould growth was reached before in "salchichón" than in "chorizo", and only when applying Dh and REO in "chorizo" a lower mould growth was detected. Thus, the biocontrol agents did not negatively affect the development of fungi. Regarding OTA, a significant reduction was observed in treatments with Dh, REO, CMR and Dh with CMR in "salchichón". Conversely, REO alone and in combination with Dh increased the OTA production in "chorizo". Biopreservation based on the use of Dh and CMR is the most effective strategy against the main ochratoxigenic moulds in "salchichón" and "chorizo".

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S4-P8: Swine-Derived Food Products as a Potential Source of *Klebsiella pneumoniae* Resistant to Critical Antimicrobials: Preliminary Findings

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Klebsiella pneumoniae (Kp) is recognized as one of the most important nosocomial pathogens. However, in recent decades, community-acquired infections associated with Kp have raised increasing concern, particularly when strains exhibit resistance to critical antimicrobials (i.e., third-generation cephalosporins – 3GCs, carbapenems). One example is represented by recent outbreaks involving highly virulent and lethal carbapenem-resistant Kp strains in Asia. Assessing potential community reservoirs of Kp has therefore become imperative. This study investigates swine-derived food products as potential reservoirs of Kp and its possible transmission through the food chain, focusing on strains resistant to critical antimicrobials (carbapenems, 3GCs). Between August and November 2025, 110 swine-derived food products (sausages, minced meat, minced meat mixture, skewers) were analysed at the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Italy, within the framework of Regional and Self-Monitoring Food Plans. *Klebsiella* spp. were isolated using standard microbiological procedures, while antibiotic-supplemented media were used to identify strains specifically resistant to carbapenems and 3GCs. Isolates were identified by MALDI-TOF. Antimicrobial resistance (AMR) profiles were evaluated by the broth microdilution method and results were interpreted according to CLSI clinical breakpoints. 3GC-resistant strains were tested for ESBL production, using the combination disk test. ESBL and carbapenem-resistant isolates were screened for the presence of associated antimicrobial resistance (AMR) genes by multiplex PCR assays. Fifty-five/110 (50.00%) samples tested positive for *Klebsiella* species. Strains belong to Kp (28/55; 50.91%) and *Klebsiella oxytoca* (27/55, 49.09%) species. Resistance to at least one antimicrobial was observed in 17/55 (30.91%) strains. The highest resistance rates were detected for tetracycline and sulfamethoxazole (9/55, 16.36% both), followed by trimethoprim (6/55, 10.91%), ciprofloxacin/amikacin/tigecycline (2/55, 3.64% all) and cefotazime/ceftazidime/chloramphenicol/ nalidixic acid (1/55, 1.82% all). Notably, two Kp strains showed an ESBL profile: one resistant to cefotaxime carrying bla_{TEM} gene and the other resistant to ceftazidime harbouring bla_{SHV} gene. Resistance to gentamycin, meropenem and colistin was not detected. Kp showed a higher association with AMR compared to *K. oxytoca*, representing 12/17 (70.59%) of resistant strains, including all MDR and ESBL isolates. Kp was frequently detected in swine-derived food products (> 50%). Considering the overall *Klebsiella* spp. population, > 30% of isolates were resistant to at least one antimicrobial, although resistance to critical drugs was limited. Nevertheless, the detection of 2 ESBL-producing Kp strains highlights swine products as a potential route of transmission for relevant antimicrobial-resistant Kp through the food chain, underlying the need to monitor their presence in the food chain.

S4-P9: Pathogen Pilot Plants: Closing the Gap Between Laboratory and Industry Testing

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When developing new, innovative tools and technologies to combat harmful microbes in food, it is crucial to evaluate their effect in conditions near real food processing, packaging and storage. While laboratories are designed to investigate the properties of harmful pathogens, they are not designed to simulate food processing. On the other hand, using industrial food processing facilities for performing experiments with harmful microbes is not allowed due to biosafety restrictions.

Pathogen pilot plants are made to fulfil the need for realistic testing conditions of new technologies in a safe way: They are carefully constructed to maintain the safety of the personnel and the environment, meanwhile containing industrially relevant machines and tools.

In Europe, a limited number of pathogen pilot plants have been established, and they are used in research and innovation projects for different food sectors. The first high security food processing facility in Europe was established at Nofima in Norway. The plant has been used to study the effect of a range of different food processes on harmful bacteria such as *Campylobacter*, *Salmonella*, *Listeria* and *Escherichia coli* and on mould spores. Some examples: 1. Steam treatment to remove *Campylobacter* and *Salmonella* from chicken carcasses; 2. Automatic peroxygen mist disinfection system to kill *Listeria* and mould on surfaces; 3. Survival of pathogenic *E. coli* in salami – effect of processing and storage conditions; 4. Examination of harborage sites of *Listeria monocytogenes* in a skinning machine. Selected examples will be presented.

In Switzerland, a partner BSL3-pilot plant is planned to become operational in 2029, with a primary focus on cheese and dairy production. This facility will support research on pathogenic microorganisms and viruses in dairy products, while also addressing challenges in meat and plant-based alternatives. The BSL3-pilot plant will enable challenge testing related to emerging risks, development and application of protective cultures, use of hurdle technologies, and the validation of processing methods.

S4-P10: Influence of Sigma Factor RpoS on Stress Resistance of *Salmonella* Typhimurium Grown at Different Temperatures

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RpoS alternative sigma factor is considered as one of the most relevant regulators involved in stress resistance in many Gram-negative bacteria. However, its precise role in the resistance of the foodborne pathogen *Salmonella* has not been sufficiently proven. In this work, we studied the survival capacity of two *Salmonella* Typhimurium strains and their Δ*rpoS* isogenic mutants under a variety of stress conditions with different modes of action on bacterial cells: acidic pH, hydrogen peroxide, NaCl, Pulsed Electric Fields (PEF), ultraviolet (UV-C) radiation and heat treatments. In parallel, the expression of the *rpoS* gene together with three genes whose expression is partly RpoS-dependent (*katN*, *otsB* and *katE*), was determined by qRT-PCR, using the *rpoZ* gene as housekeeping. Cells were grown to stationary phase at two different temperatures (10 and 37°C), in order to obtain cells with presumably different RpoS activity. Survival curves to each agent were obtained, fitted to the shoulder-tail Geeraerd model, and 2D values (time for the first two log cycles of inactivation) were calculated and used for statistical comparisons. Results obtained demonstrated that the presence of RpoS induced a clear protective response in *Salmonella* Typhimurium only against acid and oxidative stresses. UV-C and heat tolerance were unaffected by the presence/absence of RpoS. Response to osmotic stress and PEF treatments depended on the strain and growth temperature. Particularly relevant is the lack of protection against thermal treatments, since it is widely assumed that RpoS is an important determinant in heat tolerance of other Gram-negative genera. Besides, growth temperature exerted a profound effect on cell resistance to all the agents studied except UV-C, and PEF, where the effect depended on the strain and growth temperature. A raise in growth temperature from 10 to 37°C induced an increased tolerance to acid, osmotic and heat stress, but a decrease in resistance to hydrogen peroxide. The influence of growth temperature was, in general, of much higher magnitude than that of RpoS status, and very similar for the RpoS+ and RpoS- strains. qRT-PCR results showed that the expression of the RpoS-reporter genes was equal or higher for cells grown at 10°C, especially for strain ATCC 14028s. This different RpoS activity observed for cells grown at different temperatures does not seem to be the most relevant factor behind stress tolerance. These results indicate that RpoS role in stress resistance of *Salmonella* Typhimurium differs from those reported in other Gram-negative genera.

S4-P11: Synergistic Effect of Pulsed Electric Fields (PEF) and Marination on the Inactivation of *Anisakis* spp. in Fish

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Anisakis spp. is a parasitic nematode found in fish and cephalopods intended for human consumption, and the ingestion of viable larvae can cause anisakiasis, a disease characterized by gastrointestinal symptoms and allergic reactions. Among fish species with high prevalence of *Anisakis*, *anchovy* stands out, as it is commonly consumed marinated, a treatment that is insufficient to inactivate the parasite. Current European regulations (Regulation (EC) No. 853/2004) require that fishery products intended to be eaten raw or lightly cooked (≤ 60°C; ≤ 1 min at the thermal center of the product) must be frozen previously to inactivate the parasite. However, freezing can negatively affect the quality of the fish, motivating research into alternative technologies, such as Pulsed Electric Fields (PEF), which have been shown to inactivate *Anisakis* spp. while preserving the sensory quality of the product.

This study evaluated the synergistic effect of PEF combined with different concentrations of salt and acetic acid during marination on the inactivation of *Anisakis* spp.. Naturally parasitized pieces of fish belly were subjected to PEF treatments ranging from 0 to 3 kV/cm, with fixed specific energy and pulse width (20 kJ/kg; 20 µs), and subsequently marinated in solutions containing 0–6 % salt and 0–7 % acetic acid for up to 72 h at 4°C. Larval survival was assessed after the combined treatment using the mechanical stimulation technique.

Results showed that PEF treatments caused minimal inactivation (< 5 %). However, the combination of PEF with acetic acid and salt exhibited a significant synergistic effect, dependent on both the electric field strength and the concentration of the additives. Specifically, a PEF treatment of 3 kV/cm followed by marination with 3 % salt and 3.5 % acetic acid for 12 h reduced more than 90 % of viable larvae.

These findings suggest that combining PEF with marination using additives represents a promising strategy for inactivating *Anisakis* spp., providing an alternative to traditional freezing. This technology is particularly relevant for products such as marinated anchovies, consumed directly without cooking, although further studies are needed to optimize treatment parameters and ensure consumer safety.

S4-P12: Prevalence and Diversity of *Clostridium* spp. and *Clostridium botulinum* in Plant-Based Dairy Alternatives and Ingredients

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In recent years, the market for plant-based dairy alternatives has been in constant evolution, resulting in numerous new product formulations. *Clostridium botulinum* and some other *Clostridium* spp. are spore-forming microorganisms that can potentially survive and grow in this category of products and pose a risk to the consumers' health due to the production of botulinum neurotoxin (BoNT). The present study investigated the prevalence and diversity of clostridia present in plant-based products, focusing on *C. botulinum*.

A total of 103 commercially available products and powders were screened using an anaerobic enrichment procedure in tryptone-peptone-glucose-yeast extract broth and subsequent plating on McClung Toabe agar. The phenotypic diversity of the colonies was monitored systematically to facilitate the detection of potential *C. botulinum* isolates based on their unique morphology. Colonies were identified with MALDI-TOF mass spectrometry and a custom clostridia library from Health Canada with 94 reference isolates including 20 species. The mass spectra produced were used for preliminary taxonomic clustering by dendrograms. In addition, a multiplex qPCR method was established to detect BoNT toxin genes. All potentially BoNT producing *Clostridium* spp. isolates (n = 66), based on the MALDI-TOF method, were whole genome sequenced to confirm identity and presence/absence of BoNT genes.

The MALDI-TOF analysis resulted in the identification of 77 distinct species from which 20 were clostridia. Clostridia were present in powders, cheeses and spreads, but were absent from yogurt-like-desserts, drinks and crème cuisine products. Presumptive *C. botulinum* group I was present in 14 products, while the closely related *Clostridium sporogenes* was identified in 7 of them. Other identified potentially BoNT producing clostridia were *Clostridium novyi* (n = 4 products), *Clostridium butyricum* (n = 2) and *Clostridium baratii* (n = 2), while the pathogenic *Clostridium perfringens* was present in 17 of the products. MALDI-TOF dendrograms, including reference strains, successfully clustered separately the different botulinum groups. WGS analysis is the key to further investigation of the classification of the isolates.

To manage the risk of BoNT production in the studied foods, product characteristics of these plant-based dairy products must prevent outgrowth of *C. botulinum* spores. Validated predictive models may facilitate formulation of the required recipes to achieve this goal.

S4-P13: *Campylobacter* and Associated Organisms on Retail Chicken – Using Metagenomics to Investigate the Potential of Utilising Co-Resident Microbes for Pathogen Control

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Campylobacter is the leading cause of bacterial gastroenteritis, with retail chicken meat representing a major source of infection. *Campylobacter* isolation from retail meat can be difficult due to the complex growth requirements of the organism. Metagenomic sequencing may be a useful alternative approach for the direct identification of pathogens like *Campylobacter* from food samples, while also allowing characterisation of the overall sample microbiome.

Metagenomes of 58 retail chicken samples cultured for *Campylobacter* were assessed. *Campylobacter* relative abundance within the metagenome reads varied between samples (0.00086–0.47%), with overlap between culture positive and negative samples. Characterisation of *Campylobacter* to sequence type (ST) level was possible in one sample. There were no significant differences in alpha or beta diversity of taxa between the *Campylobacter* culture positive and negative samples, and individual differentially abundant taxa were not identified using ALDEx2 based on Benjamini-Hochberg adjusted Wilcoxon p-values. Preliminary analysis of metabolic reaction relative abundances indicated that several individual reactions differed in abundance between the *Campylobacter* culture positive and negative samples. However, these were found to not be statistically significant following multiple testing correction, nor through beta diversity and ALDEx2 analysis.

Metagenomic analysis of the retail chicken meat microbiome indicates that the identification of *Campylobacter* and microbial signatures associated with *Campylobacter* presence and absence on retail chicken meat is difficult, at least with current methods. Alternatively, such signatures may be absent altogether. While the small sample size in this study may have restricted our ability to identify important taxa or metabolic reactions, we outline an approach that future studies can use to investigate differences in microbial communities.

S4-P14: Species- and Strain-Specific Strategies for Survival and Persistence of *Pseudomonas* on Ready-to-Eat Spinach Leaves

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Pseudomonas species occupy diverse ecological niches and exhibit varying capacities for persistence. *Pseudomonas aeruginosa*, a clinically significant opportunistic pathogen, and *Pseudomonas fluorescens*, commonly implicated in food spoilage, are frequently detected on leafy greens, raising questions about their potential impact on food quality and safety. Post-harvest spinach provides a relevant model to study *Pseudomonas* as it is often consumed raw, allowing for human exposure, and can harbour these bacteria. To investigate *Pseudomonas* persistence and impact on the spinach microbiome, transposon-directed insertion-site sequencing (TraDIS-Xpress) and metagenomic sequencing were used. Mutant libraries of *P. aeruginosa* strains, PAO1 (clinical) and LG0328-1 (food-derived), and *P. fluorescens* SBW25 were generated and inoculated onto spinach leaves and stored at 4°C for 14 days. TraDIS-Xpress identified disrupted genes at multiple timepoints, while metagenomics assessed shifts in microbial community composition and functional potential. Both *P. aeruginosa* strains exhibited poor survival, suggesting that spinach leaves constitute a hostile environment for this species. However, TraDIS-Xpress revealed that *P. aeruginosa* can survive on leafy greens by employing a biofilm-associated strategy, which could potentially facilitate transit to the gut, whereas *P. fluorescens* exhibited niche adaptation and persisted on spinach. Metagenomic analyses showed that inoculation of *Pseudomonas* mutant libraries altered the resident spinach microbiome, causing shifts in multiple taxa and enriching specific gene families. This study highlights species- and strain-specific survival strategies of *Pseudomonas* on leafy greens and their potential to influence the native leafy green microbiome. Integrating functional genomics and metagenomics provides new insights into bacterial adaptation relevant to food safety and spoilage.

S4-P15: Characterization of the Antibacterial Activity of Cypriot Multifloral Honey in Relation to Its Phenolic Composition

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Honey is increasingly recognized as a functional food characterized by intrinsic antimicrobial properties. Its complex chemical composition is defined by high osmolarity, low water activity, acidic pH, the enzymatic generation of hydrogen peroxide and a spectrum of bioactive phytochemicals create a multifaceted defense against microbial growth. Previous research has established a correlation between antimicrobial potency and the phenolic profile of honey, which is fundamentally influenced by botanical and geographical origins. The present study evaluates the antimicrobial efficacy of Cypriot multi-floral honey against two clinically significant pathogens: the Gram-positive *Staphylococcus aureus* and the Gram-negative *Pseudomonas aeruginosa*. In the context of escalating multidrug resistance, natural products such as honey represent a promising reservoir for novel therapeutic agents. Honey samples were harvested from various ecological regions across Cyprus island. Antimicrobial activity was quantified using the broth microdilution assay, while targeted phenolic acids and flavonoids were identified and measured via High-Performance Liquid Chromatography (HPLC).

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined for all samples. Preliminary results indicated MIC values ranging from 25 to 50 µg/mL; however, as the majority of samples did not exhibit bactericidal activity, inhibitory effects were further compared at concentrations of 50 and 100 µg/mL. Inhibition rates ranged from 24.7% to 84.9% for *P. aeruginosa* and 25.6% to 71.3% for *S. aureus*. Subsequently, chemometric analyses, including Principal Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA), were employed to correlate antimicrobial activity with specific phenolic (p-coumaric, caffeic, chlorogenic, and ferulic acids) and flavonoid (catechin, epicatechin, quercetin, rutin, and naringenin) contents. Overall, this work demonstrates that Cypriot multifloral honey exerts significant antimicrobial activity against common skin pathogens. This potency is closely linked to phenolic composition, highlighting the profound impact of botanical and geographical provenance on the therapeutic potential of honey.

S4-P16: Food Matrix Ecology Shapes Strain-Specific Cereulide and Biofilm Expression in Emetic *Bacillus cereus* Across Plant- and Dairy-Based Systems

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Plant- and dairy-based foods differ markedly in composition and physicochemical properties, yet it is not fully understood how these distinct environments shape the physiology and hazard potential of the ubiquitous foodborne pathogen *Bacillus cereus*. Emetic strains of *B. cereus* produce the vomit-inducing, heat stable toxin cereulide during growth, and once synthesized, it cannot be inactivated by processing. As a result, deeper insight into how diverse food matrices influence persistence and virulence related traits in emetic *B. cereus* is crucial for accurate risk evaluation and for understanding its ecological flexibility in food associated environments.

This study examined how distinct food-matrix ecologies influence growth, germination metabolism, biofilm formation, and cereulide synthesis across seven genomically diverse emetic *B. cereus* strains. Characterization was evaluated in milk, oat drink, and liquid pea-based media at 30°C. Growth kinetics was modeled using the Gompertz equation, sporulation was monitored over 72 h, post-germination metabolic activity profiles were obtained via isothermal calorimetry, biofilm formation was assessed using a peg lid assay, and cereulide levels were quantified by LC-MS/MS. Comparative genomics was used to contextualize phenotypic divergence.

All strains exhibited similar growth and sporulation dynamics across the three matrices, demonstrating that variation in hazard potential, like cereulide expression, cannot be predicted by growth kinetics alone. The plant-based matrices, especially oat, induced elevated post-germination metabolic activity and significantly increased biofilm probability, while no biofilm was detected in milk.

Cereulide production showed variation of multiple log units across strain-matrix combinations. Statistical analyses showed a significant effect of strain and strain × matrix interaction on cereulide accumulation, whereas the matrix alone did not, indicating that cereulide expression is highly contextual. Some strains showed strong matrix-dependent shifts, accumulating substantially more cereulide in plant-based matrices, whereas others maintained similar levels across conditions. These observations could be attributed to genetic differences influencing key stress response mechanisms.

Despite comparable growth across matrices, plant-based substrates create ecological conditions that strongly modify hazard-related responses compared to dairy. The results highlight the ecological adaptability of emetic *B. cereus* and emphasize the need for updated matrix-specific food safety considerations as plant-based foods continue to expand. To strengthen the predictive accuracy of cereulide risk assessments, challenge trials would benefit from employing emetic strain cocktails that capture the phenotypic diversity in increasingly diverse food ecologies.

S4-P17: Comparative Characterization of *Salmonella* Isolated from Humans and Food Animals in South Korea

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In the context of the global spread of *Salmonella* serotypes carrying multidrug-resistant (MDR) plasmids, we investigated the prevalence and antibiotic resistance of *Salmonella* strains collected from humans and livestock in South Korea. Among human samples (*n* = 2,083), the most common serotype was *S.* 1,4,[5],12:i:- (3.0%), followed by *S.* Enteritidis (2.6%) and *S.* Infantis (1.4%). In laying hens, *S.* Thompson predominated, while in broilers, all isolates were identified as *S.* Infantis. In pigs, *S.* Typhimurium (8.2%) and *S.* 1,4,[5],12:i:- (5.2%) were the most prevalent, followed by *S.* Infantis (1.2%). Resistance rates of *Salmonella* isolates varied by serotype. Notably, *S.* Infantis isolates from broilers exhibited high resistance to cefotaxime, while human *S.* Enteritidis isolates showed high resistance to colistin. Human isolates predominantly carried the CTX-M-1 group genes ($bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-55}}$), whereas both pig and broiler isolates harbored CTX-M-9 group genes ($bla_{\text{CTX-M-65}}$). Both human and broiler *S.* Infantis isolates demonstrated resistance to six identical antibiotic classes, with most harboring $bla_{\text{CTX-M-65}}$. Comparative genomic analysis of MDR *S.* Infantis strains from human and food-animal sources revealed the presence of IncFIB-type pESI-like megaplasmids, which showed high genetic similarity and were classified as cgST267045. The detection of *S.* Infantis harboring MDR pESI-like plasmids carrying ESBL genes in chicken farms suggests that these strains may be transmitted to humans through the food chain.

S4-P18: ESKAPE Bacteria Harboured by *Musca domestica* Collected from Hospice Kitchens and a Nearby Dumping Site in South Africa

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The rise in antibiotic resistance poses a major challenge to public health, especially in healthcare settings. Key pathogens linked to multidrug-resistant hospital-acquired infections include the ESKAPE bacteria: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.. These bacteria are known for their ability to interact with various organisms, aiding their spread. The housefly, *Musca domestica*, has been identified as a mechanical carrier for multiple pathogens. This study aimed to determine the prevalence and antibiotic resistance profiles of ESKAPE pathogens carried by *M. domestica* in hospice kitchens and nearby illegal waste dumps. A total of 50 *M. domestica*, captured with sticky traps from both locations, were examined internally and externally for ESKAPE contamination. Antibiotic susceptibility testing was conducted using the Kirby-Bauer method. MALDI-TOF and species-specific gene sequencing confirmed 21 ESKAPE isolates: 38.1% from the hospice and 61.9% from the waste dump. At the hospice, isolated ESKAPE species included *E. faecium* (50%), *K. pneumoniae* (37.5%), and *P. aeruginosa* (12.5%). At the waste site, only *E. faecium* (84.6%) and *K. pneumoniae* (15.4%) were found. High vancomycin resistance was observed in *E. faecium* at both the hospice (100%) and the waste site (54.5%), along with high beta-lactam resistance at 100% and 90.9%, respectively. *M. domestica* acts as a significant mechanical vector for multidrug-resistant ESKAPE bacteria, and their presence in both hospital environments and surrounding unsanitary areas poses a risk of cross-transmission, potentially leading to infections in vulnerable patients in hospices and other healthcare settings. The study strongly recommends implementing and maintaining strict pest control measures to prevent human infections.

Keywords: ESKAPE, antibiotic-resistance, nosocomial infection, *Musca domestica*

S4-P19: Role of Vitamin B_{12} Uptake in Metabolism and Virulence of *Listeria monocytogenes*

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Listeria monocytogenes is a versatile foodborne pathogen that employs diverse mechanisms to adapt to various environments and physiological states. The formation of bacterial microcompartments (BMCs) has been identified as one mechanism for the metabolic adaptation and virulence of *L. monocytogenes*. BMCs are self-assembled intracellular protein nanostructures that confine specific metabolic reactions, such as the utilisation of ethanolamine, which is abundant in the gastrointestinal tract. The expression of BMC proteins is strictly regulated by vitamin B_{12}. Moreover, vitamin B_{12} is a key cofactor in these catabolic reactions, highlighting its pivotal role in regulating *L. monocytogenes*' physiological state.

Although vitamin B_{12} can be synthesized de novo in some bacterial species, current research indicates that *L. monocytogenes* imports it from the extracellular environment. To date, vitamin B_{12} transport systems have not been reported in *L. monocytogenes*. In this study, we identified proteins involved in vitamin B_{12} transport and further explored the impact of its impairment on metabolic adaptation, resistance, BMC formation, and virulence in *L. monocytogenes*.

Characterization of mutants lacking putative vitamin B_{12} transporter-encoding genes led to the identification of three proteins involved in vitamin B_{12} transport: Lmo2429, Lmo2430, and Lmo1073. These proteins are proposed to form an ABC transporter, consisting of an ATP-binding protein, a transporter permease, and a substrate-binding protein, respectively.

Specifically, the absence of the putative vitamin B_{12}-binding protein (Lmo1073) was shown to reduce intracellular levels of vitamin B_{12}. Consistent with this, the mutant was incapable of utilizing ethanolamine, indicating a lack of BMC-mediated metabolism. Importantly, we showed that impaired vitamin B_{12} transport reduced the virulence of *L. monocytogenes* in the presence of ethanolamine. Specifically, the absence of Lmo1073 reduced adhesion and invasion (> 90%) potential when assessed using a Caco-2 cell line model.

Overall, this study identifies proteins involved in vitamin B_{12} transport and further characterizes the importance of vitamin B_{12} for the metabolic versatility and virulence of *L. monocytogenes*.

S4-P20: Virulence Assessment of *Listeria monocytogenes* Isolated from Ready-to-Eat Seafood Products Distributed in Japan

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Listeria monocytogenes is an important foodborne pathogen in ready-to-eat (RTE) foods because it can survive and grow under refrigeration and high-salt conditions. In Japan, large documented foodborne outbreaks caused by this organism have been limited; however, the widespread consumption of raw or minimally processed seafood indicates the need to evaluate the public health significance of seafood-associated isolates. In this study, the virulence potential of 27 *L. monocytogenes* strains isolated from RTE seafood products in Japan was assessed. The isolates were recovered from five product categories purchased between April and October 2023: cod roe, seasoned cod roe, salmon roe, smoked salmon, and minced tuna. To characterize their virulence-associated properties, Caco-2 cell adhesion and invasion assays were performed in parallel with whole-genome-based analysis of virulence-associated genes.

Adhesion and invasion assays were conducted using Caco-2 intestinal epithelial cells. Bacterial suspensions prepared from stationary-phase cultures were used for infection at a multiplicity of infection of approximately 100, and adhesion and invasion rates were determined from viable counts. Adhesion rates ranged from 0.31% to 6.6% and were distributed within a relatively narrow range, with a median of 3.6%. In contrast, invasion rates ranged from 0.11% to 41.6%, with a median of 19.0%, indicating substantial strain-to-strain variability in invasive capacity. When the isolates were compared by phylogenetic lineage, lineage I strains showed significantly higher invasion rates than lineage II strains ($p < 0.05$). Comparison by serogroup showed the same tendency in both assays, with serogroup IIb isolates exhibiting the highest values, followed by serogroup IVb and serogroup IIa isolates, although these differences were not statistically significant. Six isolates showed invasion rates below 1%.

To investigate the genetic basis of low invasiveness, 12 virulence-associated genes in LIPI-1 and LIPI-2 were analysed using available whole-genome sequence data. All six low-invasive isolates belonged to lineage II. Five of these carried truncation mutations in *inlA*, which encodes a major invasion factor involved in entry into intestinal epithelial cells, consistent with the markedly reduced invasion observed in the Caco-2 assay. One additional low-invasive isolate retained all examined LIPI-1 and LIPI-2 genes without detectable truncation mutations, suggesting that reduced invasiveness cannot be explained solely by gene presence or sequence integrity. Overall, the results show that seafood-derived *L. monocytogenes* isolates in Japan differ substantially in invasive capacity, and that *inlA* truncation is a determinant of reduced invasion in a subset of isolates, while other factors are likely involved in some strains.

S4-P21: Updated Prevalence Study of *Yersinia enterocolitica* in Croatian Pig Abattoirs

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Yersinia enterocolitica is a persistent zoonotic foodborne pathogen in pork production and is listed as a priority hazard in official zoonotic reports in Europe. In this study, 50 batches representing 7,500 pigs from 41 farms in Croatia (capacities 200 to 10,000 fattening pigs) were sampled at the slaughter line to obtain current prevalence data for pathogenic *Y. enterocolitica* (winter season 2025/2026). Tonsils from 300 individual pigs were analysed following ISO method 10273:2017. Suspected bull's-eye colonies (*n* = 166) from CIN agar were subjected to MALDI-TOF mass spectrometry identification. The pathogenicity of isolates confirmed as *Y. enterocolitica* was evaluated using chromogenic agar CHROMAgarTM Y. enterocolitica and serotyping, verified by reference strains (biotype 1A, bioserotype 4/O:3 and 2/O:9). Forty-three batches (86%) were positive for *Y. enterocolitica* pathogenic biotypes, while individual prevalence was 55% (95% CI: 49.7–61.0). Animals from large farms (> 1,000 pigs) had a higher risk (RR = 2.35; 95% CI: 1.79–3.09) of being positive compared to those from small farms (< 1,000 pigs). All 166 pathogenic strains belonged to serotype O:3. The results indicate an increasing prevalence of pathogenic *Y. enterocolitica* in slaughtered pigs in Croatia compared to previous surveys. The risk of this zoonotic disease remains high due to persistent pathogen carriage in the tonsils of fattening pigs from highly intensive farms, even those with the highest biosecurity, and exposure to potentially contaminated pork. Further characterisation of collected strains will reveal genetic relatedness with deposited strains from previous years and different food-chain sources, as well as with deposited human outbreak-suspected strains.

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S4-P22: Comparative Whole Genome Analysis of LA MRSA ST398 from Humans, Pigs and RTE Foods

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a pathogen occurring in both humans and animals. The first isolation of MRSA in animals was reported in 1972 in cows with mastitis, and it was later identified in pigs as well. The term livestock-associated MRSA (LA-MRSA) was introduced following the emergence of these strains in pigs in the Netherlands in 2005, most commonly referring to clonal complex ST398, which is adapted to animal hosts. This study focuses on the whole genome characterization of MRSA ST398 isolates obtained from humans, pigs, and ready to eat (RTE) salami products, with the aim of comparing their genomic features, identifying differences, and improving our understanding of the host adaptation and spread of this clonal lineage.

A total of 31 porcine isolates (nasal swabs at slaughter), 20 human isolates (from patients without known contact with pig farms), and 4 isolates from RTE salami products collected between 2020 and 2025 were subjected to whole genome sequencing. Raw reads were quality filtered using Trimmomatic and assembled with SPAdes. Virulence and resistance genes were identified using ABRicate with the VFDB and ResFinder databases. SCC*mec* typing was performed using SCC*mec*Finder. All 55 isolates were screened for virulence determinants (IEC, PVL, TSST 1, ETA, ETB) and tetracycline resistance genes.

All isolates were negative for the screened virulence determinants. None carried the IEC, PVL, TSST 1, or exfoliative toxin ETA/ETB determinants. All isolates harbored SCC*mec* type IV or V and the tetracycline resistance gene *tet(M)*. The absence of the IEC cluster is consistent with a livestock associated genomic profile. Clonal diversity was low, with *spa* types t034 and t011 predominating across all three groups. Resistance to cefoxitin and tetracycline was detected in all isolates, in agreement with the prevalence of *mecA* and *tet(M)* genes.

The high genomic similarity among isolates from humans, pigs, and RTE food products suggests possible transmission along the food production chain or through indirect exposure routes. The consistent absence of human adaptation markers such as IEC further supports the classification of these isolates as typical LA MRSA ST398 strains adapted to animal hosts.

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S4-P23: Enrichment Dynamics of Pathogenic *Yersinia enterocolitica* Amid Diverse Background Microbiota in Food Matrices

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Yersinia enterocolitica is an important foodborne pathogen often linked to pork, vegetables and dairy. Despite standardized detection protocols, recovery of *Y. enterocolitica* from contaminated food remains challenging. A limiting factor could be the presence of competing background microbiota, which may suppress the growth of *Y. enterocolitica* during enrichment, or hinder its selective recovery on agar media. Understanding the dynamics of microbiota is therefore essential to improve detection. This study aimed to investigate the growth of *Y. enterocolitica* 4/O:3 in artificially contaminated food matrices during enrichment, while characterizing the naturally occurring microbiota.

Seventeen retail food samples (minced pork, lettuce and unpasteurized cheese) were artificially inoculated with three strains of *Y. enterocolitica* bioserotype 4/O:3 (70 CFU/25 g). Samples were stored overnight at 4°C, then incubated at 25°C using enrichment in 225 ml peptone sorbitol bile broth (PSB). At 0 h, 24 h and 48 h, aliquots were taken for culture and digital PCR analysis. Plating was performed on tryptic soy agar (TSA) to assess the microbiota composition, and cefsulodin–irgasan–novobiocin (CIN) for the detection of *Y. enterocolitica* according to ISO 10273. At each time point, 30 colonies from the TSA plates and CIN plates were identified using MALDI-TOF MS.

Despite spiking with 70 CFU/25 g, pathogenic *Y. enterocolitica* could not be isolated from over half of the samples, while digital PCR confirmed its presence in all spiked samples. Pathogen recovery consistently coincided with peak *Y. enterocolitica* concentrations, which occurred variably after 24 h or 48 h of enrichment, suggesting that sampling at a single fixed time point may not capture optimal recoverability. Across the three food matrices, enrichment was characterized by highly variable, matrix- and sample-dependent shifts in background microbiota composition. In addition, CIN agar frequently yielded closely related species such as *Yersinia intermedia* and *Serratia fonticola*, producing colonies indistinguishable from those of pathogenic *Y. enterocolitica*.

Together, these findings indicate that culture-based detection occurs within a dynamic microbial environment, suggesting that standardized enrichment and plating protocols may not fully account for variability across food matrices and samples.

S4-P24: Dynamics of *Yersinia enterocolitica* Growth Under Stress and Temperature Variations

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Yersinia enterocolitica remains an important foodborne pathogen associated with the consumption of pork, vegetables and unpasteurized cheese. Despite standardized detection protocols, its recovery from contaminated food remains challenging. An important challenge is the presence of competing background microbiota during enrichment, where rapidly growing species frequently outcompete *Y. enterocolitica* across diverse food matrices. Consequently, the pathogen remains detectable by molecular methods while unrecoverable through culture-based approaches.

Y. enterocolitica is a psychrotrophic bacterium capable of growth at relatively low temperatures. Incubation temperature therefore offers a potential strategy to influence these competitive interactions and forms the basis of traditional cold enrichment procedures. However, their applicability to routine diagnostics is limited due to long incubation periods. A more moderate reduction in enrichment temperature, such as 15°C, may provide a competitive advantage to *Y. enterocolitica* by slowing the growth of competing microbiota while still allowing pathogen proliferation, compared to the standard enrichment temperature of 25°C (ISO 10273:2017).

However, the effectiveness of this approach is likely strain-dependent, as variability between strains may affect both the lag phase (λ) and the maximum growth rate (μ_{max}). In addition, food-related stresses may also prolong the lag phase, potentially counteracting the expected competitive advantage at lower temperatures.

A diverse collection of approximately 90 *Y. enterocolitica* strains was used to assess variability in growth behaviour. A subset of representative strains was selected to investigate the combined effect of temperature and food-related stresses on growth kinetics. To mimic realistic contamination scenarios, strains were exposed to defined stress conditions: freezing and lowering the water activity. Growth curves were established by enumeration through plate counting after enrichment at 25°C and 15°C. This enables the quantification of lag phase and maximum growth rate.

Linking strain variability and stress adaptation to temperature-dependent growth kinetics provides insight into favorable growth conditions for *Y. enterocolitica* during enrichment. This contributes to the development of more effective enrichment strategies and improved culture-based detection in complex food matrices.

S4-P25: Investigating the Molecular Mechanisms of *Listeria monocytogenes* Strains in Response to Antimicrobial Effects of Carrot Juice

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Listeria monocytogenes is one of the most important foodborne pathogens, characterized by a high hospitalization and fatality rate. Its ability to adapt to diverse environmental stresses allows it to persist in both food matrices and processing plants. Driven by increasing consumer demand for natural alternatives to chemical preservatives, research into natural antimicrobial compounds has become increasingly important.

While the antimicrobial properties of carrots against *L. moncytogenes* have long been documented, the specific bioactive compound(s) responsible and the molecular mechanisms behind this effect remain unclear. This study aimed to (*i*) screen a diverse collection of 52 carrot accessions to identify those with the highest antilisterial effect and (*ii*) elucidate the molecular response mechanisms of three different *L. monocytogenes* strains to carrot-induced stress.

Initially, we screened these 52 carrot accessions, including commercial cultivars, landraces and wild relatives, for their antilisterial activity. The accessions were selected based on their differing concentrations of potential antimicrobial compounds. The screening identified three accessions capable of reducing *L. monocytogenes* from an initial inoculum of 7-$\log_{10}$ CFU/mL to below the limit of detection within 15 min of inoculation. In contrast, three accessions showed no observable antimicrobial effect after 120 min of incubation.

These six accessions were selected for further investigation of the molecular response of *L. monocytogenes* to carrot-induced stress. RNA-seq analysis revealed significant transcriptomic changes. Notably, pathways involved in cell wall structure and integrity, two-component systems, and ABC transporters were strongly upregulated. Interestingly, genes previously associated with the response to nisin-induced stress were also differentially expressed in response to carrot-induced stress, suggesting a partially overlapping mechanism of action.

Overall, these results provide new insights into the molecular response mechanisms of *L. monocytogenes*, suggesting that the cell wall is a primary target of carrot-drived antimicrobial activity and supporting the potential of carrots as natural antimicrobials to mitigate the contamination risk with *L. monocytogenes*.

S4-P26: Phenotypic and Genotypic Characteristics of Carbapenem Resistant *Campylobacter coli* Isolated from Poultry in Poland

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Campylobacteriosis is currently the most frequently reported zoonosis in humans. Poultry is a natural reservoir for *Campylobacter*. The overuse of antibiotics in human medicine and animal breeding has led to the emergence of antimicrobials resistance in bacteria and spread of multidrug resistant (MDR) strains in environment. In recent years, there has been an increasing number of cases of resistance to carbapenems. These antibiotics are used to treat severe invasive *Campylobacter* infections in humans, and any reports of resistance to this group of antimicrobial agents are a cause for concern.

The aim of the study was to phenotypic and genotypic characterization of carbapenem resistant *Campylobacter coli* isolated from poultry carcasses in Poland using microbroth dilution method and whole-genome sequencing (WGS).

A total of 50 *C. coli* isolates tested were collected during 2024 from poultry carcases. Antimicrobial susceptibility testing was performed using the microbroth dilution method and determination of the MIC (Minimum Inhibitory Concentration), with the use of EUCAMP3 plates. In addition, isolates were characterized towards the presence of resistance genes using WGS.

Among the tested isolates, resistance was most frequently observed to ciprofloxacin (*n* = 48; 96.0%), ertapenem (*n* = 45; 90.0%), and tetracycline (*n* = 38; 78.0%). Only 2 strains (4.0%) were resistant to erythromycin. These isolates were found to be susceptible to chloramphenicol and gentamicin. Most of *C. coli* isolates (*n* = 34; 68.0%) showed a resistance profile of ciprofloxacin + ertapenem + tetracycline. Only one strain exhibited resistance to ciprofloxacin + ertapenem + tetracycline + erythromycin. Analysis of the correlation between genotype and phenotype showed that no resistance genes for ertapenem, ciprofloxacin, and erythromycin were detected in any of the isolates. Such correlation was observed only for tetracycline, where 97.4% of phenotypically resistant strains possessed the *tet(O)* gene. Technical issues, such as assembly errors, use of automated annotation processes, may contribute to differences in phenotypic and genotypic patterns of antibiotic resistance. Furthermore, the presence or absence of efflux pumps, plasmids, cell membrane permeability, frameshift mutations, or existence of mosaic or novel resistance genes may influence the phenotypic resistance and lack of the correlation with the genotypic pattern.

The results of the study showed a high prevalence of *C. coli* isolates resistant to antibiotics used in treatment of campylobacteriosis, including carbapenems. Furthermore, multidrug resistant strains were also observed. The high rate of antimicrobial resistance among *C. coli* highlights the need to monitor these bacteria in food and food products.

S4-P27: Prevalence of *Clostridium botulinum* in Food Raw Materials and Products Available on the Finnish Market

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Clostridium botulinum is an anaerobic, spore-forming bacterium commonly found in the environment. The spores prevalent in soils and sediments may contaminate food raw materials and, under suitable anaerobic conditions, can germinate and outgrow into toxinogenic bacterial cultures. The botulinum neurotoxin (BoNT) is the most potent known biological toxin. Consumption of food containing preformed BoNT can lead to foodborne botulism, a potentially life threatening intoxication characterized by descending flaccid paralysis that may progress to respiratory failure and death.

Vacuum packaged, minimally processed chilled foods with extended shelf life are of particular concern as the spores of non-proteolytic *C. botulinum* Group II can survive mild to moderate heat treatments and can grow and produce BoNT at refrigeration temperatures. This highlights the need for careful risk assessment and effective control strategies. At present, risk management often relies on intense heat treatments combined with cold storage. Such harsh heat processing can negatively affect the nutritional and sensory quality of the food products and increase product loss and raise energy consumption thus challenging sustainability.

For accurate food safety risk assessment, it is important to gain a detailed understanding of the natural prevalence of *C. botulinum* spores in food raw materials. Here we analyzed 1037 samples from a range of food categories for the presence of Groups I and II *C. botulinum* spores. We examined commonly used industrial ingredients such as meats (pork and poultry), vegetables (carrot and onion), spices (black pepper, parsley, basil, carrot, celery and onion) and salt. In addition, we studied plant-based meat alternatives such as vegetarian sausages and vegetarian cold cuts. Detection of *C. botulinum* Groups I and II was carried out using enrichment protocols optimized for different food categories followed by real time PCR targeting the botulinum neurotoxin gene.

We observed a very low prevalence (0.1 %) of *C. botulinum* in food raw materials and products available on the Finnish market. A single dried parsley sample tested positive by PCR. From this sample, we successfully isolated a *C. botulinum* Group II strain encoding BoNT subtype B4. The strain was further characterized genomically and phenotypically.

In conclusion, although *C. botulinum* appears to be a rare finding in food raw materials and products on the Finnish market, occasional contamination does occur. This poses a relevant food safety risk and should be considered when evaluating food safety hazards and designing control strategies, especially for minimally processed foods.

S4-P28: Phenotypic Characterization of *Campylobacter jejuni* Stress Responses as a Basis for Genome-Wide Aassociation Studies

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Campylobacter jejuni is a major cause of foodborne enteritis, responsible for 90% of human campylobacteriosis, with 246,000 cases reported annually in France. Although widespread in the environment, poultry meat is the primary transmission source, and its persistence along the food chain remains a key public health concern, with nearly one-quarter of carcasses exceeding regulatory limits at slaughter. Survival under these conditions appears limited to the most stress-tolerant strains, highlighting phenotypic heterogeneity likely driven by genomic variation. Identifying genetic biomarkers of stress resistance by integrating omics and statistical approaches can be done through genome-wide association studies (GWAS).

The first step prior to GWAS is the phenotypic characterisation of *C. jejuni* ability to survive to stress-related process conditions. Fifty-three sequenced strains (29 clinical, 24 food-related) representing ST21, ST45, ST354, ST464, and ST10846, were submitted to four stresses encountered in slaughterhouses, selected through multicriteria analysis: heat stress (55°C for 3 h), acid stress (pH 3.8 for 40 min), aerobic stress (ambient atmosphere) at 25°C for 168 h and at 42°C for 30 h. Surviving cells were enumerated at time intervals on Columbia agar. Experiments were conducted in at least three independent replicates.

For each stress condition, bacterial responses were expressed as Δlog (Δlog = logN1 – logN0). The most critical intervals for each stress were selected for subsequent analysis (20 min for acid stress and 168 h for aerobic ambient stress). Because the study focused on strains exhibiting resistance to multiple stresses, the $\Delta\log_{10}$ values obtained for each replicate under these conditions were jointly analyzed using a multivariate approach to characterize overall resistance profiles. Principal component analysis (PCA) was applied to summarize the dataset and reduce its dimensionality. The resulting coordinates were then used for hierarchical clustering based on Euclidean distance, enabling classification of strains based on their combined responses to the different stresses. The significance of the resulting clusters was assessed using multivariate analysis of variance (MANOVA). Three distinct groups were identified, corresponding to highly resistant, intermediate, and low-resistance strains, including 8, 19, and 26 strains, respectively ($p < 0.001$).

The variability in strain responses under each stress condition led us to perform a GWAS analysis in subsequent steps to identify genetic biomarkers associated with stress persistence. While GWAS has so far been mainly applied to clinical traits such as virulence and antibiotic resistance, its application in this context could improve risk assessment models and guide control strategies in food production.

S4-P29: Occurrence and Characteristics of Methicillin-Resistant *Staphylococcus argenteus* in Food Products in Germany

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Staphylococcal species are well-known zoonotic opportunistic pathogens leading to clinical symptoms that range from skin and soft tissue infections to severe blood stream infections as well as human food poisoning. In 2015, *Staphylococcus argenteus* was defined as a separate species within the *Staphylococcus aureus* complex but with a similar pathogenicity in humans, proven by occasional infectious disease and foodborne poisoning outbreaks especially in Asia and Australia.

To identify methicillin-resistant *S. argenteus* in the German food chain, we analysed approximately 6000 presumptive methicillin-resistant *S. aureus* (MRSA) isolates that have been provided to the German NRL-Staph between 2014 and 2024, mostly in frame of the national zoonoses monitoring. In total, we identified 11 *mecA*-positive *S. argenteus* strains and one *mecA*/*mecC*-negative *S. argenteus*. Because all identified *S. argenteus* isolates originated from Asian seafood, a targeted investigation of fish and seafood imported into Germany in 2024/2025 followed which resulted in further 23 isolates. All isolates were further characterised by whole genome sequencing and phenotypic antimicrobial resistance (AMR) testing. We found a highly clonal structure and narrower AMR-profiles than in MRSA from similar food origin, but most of the strains harbored relevant virulence genes like staphylococcal enterotoxin and immune evasion cluster genes. Core genome multilocus sequence typing including genomic sequences from human clinical strains which are available in public databases do not show clustering according to their human or food origin indicating a common human origin or a missing species adaptation. On the other hand, based on our limited data we could not prove direct transmission events which would be indicated by no or very low allelic differences.

In conclusion, antimicrobial resistant and potentially virulent *S. argenteus* strains frequently occur in fish and seafood from Asian regions, but have not yet been observed in German food products. The strains might represent a threat to human health, although direct transmission from food to humans could not be proven yet.

S4-P30: Synergistic Effect of ZnO Nanoparticles and Sodium Hypochlorite on Reducing Pathogenic Bacterial Adhesion to Stainless Steel Surfaces

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The use of commercial disinfectants in combination with other antimicrobial agents, such as ZnO nanoparticles, represents a promising strategy for enhancing disinfection efficacy and controlling pathogenic bacteria. In this study, the minimum inhibitory concentrations (MICs) of sodium hypochlorite, pure ZnO nanoparticles, and Mn-, Ce-, and Co-doped ZnO nanoparticles (doping concentrations 10%, 20%, 30%) were determined against gram-negative bacteria *Escherichia coli* and *Salmonella* Typhimurium, and gram-positive bacteria *Staphylococcus aureus* and *Listeria monocytogenes* using the broth microdilution method (CLSI M07-A10). In addition, the checkerboard assay was employed to evaluate the type interaction of sodium hypochlorite in combination with pure ZnO nanoparticles. The results indicated that ZnO nanoparticles required higher concentrations to inhibit bacterial growth compared to sodium hypochlorite, whereby their combination exhibited a synergistic effect. It was also revealed that doping of Mn and Co in ZnO nanoparticles improved antibacterial activity against gram-positive bacteria. The study also evaluated the effectiveness of individual treatments and their combination in reducing initial bacterial adhesion to stainless steel surfaces (AISI 304) under different environmental conditions (7°C, 25°C, and 37°C; pH 4.5, 7.0, and 8.5), using the colony-forming unit (CFU) count method. ZnO nanoparticles were more effective than sodium hypochlorite in reducing bacterial adherence, while the combined treatment showed superior performance compared to either treatment alone, highlighting its potential as a novel approach for preventing bacterial biofilm formation. Furthermore, data that temperature and pH affected bacterial adhesion provide valuable insight how bacteria survive in the food processing environments, which could assist in assessment the risk of contamination.

S4-P31: Mediating Interactions: Multifunctional Roles of Extracellular Vesicles in *Listeria monocytogenes*

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Listeria monocytogenes is a foodborne Gram-positive pathogen posing significant risks to the elderly, immunocompromised, pregnant, and infant populations. A major challenge in combatting this pathogen is its ability to persist in the food chain and transmit to host systems. Resistance to environmental stresses and surfaces attachment contribute to its persistence.

Extracellular vesicles (EVs) have been proposed as mediators of bacterial interactions that support survival and adaptation across environments. Bacterial EVs have been shown to enhance stress tolerance, promote biofilm formation and facilitate pathogenesis in some species, with limited evidence collected for *L. monocytogenes* so far. Therefore, we investigated the effect of *L. monocytogenes* EVs in mediating interactions with antimicrobial compounds, surface materials and host cells to elucidate their role in transmission and pathogenesis.

We collected EVs from cell fractions and culture supernatant under defined conditions. To characterize EVs, we employed Nanoparticle Tracking Analysis, Transmission Electron Microscopy. To examine antimicrobial interaction effect, we applied time-kill assays with various concentrations of EVs. To examine surface interaction effect, bacterial attachment assays to different abiotic surface materials in presence of EVs were performed. To evaluate pathogenesis effect, bacterial adhesion, invasion and translocation assays were conducted on intestinal epithelial model exposed to EVs.

EV-mediated antimicrobial interaction. Membrane-targeting antibacterial peptide Nisin is widely used in food preservation. We observed that EVs addition significantly reduced Nisin inhibition on *L. monocytogenes* growth and enhanced bacterial survival. This result suggests that EVs can act as decoys to protect bacteria from (membrane-targeting) antimicrobial compounds. .

EV-mediated surface interaction. Stainless steel and PVC were employed as model food-processing surfaces. The bacterial attachment was significantly different in presence of EVs. EVs concentration, temperature, production phase and surface type all influenced the bacterial attachment. This result indicates that EVs can modulate surface-associated bacterial behaviors.

EV-mediated host interaction. In Caco-2 cells, direct EVs treatment did not damage cell membrane integrity or metabolic activity. In addition, EVs exposure also did not significantly affect bacterial attachment, invasion or translocation in this model. This result suggests EVs do not directly influence host intestinal cell viability or susceptibility to infection under tested conditions.

Our results suggest that *L. monocytogenes* EVs play a role in pathogen transmission by mediating interactions with antimicrobial compounds and abiotic surfaces, while their direct effect on host epithelial intestinal cells is limited. Further mechanistic exploration will help deepen our understanding on the ecological and physiological relevance of bacterial EVs.

S4-P32: Fighting Resistance Naturally: Essential Oils Restore Antibiotic Power Against Foodborne Pathogens

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Antibiotic resistance reduces available therapeutic options, increasing the risk of morbidity and mortality, and placing an additional burden on healthcare system. Essential oils (EOs), due to their complex composition and multiple mechanisms of action targeting microbial cells, may act synergistically with antibiotics, hence enhancing or restoring antimicrobial susceptibility.

This research aimed to evaluate the efficacy of selected EOs, either in combination with antibiotics or as a pre-treatment, in restoring susceptibility to commonly used antibiotics. The effect on *Listeria monocytogenes* and *Salmonella enterica*, two pathogens of major relevance in food safety and veterinary context, is proposed as a case study.

In detail, eleven tetracycline-resistant *S. enterica* strains, isolated from the swine food supply chain, were treated with EOs derived from *Thymbra capitata*, and *Thymus serpillum* (Flora Srl, Lorenzana, Italy), in combination with tetracycline, to determine the Minimum Inhibitory Concentration (MIC) and Fractional Inhibitory Concentration Index (FICI) at 37°C for 48 h. Additionally, two food derived and two clinical isolates of *L. monocytogenes* were exposed to *T. capitata* EO (Exentiae, Italy), both as a pretreatment (1 h at 37°C at MIC/2, followed by removal) and in combination with penicillin, ampicillin, and gentamicin, at 37°C for 48 h. Growth and inhibition dynamics were monitored using the Omnilog system (Biolog Inc., USA).

The combination of EOs with tetracycline reduced the antibiotic MIC to 4 µg/mL for *Salmonella* strains, restoring susceptibility according to CLSI and EUCAST standards. The effect was strain-dependent, with interactions ranging from additive to synergistic.

In *L. monocytogenes*, *T. capitata* EO combined with antibiotics, resulted in up to a seven-fold decrease in MIC and MBC values, thus restoring susceptibility. Pre-treatment with EO also produced a bacteriostatic effect when the cells were subsequently exposed to sub-inhibitory antibiotic concentrations (MIC/2), with stronger effects observed in clinical strains.

The major component of the tested EOs was carvacrol, known to disrupt cytoplasmic membranes. Other phenolic compounds likely enhance its efficacy by increasing membrane permeability and facilitating antibiotic uptake. EO-induced cellular stress may deplete cellular energy and further increase susceptibility to antimicrobial agents, including antibiotics.

In conclusion, essential oils reduce the concentrations of antibiotic required to inhibit *S. enterica* and *L. monocytogenes*, highlighting their potential as adjuvants in strategies to combat antimicrobial resistance. Further studies are necessary to explore their integration into antimicrobial therapies targeting multidrug-resistance bacteria.

S4-P33: The Osmotic Stress Response as an Essential Survival Mechanism in STEC O121

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The persistence of Shiga toxin-producing *Escherichia coli* (STEC) in low-moisture foods like wheat flour poses a significant public health risk. Understanding the genetic mechanisms that enable this persistence is critical for developing control strategies. Previous transcriptomic analyses revealed strong upregulation of genes associated with osmotic stress in wheat flour. This study aimed to validate the functional roles of the pro and bet operons in the osmotic stress tolerance of STEC O121 during persistence in wheat flour. To achieve this, deletion mutants were constructed targeting proline biosynthesis (*proC*), glycine betaine biosynthesis (*betB*), the glycine betaine/proline transporter (*proU*), and a Δ*proU* Δ*betB* double mutant. The growth kinetics of these mutants were compared with the wild type (WT) strain in M9 minimal medium supplemented with 0.2 M NaCl. The WT strain exhibited a maximum specific growth rate (μ_{max}) of approximately 0.41/h. While the Δ*betB* (0.46/h) and Δ*proU* (0.41/h) mutants grew at near WT rates, the Δ*proC* (0.33/h) and Δ*proU* Δ*betB* (0.32/h) ($p < 0.05$) mutants showed a severe growth defect, confirming their sensitivity to high osmolarity conditions. Notably, the growth kinetics of all tested mutants improved significantly when the medium was supplemented with 1 mM glycine betaine, either by increasing the μ_{max} or reducing the lag phase. These results provide strong functional evidence that the pro and bet operons are critical for tolerating osmotic stress. Future work will involve constructing a *proP* mutant to further dissect these transport systems and challenging the mutants directly in wheat flour to confirm their role in persistence within the food matrix.

S4-P34: Beyond the Bite: Tick-Borne Encephalitis Virus (TBEV) in Raw Milk in Italy

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Tick-borne encephalitis (TBE) is a viral infectious disease of the central nervous system caused by the tick-borne encephalitis virus (TBEV), a member of genus *Flavivirus*. The clinical progression of TBEV infection typically follows a biphasic course: an initial viremic phase, which may subsequently progress to a second neurological phase. The latter phase may present in meningeal or focal forms, including meningoencephalitis, meningoencephalomyelitis, and encephaloradiculitis. TBEV has three main subtypes – European, Siberian, and Far Eastern. The case fatality rate for the European subtype is approximately 1–2%.

In recent decades, TBE has emerged as a significant public health concern in Europe, exhibiting a distinct endemic distribution. Global climate change is contributing to the introduction and spread of tick populations and TBEV to new areas and higher altitudes. The Lombardy region is considered a potentially at-risk area for tick-borne encephalitis virus circulation and emergence due to its geographic proximity to endemic areas and the widespread presence of *Ixodes ricinus* ticks. While the virus is primarily transmitted to humans through the bite of infected ticks, fewer than 1% of cases occur via the alimentary route through the consumption of unpasteurised milk or dairy products derived from infected goats, sheep, or cows. However, limited data are available on TBEV-contaminated food and foodborne TBE cases in Italy.

The aim of the study was to evaluate the presence of TBEV in raw milk from goats and cows reared in the mountainous provinces of Lombardy, Italy. Throughout 2024, a passive surveillance program was implemented, with a peak in summer during the activity of alpine pastures. This effort yielded a total of 94 raw milk samples from the study area. Mengovirus was employed as a process control. TBEV and Mengovirus detection was performed via two specific one-step real-time RT-PCR assays.

Two goat milk samples from the same herd tested positive for TBEV, with a one-month interval between detections. The recovery rate of process control RNA validated the reliability of these results.

The presence of the viral genome was subsequently confirmed via Sanger sequencing analysis. No serological investigation on animals was done.

These findings underscore the need for a continuous monitoring framework involving both human and animal healthcare systems (One-Health approach) to track TBEV circulation. Future efforts should prioritize assessing viral prevalence in ticks, ruminant populations, and susceptible dairy products to ensure food safety and protect public health.

S4-P35: Efficacy of High-Pressure Processing on Foodborne Pathogens in a Nut-Based Cheese Analogue

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The expansion of the plant-based food sector has led to the development of cheese alternatives. The absence of lethality steps in the production process may pose a risk to microbiological safety, because the physicochemical properties of these products, such as high water activity (aw) and mildly acidic pH, may support the growth of foodborne pathogens. This study aimed to validate the efficacy of high-pressure processing (HPP) on a spreadable, fermented nut-based cheese analogue in reducing pathogen levels and preventing their regrowth following sublethal injury during refrigerated storage.

The efficacy of HPP was assessed through a challenge test following ISO 20976-2:2022, using samples inoculated pre-treatment with *Listeria innocua* (surrogate for *L. monocytogenes*), *Salmonella* spp., *Bacillus cereus* and *Staphylococcus aureus*. The growth potential (GP) of target microorganisms post-treatment was evaluated following ISO 20976-1:2019 as the difference (Δ) between the highest observed concentration and the initial concentration.

Test units were inoculated (10^7 CFU/mL) with 1% (w/v) multi-strain cocktails of target microorganisms; control units inoculated with 1% (w/v) physiological solution and uninoculated food control samples (FCS) were included. Samples were vacuum-packed, then subjected to HPP (593 MPa, 180 s). Pathogens were enumerated on three samples before treatment and ten samples after treatment to assess log reductions. Subsequently, GP was evaluated at 7°C for 18 days, then at 10°C until the end of shelf-life (extended to 40 days). Three test units were analysed for pathogen enumeration at T0, T7, T14, T21, T30 and T40; pH and water activity (aw) were monitored in control units and FCS.

The impact of HPP, under the worst-case scenario, resulted in reductions of 4.89 $\log_{10}$ CFU/g for *L. innocua* and 6.28 $\log_{10}$ CFU/g for *Salmonella* spp.; limited reductions emerged for *B. cereus* and *S. aureus* (0.55 $\log_{10}$ CFU/g), consistent with the high resistance of *S. aureus* and the spore-forming nature of *B. cereus*. Regarding GP, no regrowth was observed: *L. innocua* and *Salmonella* spp. remained detectable, although below the limit of quantification (< 10 CFU/g); *S. aureus* gradually declined ($\Delta = -0.75$ $\log_{10}$ CFU/g) and *B. cereus* remained stable ($\Delta = 0.14$ $\log_{10}$ CFU/g), suggesting persistence of spores rather than vegetative growth. pH (5.28) and aw (0.99) remained stable, indicating no matrix changes affecting pathogen behaviour.

These findings demonstrate that HPP is an effective preservation strategy for plant-based cheese analogues, contributing to microbial safety in products not re-exposed to post-process contamination and supporting safe shelf-life extension.

S4-P36: Bacteriophage Intervention Targeting *Salmonella* in Minced Meat Matrices: Effective Control in *in vitro* Application and Industrial Processing

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Despite major efforts made by industry, *Salmonella* continues to be a major public health risk associated with food production and consumption. This risk is particularly pronounced in minced meat products, due to the product being a homogenous blend of multiple carcasses, parts, and sometimes even flocks, giving single *Salmonella* positive carcasses the potential to contaminate entire batches of minced meat. Bacteriophages offer a promising tool for *Salmonella* control in addition to conventional antimicrobial methods. The ability to specifically kill pathogenic bacteria with minimal impact on organoleptic properties and without harm to the beneficial microbiota makes them an attractive measure for enhancing food safety.

Host-range spectrum of anti-*Salmonella* bacteriophage product, Phageguard S (PGS), was tested on over 150 *Salmonella* strains belonging to 43 different serotypes using the traditional spot plate method. The efficacy of the bacteriophage product was tested on various refrigerated minced meat matrices such as pork, beef, and chicken, which were contaminated with a 5-strain cocktail of relevant *Salmonella* strains. In addition, similar experiments were performed in three types of minced raw pet food matrices. *Salmonella* reductions were measured by retrieval and plating on selective XLD agar. To highlight practical relevance, the *in situ* efficacy of PGS against naturally occurring *Salmonella* in turkey was assessed during a ten-day industrial trial at a ground turkey processor.

The host range spectrum of the anti-*Salmonella* bacteriophage is very broad, as all major *Salmonella* serotypes were effectively lysed on plate. In addition to its broad host, the efficacy of PGS also spans across multiple protein matrices with fine particle size, as PGS was able to significantly reduce *Salmonella* in the tested minced meat and raw pet food matrices ($p < 0.05$). The reductions were dependent on the food matrix and phage concentration applied. Interestingly at a commercial-scale trial, the *Salmonella* incidence in a ground turkey dropped from an average 56% *Salmonella* incidence in control group to 10% *Salmonella* incidence in the PGS-treated group.

The data gathered in this study show that the anti-*Salmonella* bacteriophage product PGS acts as a targeted, scalable intervention that can reduce a broad range of *Salmonella* serotypes in minced meat products and raw pet food matrices. The use of anti-*Salmonella* bacteriophages offers an additional effective tool for *Salmonella* control in minced meat processing as well as in raw pet food production.

S4-P38: Molecular and Environmental Indicators of *Listeria monocytogenes* Persistence: A Multi Sector Approach

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Listeria monocytogenes is a bacterial foodborne pathogen able to persist for long periods in food processing environments, particularly through biofilm formation. This persistence results from complex interactions between strain genetic characteristics and environmental conditions. The aim of this study was to identify molecular and environmental indicators associated with the persistence of *L. monocytogenes* across several high-risk food production sectors.

Three environmental sampling campaigns were conducted in nine food processing facilities belonging to three high-risk sectors (seafood, vegetables, and pork). A total of 1,097 samples were analyzed for the presence of *L. monocytogenes*, along with the collection of environmental and microbiological data (total aerobic mesophilic flora, pH, relative humidity, temperature, biocide residues, presence of debris, surface type and condition…). *L. monocytogenes* isolates were characterized by WGS and classified as persistent or transient (≥ 2 campaigns, ≤ 7 allelic differences by cgMLST). Machine learning approaches and pangenome analysis were used to identify determinants associated with persistence.

L. monocytogenes was detected in 114 out of 1,097 samples (10.40%), with higher prevalence in the vegetable sector (51/357), followed by pork (35/360) and seafood (28/380). From these 114 samples, 12 persistent clusters, comprising 67 strains, were identified. The clonal complex CC121 was particularly represented, although other CC (CC31, CC101, CC5, CC412, and CC155) were also associated with persistence profiles. Pangenome analysis showed that persistence is not linked to a single genetic determinant but rather to a combination of biological functions, including prophage-related loci, transport systems (TatA, TatC, EfeO), and stress adaptation mechanisms. In contrast to previous assumptions, no direct association was observed between biofilm formation and persistence phenotype through quantitative crystal violet characterization. Persistent isolates were mainly found in environments with higher total aerobic mesophilic flora levels, typically above ~5.3–6.0 $\log_{10}$ CFU/mL (e.g., non-food-contact surfaces), compared to ~3.8–4.7 $\log_{10}$ CFU/mL for transient isolates. Similarly, persistent isolates were associated with higher relative humidity levels, often around ~80–85% (e.g., non-food-contact surfaces), compared to ~68–78% for transient isolates. These results suggest that the high levels of total aerobic mesophilic flora and humid conditions favor the establishment and maintenance of persistent clones. Overall, these findings indicate that *L. monocytogenes* persistence results from the interaction between strain genetic characteristics and site-specific environmental conditions. This study highlights the value of an integrated approach combining genomic data and detailed environmental characterization to improve monitoring and control strategies of *L. monocytogenes* in food processing environments.

S4-P39: Enhanced Antimicrobial Activity of Sodium Hypochlorite Combined With Essential Oils Against Pathogenic Bacteria

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Insufficient hygiene of food-contact surfaces and medical devices contributes to contamination by pathogenic bacteria, posing a significant public health risk and economic challenge. The increasing prevalence of multidrug-resistant bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella* spp., further complicates infection control. In particular, *Salmonella* remains a leading cause of foodborne illness, while *P. aeruginosa* is recognized as a priority pathogen due to its resistance mechanisms and clinical impact in vulnerable populations. This study aimed to evaluate the combined antimicrobial effects of disinfectant based on sodium hypochlorite and essential oils from *Lavandula officinalis* (lavender), *Melaleuca alternifolia* (tea tree), *Mentha piperita* (mint), and *Rosmarinus officinalis* (rosemary). The activity was tested against Gram-positive bacteria *Bacillus cereus* ATCC 11778 and *Staphylococcus epidermidis* ATCC 12228, and Gram-negative bacteria *P. aeruginosa* ATCC 10145 and *Salmonella* Typhimurium ATCC 14028. Interactions between disinfectant and essential oils were assessed using an improved checkerboard method to determine synergistic to antagonistic effects. The germicidal efficacy of selected combinations was further evaluated using a quantitative suspension test. Results demonstrated that most combinations exhibited synergistic or additive effects, enhancing antimicrobial activity compared to the disinfectant alone. A combination of disinfectant at 1/4 × MIC with lavender essential oil (from 1/4 to 1/8 × MIC) showed synergistic effects in Gram-positive bacteria, while additive effects were observed in Gram-negative bacteria (FIC = 1). The combined treatments showed increased bactericidal activity even at shorter exposure times of 1 min, with a complete germicidal effect achieved after 10 min for all tested bacteria. The strongest effects were observed against *S. epidermidis* ATCC 12228, while slightly lower susceptibility was noted for *Salmonella* Typhimurium ATCC 14028 at shorter exposure times. These findings highlight the potential of combining conventional disinfectants with natural antimicrobial agents to improve efficacy and reduce the risk of resistance development. The integration of essential oils into disinfection strategies represents an environmentally friendly and effective approach for controlling pathogenic bacteria in medical and food-processing environments.

S4-P40: Insights into Virulence Characteristics Across the *Cronobacter* Genus

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Cronobacter spp. are opportunistic foodborne pathogens associated with severe neonatal infections, including necrotizing enterocolitis, sepsis, and meningitis, commonly linked to contaminated powdered infant formula. Among the genus, *C. sakazakii* is the primary species implicated in fatal neonatal infections, with certain sequence types (STs) showing stronger associations with specific clinical outcomes. However, the underlying mechanisms driving these differences remain poorly understood.

This study aimed to explore *in vitro* virulence characteristics across the *Cronobacter* genus focusing on inter- and intra-species variability in *C. sakazakii*. Therefore, key infection-related traits were assessed, including the adhesion, invasion, and translocation using differentiated Caco-2 intestinal epithelial cells. The integrity of the epithelial layer was evaluated via transepithelial electrical resistance (TEER) measurements to assess intestinal barrier disruption during infection. In addition, bacterial motility (swimming, swarming, and twitching) was characterized using semi-solid agar assays to determine the capacity for host colonization and surface-associated movement. Furthermore, the ability to utilize sialic acid – a host-associated compound abundant in breast milk, the intestine, and the brain – was investigated by analyzing bacterial growth and surface adhesion in the presence of this substrate to evaluate a potential metabolic advantage in host environments.

The capacity to adhere to, invade, and translocate through Caco-2 cells was observed with significant variability between species and strains (belonging to ST1, ST4, and ST8). Differences in motility were observed, particularly in swimming behavior, suggesting a variability in the colonization potential. Notably, *C. sakazakii* strains were able to utilize sialic acid, but only in its free form. When bound to biologically relevant substrates such as mucin (in the intestine) or gangliosides (in the brain), sialic acid was not accessible, indicating that *C. sakazakii* may rely on exogenous sialidase activity to exploit this nutrient *in vivo*. Other *Cronobacter* species lacked the ability to utilize sialic acid, except one single *C. turicensis* isolate. Genomic analysis revealed that this isolate, alongside all *C. sakazakii* isolates, possessed the *nanAEKRT* gene cluster associated with sialic acid metabolism.

In conclusion, this study highlights significant species- and strain-dependent differences in virulence-associated traits within the *Cronobacter* genus, suggesting a variable virulence potential. While all strains exhibited the capacity for host cell interaction, the ability to utilize sialic acid appears largely restricted to *C. sakazakii*, potentially conferring a competitive advantage in host environments and contributing to its predominance in clinical infections.

S4-P41: 3D Growth of *Salmonella enterica*: From Food Tolerance to Digestive Resilience

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Understanding how foodborne pathogens survive along the food chain and through the host digestive tract remains a major challenge for food safety. While most studies rely on liquid cultures, real food systems are heterogeneous, spatially structured environments in which bacteria predominantly grow as microcolonies. This spatial organisation generates physicochemical gradients, and phenotypic heterogeneity can critically influence bacterial physiology and stress responses.

Here, we investigated how growth in food-like semi-solid matrices impacts bacterial behaviour compared to planktonic conditions. Across several major foodborne pathogens exposed to food-related and gastrointestinal stresses, spatial growth significantly altered growth dynamics in a stress- and species-dependent manner. Notably, microcolony growth enhanced tolerance to acidic conditions mimicking digestion, an effect further validated *in vivo* within the acidic midgut of *Hermetia illucens* larvae.

Focusing on *Salmonella enterica*, we explored the underlying molecular mechanisms using transcriptomics and spatial approaches. RNA-seq analysis revealed extensive transcriptional reprogramming, with over 1,400 genes upregulated in the spatial lifestyle, mainly associated with metabolism (carbohydrate: *otsB*, *treA*; amino acids: *astC*, STM1633, *trpB*; and ions: *focA*, *katE*), transcriptional regulation (*csgD*, *rpoS*), and functions of unknown role. Key genes involved in biofilm formation (*csgD*), motility (*fliA*), and stress response (*katE*, *rpoS*) were identified.

To investigate intra-colony heterogeneity, we combined laser capture microdissection (LCM), RT-qPCR, and GFP-based reporter imaging using confocal microscopy. Our results demonstrate a clear spatial organisation: peripheral cells are more metabolically active but also more exposed to stress, whereas central cells display a more protected phenotype.

Overall, this study identifies spatial structuring as a key determinant of pathogen stress tolerance and digestive resilience, bridging food matrix and host survival. These findings open new perspectives for improving risk assessment and developing more effective strategies to control foodborne pathogens.

S4-P42: Mobile Genetic Elements as Drivers of AMR Dissemination in the Brazilian Animal Production Chains

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Antimicrobial resistance (AMR) is a global health threat and the animal production chain is a major contributor due to the misuse of antibiotics, including subtherapeutic dosing and prophylactic overuse. This study investigates the role of mobile genetic elements (MGEs) in spreading antibiotic resistance genes (ARGs) in commensal *Escherichia coli* from five Brazilian animal production chains: beef, dairy, fish, pork, and poultry. Based on whole-genome sequencing and bioinformatics, we characterized 243 *E. coli* isolates, identifying 1,831 ARGs associated with plasmids, transposons, and prophages. Plasmids were the primary vectors of ARGs, with 226 isolates (93.0%) harboring plasmids conferring resistance to major antibiotic classes, including β-lactams, tetracyclines, aminoglycosides, and sulfonamides. Extended-spectrum β-lactamase (ESBL) genes (*bla*$_{CTX-M}$, *bla*$_{SHV}$) and the colistin resistance gene *mcr-1.1* were associated with specific plasmid types (e.g., IncFII, IncX4). Transposons, particularly IS26 and ISKpn26, were also significant in mobilizing ARGs, with co-localization analysis revealing their role in enhancing resistance gene expression. Prophages contributed minimally to ARG dissemination, with only 0.16% of ARGs identified within complete prophage regions. The dairy cattle chain showed the highest ARG richness and abundance per isolate (median 9.0 genes/isolate, IQR: 6–12; mean H' = 1.91 ± 0.70). Meanwhile, the pork and poultry chains exhibited the highest absolute number of ARGs (522 and 621, 28,51% and 33,91%, respectively), as a consequence of the intensive antibiotic use in these systems. Beef (median 2.0 genes/isolate, IQR: 1–4.5) and fish (median 1.0 genes/isolate, IQR: 1–4.25) exhibited the lowest richness, with no significant difference between them ($p > 0.05$). PERMANOVA $R^2 = 0.0811$ ($p = 0.001$) indicates the production chain explains only 8.11% of ARG variation, indicating that intra-chain heterogeneity must be due to farm-specific patterns of biosecurity and antibiotic prescription. These findings underscore the role of MGEs in the spread of AMR within food production chains and demonstrate the need for targeted surveillance beyond ARGs only and on-farm mitigation strategies to curb the global AMR crisis.

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S4-P43: Genomic Diversity, Antimicrobial Resistance and Virulence Potential of *Bacillus cereus* Group Isolates from Food and Human Milk

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The *Bacillus cereus* group includes spore-forming, ubiquitous, opportunistic foodborne pathogens frequently detected in foods, causing diarrheal and emetic illnesses. It can harbour multiple antimicrobial resistance and virulence genes, representing a potential concern for food safety. Other than in food, *B. cereus* is one of the most frequent contaminants of pasteurized donated human milk, posing a potential risk for neonates and leading to significant losses of this precious resource.

The aim of this study was to characterize the genomic diversity of *B. cereus* isolates from foods and donated human milk using whole genome sequencing (WGS) and to investigate possible links with food-associated strains.

A total of 212 isolates were collected from cereal-based products (*n* = 25), dairy products (*n* = 2), plant-based foods (*n* = 19), fish and seafood (*n* = 4), meat products (*n* = 3), ready-to-eat foods (*n* = 20), and human milk (*n* = 139), and were subjected to WGS. Isolates were taxonomically classified and sequence types (STs) were assigned using MLST. Genomes were screened for antimicrobial resistance and virulence genes.

The 24% of the isolates were *B. cereus* sensu stricto, while the rest belonged to *Bacillus mosaicus*. MLST showed high diversity across all sources, with over 50 distinct STs identified, the most frequent being ST26, ST164, ST24 and ST144, predominantly found in human milk. Most STs were shared between food and human milk.

All isolates carried β-lactamase genes, while *fosB* was detected in 79% of isolates. The streptothricin resistance gene (*satA*) was found in 23% of isolates, the disinfectant resistance gene (*qac*) in 7%, whereas other resistance determinants were sporadically detected.

Virulence gene analysis showed that the non-hemolytic enterotoxin cluster (*nheABC*) and *sph* were present in all isolates. The *cytK-2* gene was detected in 39% of isolates and the hemolysin BL complex (*hblACD*) in 26%, both mainly associated with *B. cereus* sensu stricto. The emetic toxin genes (*cesABCD*) were identified in 16 isolates, mainly ST26 (*n* = 12) and ST164 (*n* = 4), from both foods and human milk.

This study highlights the high genomic diversity and heterogeneous distribution of antimicrobial resistance and virulence determinants within the *B. cereus* group across food matrices and human milk. Although some genomic similarities were observed between food and human milk isolates, the limited number of food samples, due to the lack of structured surveillance for this pathogen, prevents robust source attribution. Different contamination routes are suggested, but linking milk contamination to food sources requires further analyses, emphasizing the need for enhanced genomic surveillance.

S4-P44: Population Genetic Structure of *Listeria monocytogenes* Strains Isolated from the Food Production Chain in Ten Central and Southeastern European Countries

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Listeria monocytogenes (Lm) is a ubiquitous bacterium responsible for listeriosis, a rare but severe foodborne disease. Most strains can be classified into major clonal complexes (CCs), which are associated with the majority of outbreaks and sporadic cases across Europe. Frontline identification of CCs is essential for epidemiological surveillance and the control of contamination in food production. In this study, a real-time PCR method developed by the European Union Reference Laboratory for Lm was used for rapid and cost-effective CC typing, supplemented by whole genome sequencing (WGS) when necessary.

The present study shows for the first time MLST-based typing data for the participating countries. A total of 1030 strains were isolated from food product across ten central and southeastern European countries: BG (*n* = 173), BS (*n* = 1), CY (*n* = 49), GR (*n* = 59), HR (*n* = 162), HU (*n* = 69), MK (*n* = 55), RO (*n* = 125), RS (*n* = 220), XK (*n* = 117), between 2005 and 2025. Twenty one different CCs were identified, reflecting considerable genetic diversity. Most isolates originated from meat (*n* = 585), fish (*n* = 143), milk (*n* = 86), combined (*n* = 70) and vegetable (*n* = 59) food products. CC9, CC121, CC8, CC155, and CC37, collectively account for approximately half of the dataset.

This massive dataset is presented, with a particular focus on 163 isolates from Croatia. Primarily isolated from pork meat products, they belong predominantly to CC37, CC9, CC8, CC29 and CC121. Notably, both CC37 and CC29 are prevalent in Croatia, distributed across eleven cities, over ten years, but not prevalent in the other countries. Furthermore, CC29 appears largely specific to Croatia; except in Kosovo where eight strains were isolated, from meat (*n* = 2) milk (*n* = 6) products. At the regional level, CC8 and CC9 were consistently associated with meat products across Croatia and other countries.

The entire dataset was compared with eight recent publicly available European multi-country outbreaks. All strain matching the outbreaks for product vehicle and CC were typed by WGS and compared. To illustrate one of the analysis conducted, for one of the outbreak related to CC6 strain and beef meat, eight isolates from the same CC and same food type were isolated in seven countries, one was from Croatia. The sequencing work on these isolates is in progress.

These findings highlight the persistence, geographic specificities, transboundary genetic similarities and potential linkages of certain strains. These data are of primary importance for developing public health and investigation guidance, as well as fostering a better understanding of the international circulation of *Listeria monocytogenes*.

S4-P45: *Salmonella* Enteritidis Outbreak Linked to Steak Tartare in Slovenia in 2022

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In November 2022, the National Institute of Public Health of Slovenia (NIJZ) confirmed an increased number of patients with gastroenteritis caused by *Salmonella* Enteritidis. Patients were identified in all regions of Slovenia except one.

The initial epidemiological investigation indicated that affected individuals had consumed steak tartare produced by a single meat-processing plant in Slovenia one to several days prior to symptom onset. After suspecting a foodborne infection, NIJZ promptly notified the Administration for Food Safety, Veterinary and Plant Protection (UVHVVR).

An extensive epidemiological investigation was led by the NIJZ. A total of 138 cases were reported in the period between 10th November and 2nd December 2022, of which 70 were women and 68 men. The average age was 33 years. Thirty-three patients were hospitalized, no deaths were recorded.

In November 2022, officials from UVHVVR carried out several inspections and took samples from raw materials and finished products at the identified meat-processing plant. They also assessed the general hygiene conditions, hygiene practices, and the implementation of HACCP procedures. A recall of two lots of steak tartare was ordered to prevent possible further infections. Employees were additionally referred for health assessments. The meat-processing plant was temporarily restricted from selling produce that posed a risk of *Salmonella* infection. It had been classified as high risk by the UVHVVR, which meant that more inspections and official samplings were planned.

The steak tartare was produced and distributed only in Slovenia, but NIJZ also informed other EU countries about the outbreak via European surveillance portal for infectious diseases (Epipulse). No other country confirmed similar *Salmonella* strains.

The source and mechanism of *Salmonella* contamination in the steak tartare could not be determined. However, the link between the origin of the infection – steak tartar and *Salmonella* infection was confirmed by sequencing the entire genome of human and food isolates. Comparison of the sequence profiles of isolates from stool samples of patients and steak tartare demonstrated that the isolates were closely genetically related and belonged to the same genetic cluster.

S4-P46: Effect of Nanoemulsified D-Limonene on *Listeria monocytogenes* Wild Type and Heat-Adapted Variants During Washing of Cherry Tomatoes

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This study evaluated the antimicrobial efficacy of nanoemulsified D-limonene during the washing of cherry tomatoes artificially contaminated with *Listeria monocytogenes* 4032 wild-type and two heat-adapted variants (58°C and 62°C). The performance of D-limonene was compared with a conventional chlorine-based sanitizer and water as control, focusing on microbial reductions and susceptibility differences among strains.

Washing treatments revealed that chlorine consistently achieved the highest reductions in bacterial load, confirming its strong antimicrobial performance under the tested conditions. In contrast, nanoemulsified D-limonene at 50 mM produced modest reductions, generally below 0.5 log cycles, while essential oil solutions, composed of a mixture of bioactive compounds, showed effects comparable to water. Despite observable numerical differences among treatments, statistical analysis (two-way ANOVA with Tukey's test) did not indicate significant differences between variants within the same treatment ($p > 0.05$), suggesting similar responses across wild-type and adapted populations under these conditions. However, descriptive trends between the adapted variants were apparent, and although not statistically significant, they may reflect subtle differences in stress response that could become relevant under different conditions. The variant adapted at 58°C exhibited slightly higher reductions with chlorine (~1.2 log), whereas the 62°C variant showed lower reductions (~0.7 log), reflecting potential variability in stress response phenotypes. However, both adapted strains did not display markedly enhanced resistance to nanoemulsified D-limonene compared to the wild-type, indicating a lack of strong cross-protection between thermal adaptation and susceptibility to this antimicrobial.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays further supported these observations. The wild-type strain exhibited a lower MIC (3.33 mM), whereas both adapted variants required higher concentrations (6.66 mM), indicating reduced sensitivity following thermal adaptation. This shift suggests that adaptation to heat stress may partially alter membrane or physiological properties affecting antimicrobial susceptibility, although without fully compromising the effectiveness of nanoemulsified systems.

Overall, the results demonstrate that while chlorine remains the most effective sanitizer, nanoemulsified D-limonene retains antimicrobial activity against both wild-type and heat-adapted *L. monocytogenes*. Importantly, thermal adaptation did not confer substantial cross-resistance to the nanoemulsion, highlighting its potential as a complementary or alternative natural sanitizing strategy for fresh produce.

S4-P47: Behaviour of Non-Proteolytic *Clostridium botulinum* in Cooked Ham Model System for the Safety Assessment of Nitrite-Free Meat Products Made with Addition of Natural Ingredients or Specific Starter Cultures

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The aim of this study was to verify the safety of different formulations commercially proposed as substitutes of nitrites in cooked meat products. The experiments focused on non-proteolytic *C. botulinum strains* (*C. botulinum* 8550 and *C. botulinum* 11219) examining their growth, and toxin production in meat-based model systems.

To investigate the behaviour of non-proteolytic *C. botulinum* during the shelf life of cocked ham model systems, five different formulations were prepared: Positive control (without additives), Negative control (with nitrites), Samples with addition of starter culture (*Staphylococcus xylosus* and *Staphylococcus vitulinus*), Samples with addition of starter culture (*Staphylococcus carnosus*) and nitrate-rich vegetable extract, Samples with addition of polyphenol-rich plant extract.

Minced pork meat was inoculated with a mixed spore suspension of the two non-proteolytic strains of *C. botulinum* and added with saline for each formulation. Samples were vacuum-sealed and thermally treated at 70°C for 40 min to simulate industrial processes. After treatment, samples were stored at 8°C, 12°C, and 15°C to simulate refrigerated and abuse storage temperatures. Microbiological and chemical-physical analyses on non-inoculated samples were performed before and after thermal treatment and after 40 days of storage. Analyses for the count of *C. botulinum* on inoculated samples were performed before and after thermal treatment, and after 10, 20, 30 and 40 days of storage at the three different temperatures (8°C, 12°C and 15°C).

To verify the presence of the toxin of *C. botulinum*, the Tetracore ELISA Antigen Capture system was performed, a sensitive assay designed to detect *C. botulinum* neurotoxin E.

To evaluate the *C. botulinum* concentration, meat samples were analyzed at each deadline, up to 40 days of incubation. No typical colonies were seen in any of the formulation at any deadline. The ELISA method was fundamental in confirming the presence of *C. botulinum* E toxin, offering a rapid alternative to the traditional mouse bioassay.

In meat formulations without additives, non-proteolytic *C. botulinum* toxin production occurred more rapidly at higher temperatures, whereas at 8°C, toxin formation was delayed by approximately 10 days. Formulations with nitrites (negative control) did not show toxin production in meat samples. Conversely, formulations containing *S. carnosus* and nitrate-rich extracts (S2) effectively prevented toxin production at all tested temperatures, attributed to the nitrate reductase activity of *S. carnosus*. Polyphenol-rich formulations (P) also inhibited toxin production, likely due to their antimicrobial properties.

S4-P48: Strategies for Controlling the Growth of *Listeria monocytogenes* During the Shelf Life of Smoked Salmon

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If smoked salmon is contaminated with *Listeria monocytogenes*, microbiological risks associated with the growth of this pathogen may arise during storage because the chemical and physical characteristics of the product support its proliferation.

To reduce the risk associated with the distribution of vacuum-packaged pre-sliced smoked salmon, the objective of this study was to identify and test treatments with potential inhibitory effects to control the growth of *L. monocytogenes*.

Microbial Challenge Tests were conducted to evaluate, up to 45-day shelf life, the behavior of *L. monocytogenes* in smoked salmon sprayed with bioprotective cultures (*Lactobacillus sakei*, *Lactobacillus curvatus*, *Lactococcus lactis* and *Leuconostoc carnosum*) and with different concentrations of organic acids (8.5%, 12%, 17%, 28%, 57% lactic acid and 100%, 50% and 20% diacetate-lactate). The studies were conducted in accordance with the ANSES 2021 guidelines.

The Microbial Challenge Tests were conducted using pre-sliced smoked Norwegian salmon produced by two different companies and from different production batches. After an initial characterization to verify the main microbiological and chemical-physical parameters, the salmon were portioned into 25-g aliquots and they were inoculated and treated in the various challenge tests. For each test, control samples inoculated with *L. monocytogenes* and not subjected to any treatment were prepared.

At the same time, treated and uninoculated samples were also prepared to monitor the main microbiological and physicochemical parameters during storage. All samples were incubated at 7°C for the first 15 days and at 10°C for the remainder of the shelf life.

The results obtained show that among the treatments tested in this study, applications of lactic acid starting from a solution with a minimum concentration of 17% and treatments with a commercial diacetate lactate solution used in original state or diluted to 50% proved effective for controlling *L. monocytogenes*. The addition of lactic acid at concentrations proven effective for controlling the pathogen, however, resulted in noticeable discoloration of the slices and an inevitable lowering of the pH, factors that could compromise the acceptability of the product. Spraying the slices with the culture consisting of *L. lactis* in combination with *L. carnosum* and treatment with *L. sakei* allowed to control the growth of *L. monocytogenes* throughout the shelf-life.

S4-P49: Validating Meat Dry Aging Processes Beyond EU Regulatory Conditions: Procedures Based on Literature, Predictive Models and Challenge Testing

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Dry-aged meat is growing in popularity, boosting production and making dry aging a major industry trend. However, the extended ageing period and specific environmental conditions required raise relevant food safety concerns. After the 2023 EFSA scientific opinion, Regulation (EU) 2024/1141 established specific requirements for dry aging conditions (i.e. maximum 3.0°C, 85% relative humidity [RH], and 35 days). However, when producers deviate from these conditions, microbiological safety must be validated. Scientific literature, predictive microbiology, experimental challenge tests, or combined approaches can be used to validate control measures within the food safety management system. Predictive microbiology models used so far focus mainly on growth of *Listeria monocytogenes* and do not cover inactivation dynamics that have been reported during dry-ageing. Moreover, they do not account for product specific factors such as breed. Due to scarce experimental data linking microbial behaviour with the changes in the physicochemical factors, this study generated new challenge test data to characterize *L. monocytogenes* dynamics and evaluated stochastic predictive models considering both growth and inactivation. The study assessed dry-aged beef from three breeds selected for their contrasting meat quality traits: leanness and flavor intensity (Bruna dels Pirineus), balanced marbling (Angus), and extreme intramuscular fat and tenderness (Wagyu). *L. monocytogenes* was inoculated on the surface and inside (simulating hook/knife punctures) of beef loin cuts, at ca. 4.5 log CFU/g. Meat was aged for 90 days at 2.4 ± 0.6°C and 59.3 ± 8.3% RH in a BSL2 facility while microbiological and physico-chemical parameters were monitored. On the surface of Angus and Wagyu beef, *L. monocytogenes* levels decreased > 1.5 log by the end of the process, whereas in Bruna dels Pirineus an initial increase returned to initial concentrations. In punctures, the pathogen grew up to 3.5 log in all breeds, which can be explained by the higher aw. Stochastic predictive models incorporating both growth and inactivation broadly reproduced the experimental data trends, although with substantial uncertainty, highlighting both their potential and limitations for quantitative risk assessment. Challenge testing proved valuable for quantifying the behavior of *L. monocytogenes* under different conditions, including realistic high-risk scenarios. The findings indicate that the evaluated dry-ageing process can produce microbiologically safe meat and highlight the critical influence of breed related characteristics on pathogen dynamics. In industrial production, the monitoring/verification of key parameters, including environmental temperature and RH and meat pH, aw decrease and microbial indicators, is essential for safety assurance and provides critical input for predictive microbiology models.

S4-P50: High-Throughput Microbial Typing Via Machine Learning-Enhanced FTIR Spectroscopy

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Fourier-transform infrared (FTIR) spectroscopy has emerged as a transformative tool in microbial diagnostics, enabling rapid differentiation of microorganisms based on their unique spectral "fingerprints." These fingerprints arise from infrared absorption patterns of microbial cells that reflect their overall biochemical composition, including lipids, proteins, carbohydrates, and nucleic acids. While FTIR has been applied in microbiology for decades, the modern integration of machine learning algorithms and hierarchical classification strategies has significantly enhanced its discriminatory power. This combination allows for precise, stepwise identification—moving from broad taxonomic groups down to specific serovars, with minimal hands-on time and low analytical costs. The utility of this approach was evaluated using *Salmonella enterica* subsp. *enterica*, a foodborne pathogen of major relevance to One Health surveillance. Because distinct serovars require specific monitoring and control strategies, their rapid identification is essential. However, targeted control measures often lead to bacterial adaptation and epidemiological shifts. In the case of *Salmonella*, multidrug-resistant (MDR) *S*. Infantis has become increasingly prevalent, superseding traditionally dominant serovars like *S*. Enteritidis and *S*. Typhimurium. This emergence underscores the urgent need for continuous, high-resolution surveillance. Although labour-intensive and poorly suited for modern diagnostic demands, traditional serotyping is still considered to be the current gold standard. To address this, we applied FTIR spectroscopy (IR Biotyper, Bruker) integrated with machine learning to a comprehensive One Health dataset. Our results demonstrate an accuracy exceeding 95% in serovar identification. By offering a rapid, cost-effective, and scalable alternative to traditional techniques, this methodology provides a robust solution for routine diagnostic workflows. Ultimately, these findings position FTIR spectroscopy as a pivotal diagnostic bridge, harmonising data across the human, animal, and environmental sectors to strengthen the entire One Health mission.

Key words: FTIR spectroscopy; machine learning; *Salmonella enterica* subsp. *enterica;* One Health; IR Biotyper

S4-P51: From Lab to Industry: A Thermal Process Validation Study for Kale Pasteurization

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Validation of a heat inactivation process in the food industry is a crucial step to ensure microbial safety of heat-treated food products. In this study, the effectiveness of heat inactivation during kale pasteurization at lab and industrial scales against two relevant vegetative pathogens, i.e., *Salmonella* Senftenberg and *Listeria monocytogenes*, was validated using a surrogate.

In the first step, the heat resistance of *S.* Senftenberg and *L. monocytogenes* was determined in kale using the Thermoresistometer Mastia, and *S.* Senftenberg was found to be more heat resistant. Similar experiments were conducted on two potential *Enterococcus faecium* surrogate strains, concluding based on predictive microbiology that *E. faecium* LMG11397 shows a heat resistance comparable to *S.* Senftenberg, demonstrating its ability to act as a surrogate.

Secondly, a lab scale pasteurization (5 min at 78°C) was performed on kale in a warm-water bath with co-inoculation with *E. faecium* LMG11397, *S.* Senftenberg, and the *L. monocytogenes* cocktail. The lab scale pasteurization ensured the inactivation of the surrogate and pathogenic strains of 6 log CFU/g.

Finally, at the industrial scale, four validation runs of kale pasteurization (5 min at 78°C) were performed with *E. faecium* LMG11397: one in the cooking kettle using water as the heating medium and three consecutive runs using glycol. For each run, the cooling process was conducted in two ways: either on ice or standing time with industrial cooling. Results demonstrated that the second and third pasteurization runs in the glycol kettle and cooling on ice did not guarantee 6-log CFU/g inactivation of vegetative pathogens did not reach a 6-log reduction of the surrogate, providing actionable recommendations to the industry. All other industrial pasteurization scenarios ensured inactivation of vegetative pathogens of 6 log CFU/g or more.

The results of this study highlight the importance of validating an industrial heat inactivation process using an appropriate surrogate strain to enhance the reliability of heat inactivation under industrial conditions.

S4-P52: Evolution of Pathogenic *Escherichia coli* Carrying the Transmissible Locus of Stress Tolerance in Brazil: From Food Sources to Clinical Settings

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Escherichia coli carrying the transmissible locus of stress tolerance (tLST) are able to overcome numerous environmental challenges. In our in-silico study, we aimed to characterize tLST in terms of its variants in 793 genomes of *E. coli* from Brazil originating from food, environmental and clinical (animal and human) sources, and to perform a temporal analysis in order to identify the historical moment of its emergence. We also analyzed the presence of two *Yersinia* high pathogenicity island (HPI) variants in *E. coli* genomes, describing other genes and accessory for resistance, persistence, mobile elements (plasmids) and sequence types. The prevalence of the tLST was 10% in *E. coli* from Brazil, predominantly observed in milk-originating genomes, within the prevalent $tLST_{CP010237}$ variant. In *E. coli* from other sources (clinical/environmental), only part of the tLST was present. Remarkably, our temporal analysis pinpointed the emergence of tLST back to around 1914, coinciding with major societal events. Regarding virulence genes, we found a prevalence of 38.5% for HPI of *Y. pestis* across genomes from all sources. Our global analysis also showed a high diversity of other virulence genes for milk *E. coli* (+ 100 genes). These genomes also stood out from the overall metadata for presenting a greater variety of resistance genes to other stresses, such as metals, biocides and acids, as well as persistence genes (biofilm formation). This study demonstrated the historical background of *E. coli* with tLST genes dating back more than 100 years, and the acquisition of a wide range of virulence and resistance genes that allow it to circulate in different environments: from food to clinic or from clinic to food, making this bacterium a pathogen that requires rigorous surveillance and strategic interventions to mitigate potential risks.

S4-P53: Effect of the Order of Food Residue and Bacterial Deposition on the Long-Term Survival of *Listeria monocytogenes*

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Listeria monocytogenes persists in food processing facilities for long periods because of its tolerance to environmental stresses. While food residues are known to affect its survival, it remains unclear whether the deposition of food residues before or after bacterial attachment influences its long-term persistence. Therefore, this study investigated how different deposition sequences affect the survival of *L. monocytogenes* on stainless steel surfaces.

Two *L. monocytogenes* strains with different tolerances to osmotic and desiccation stress, isolated from ready-to-eat seafood products, were used. Bacterial suspensions of each strain were prepared in phosphate buffered saline, and sterile food filtrate was obtained from minced tuna. To compare different contamination scenarios, the bacterial suspension and the food filtrate were applied to stainless steel coupons in four ways:
(*i*) bacterial suspension only, (*ii*) bacterial suspension dried before the addition of food filtrate, (*iii*) food filtrate dried before bacterial inoculation, and (*iv*) an equal-volume mixture of bacterial suspension and food filtrate applied simultaneously. An initial contamination level of approximately 8 log CFU/coupon was achieved by applying multiple 5 µL spots. The coupons were dried for 3 h at < 10% RH and 15°C. After drying, they were stored for 21 days at 75% RH and 15°C to simulate wet food production environments. Viable counts of *L. monocytogenes* were determined every 7 days.

Immediately after drying, conditions in which the food filtrate was present during inoculation ((*iii*) and (*iv*)) showed smaller reductions (0.3–0.9 log CFU/coupon) than those without the food filtrate ((*i*)and (*ii*)), which showed reductions of 1.9–2.2 log CFU/coupon. This trend persisted through day 7, with reductions of 0.7–1.4 log CFU/coupon in (*iii*) and (*iv*) compared with 1.5–2.4 log CFU/coupon in (*i*) and (*ii*). After day 7, however, the rate of decline in viable counts increased, resulting in total reductions of 5.0–5.6 log CFU/coupon by day 21 in (*iii*) and (*iv*). Interestingly, under condition (*ii*), viable counts decreased to 1.8–2.2 log CFU/coupon by day 21, resulting in lower counts than under condition (*i*) for both strains. This suggests that components in the food filtrate may contribute to the accelerated decline observed during long term storage.

Overall, these results indicate that food residues may protect *L. monocytogenes* during drying and short-term storage but accelerate the decline in viable counts during long-term storage. In this presentation, we will also discuss survival under different humidity conditions and the mechanisms by which food residues contribute to the accelerated decline in viable counts.

S4-P54: Antimicrobial Resistance Reshapes Growth Dynamics in *Salmonella*: Fitness Trade-Offs and Implications for Food Safety Risk Assessment

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Research has increasingly focused on the mechanistic basis of antimicrobial resistance (AMR) and its implications for food safety. Ciprofloxacin (CIP) resistance can alter bacterial physiology and fitness in *Salmonella* Typhimurium, potentially affecting its behavior along the agri-food chain. AMR-associated mutations often impose fitness costs that reduce competitiveness in the absence of selective pressure. However, resistant variants (RVs) may outcompete susceptible populations under antibiotic stress, increasing their likelihood of persistence and transmission. This is why assessing their competitiveness across selective pressure gradients is essential.

The objective was to evaluate the fitness and growth of CIP-resistant *Salmonella* Typhimurium variants across antibiotic gradients and to determine the contribution and interaction of key resistance-associated mutations (*rpoD*, *cyaA*, and *ramR*).

Five CIP-RVs (SeTRV1–SeTRV5) were obtained by exposing parental strain *S.* Typhimurium LT2 (SeT) to increasing CIP doses. Isogenic mutants were also generated harboring the SeTRV1-associated mutations in *rpoD*, *cyaA*, and *ramR*. Bacterial cultures were serially diluted in 96-well plates with TSBYE and increasing CIP doses. Growth kinetics were obtained at 37°C via optical density at 595 nm (OD_{595}) measured over time. Detection times (td) to reach a defined OD_{595} threshold were used to estimate μ_{max} from linear regressions of Ln(dilution) versus td ($R^2 \geq 0.950$). μ_{max} values were modeled using the Lambert-Pearson approach in R to obtain the non (NIC) and minimum inhibitory concentrations (MIC) for comparison.

SeT showed a concentration-dependent decline in μ_{max} values, from ≈ 2.35 h^{-1} to no growth at 0.2 mg/L (NIC 0.055; MIC 0.091 mg/L). SeTRV1 displayed clear basal fitness cost ($\mu_{max}0 \approx 1.66$ h^{-1}) but maintained growth up to 0.2 mg/L, with increased NIC and MIC (0.205 and 0.235 mg/L). Other RVs tolerated higher CIP concentrations, sustaining growth up to 0.5–2.0 mg/L, showing higher NIC and MIC estimates than SeT.

Isogenic mutants pointed to *rpoD* as the main contributor to the fitness cost, reducing $\mu_{max}0$ and limiting growth (NIC-MIC ≈ 0.083–0.095 mg/L). In contrast, *cyaA* and *ramR* mutants retained high μ_{max} values and remarkably extended growth (> 0.4 mg/L). Combining these mutations partially compensated *rpoD* costs, particularly with *ramR*, indicating interactions that enhance growth while maintaining resistance.

CIP resistance caused context-dependent fitness trade-offs, with RVs outperforming the parental strain above a threshold concentration. The *rpoD* mutation imposed a fitness cost while enhancing resistance, partially compensated by additional mutations. These findings indicate that AMR can reshape bacterial physiology and persistence, influencing *Salmonella* behavior in food-related environments and impacting food safety.

S4-P55: Comparison of *Enterococcus faecalis* Isolates Across the Chicken Production Chain from Poultry Farms to Retail Chicken Meat in Korea

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This study investigated the prevalence, antimicrobial resistance (AMR), and dissemination of *Enterococcus faecalis* across the chicken production chain from poultry farms to retail products in Korea. A total of 1,043 *E. faecalis* isolates were obtained with an overall isolation rate of 72.2%, and were subsequently characterized using antimicrobial susceptibility testing and multilocus sequence typing. High resistance rates were observed against macrolides (66.9–69.9%), tetracycline (62.4%), phenicols (55.7–56.1%), fluoroquinolones (53.1%), and linezolid (41.1%). In contrast, resistance to clinically important antimicrobials, such as daptomycin and vancomycin, was low or not detected. Comparative analysis revealed similar resistance patterns across isolates, with resistance to multiple antimicrobial classes consistently observed at all production stages, from farms to retail, indicating the persistent presence of antimicrobial-resistant *E. faecalis* throughout the production chain. Genotypic analysis identified several dominant sequence types (ST1718, ST476, ST444, and ST314) distributed across different production stages, suggesting widespread dissemination of *E. faecalis*. Among these, ST1718 was the most frequently detected and exhibited relatively consistent antimicrobial resistance patterns across stages, indicating its potential role in clonal dissemination. Overall, the presence of shared sequence types and similar AMR profiles suggests the dissemination of multidrug-resistant *E. faecalis* throughout the production chain. These findings highlight the need for integrated surveillance, as well as strengthened hygiene and control strategies, to mitigate the spread of antimicrobial resistance.

S4-P56: Phenotypic Differentiation of Macrolide Resistant Determinants - *erm(B)* and Copy Number Variable 23S RNA A2075G - in Thermotolerant *Campylobacter* spp.

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Thermotolerant *Campylobacter* spp. are the leading cause of foodborne infections in the European Union (EU). For treatment of severe campylobacteriosis cases, macrolides are the antibiotics of choice due to widespread high-level fluoroquinolone resistance in *Campylobacter* spp.. Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli* remains rare to moderate in the EU and is predominantly caused by the 23S rRNA A2075G point mutation. However, the emergence and spread of the resistance determinant *erm(B)*, highly prevalent in some Asian countries, causes concerns. Since current phenotypic monitoring cannot differentiate between underlying resistance determinants, the occurrence of *erm(B)* remains obscured.

We tested a panel of 192 *C. jejuni* and *C. coli* isolates from Germany and Vietnam using broth microdilution to assess antimicrobial susceptibility to erythromycin and clindamycin. Whole-genome sequencing revealed that isolates harboured either the 23S rRNA A2075G or A2074C point mutation, *erm(B)*, or neither of these determinants. As expected, isolates with either *erm(B)* or 23S rRNA point mutations showed indistinguishable resistance levels to erythromycin. Few putative wild-type isolates, which displayed ambiguous results with variable intermediate to high levels of erythromycin resistance, were analysed more in detail. First, resistance genotypes derived from genome assemblies appeared to be incorrect for these isolates. Long-read sequencing indicated that the presence of fewer than three copies of the 23S rRNA A2075G mutation per chromosome resulted in intermediate erythromycin resistance. Second, under selection pressure, the presence of one or two mutated copies facilitated the acquisition of the A2075G mutation in the residual 23S rRNA gene copy, thereby conferring high-level erythromycin resistance.

In the absence of selection pressure, the number of mutated 23 rRNA gene copies (one to three) remained stable even after 55 *in vitro* passages. Intriguingly, the presence of *erm(B)* led to high-level clindamycin resistance (≥ 256 mg/L), whereas the 23S rRNA point mutation – even with full three 23S rRNA A2075G copies – conferred only intermediate resistance (4–128 mg/L). In conclusion, our study revealed that only three copies of the 23S rRNA gene with the A2075G point mutation conferred full resistance to erythromycin, indistinguishable to resistance mediated by *erm(B)*. Furthermore, clindamycin may serve as a suitable phenotypic marker to distinguish between *erm(B)*-mediated resistance and 23S rRNA point mutations and could therefore be considered for inclusion in future monitoring programs.

S4-P57: Climate-Driven Thermal Adaptation and Prevalence: A Study of *Salmonella enterica* Serovars Isolated from Imported Low-Moisture Foods in Greece

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Salmonella remains a food safety concern, particularly in low-moisture foods (LMFs), where it can persist throughout the supply chain and pose public health and economic burdens. Climate change—rising temperatures, altered humidity—may further enhance *Salmonella* survival or enable growth in previously inhibitory environments. Thus, this study aimed to: (*i*) conduct a meta-analysis of *Salmonella* prevalence of imported LMFs in Greece and (*ii*) assess *in vitro* the capacity of specific isolated *Salmonella enterica* serotypes to undergo thermal acclimation—a critical survival mechanism in warming environments.

Over 3,400 LMF samples, collected between January 2024 and May 2025, were assigned unique IDs with associated metadata (origin, country, and sampling date). *Salmonella* was detected using the VIDAS® ELFA system and confirmed by selective media, biochemical, and serological tests (EN ISO 6579-1:2017). For heat exposure experiments, three serotypes isolated from sesame (*S.* Enteritidis, *S.* Johannesburg, *S.* Agona) were examined. Cultures (~7.5 log CFU/mL) were exposed *in vitro* to isothermal temperatures (42°C–52°C) for 2 h to estimate maximum growth temperature (T_{max}). Relative bacterial growth was calculated as the ratio of the population at each isothermal temperature to the room-temperature control. To study the thermal acclimation potential, cultures were inoculated in TSB (~8.0 log CFU/mL) and incubated from 40°C to 52°C with daily increases of 2°C. Every 24 h, 4 mL was sub-cultured into 36 mL of fresh, pre-warmed TSB, and populations were enumerated before and after transfer by plating on TSA (37°C/24h). Controls were incubated at 37°C following the same transfer protocol.

Most samples were sesame seeds and derived products (*n* = 3,255), followed by nuts (*n* = 135) and spices (*n* = 50). 104 samples (3.0%) were positive for *Salmonella*, with 101 being isolated from sesame seeds, 2 from halva/tahini, and 1 from pistachios. The highest prevalence was observed in samples from Nigeria (44.2%) and Turkey (33.7%), while India accounted for 11.5% of positives. No clear seasonal pattern was observed. In heat exposure experiments, *S.* Enteritidis exhibited thermal acclimation, growing up to 46°C compared to the estimated maximum growth temperature of 44°C during the isothermal treatment. In contrast, *S.* Johannesburg and *S.* Agona showed no acclimation ability, with growth limited to 42°C and 44°C, respectively. These findings indicate that thermal adaptation may be serotype-specific, with *S.* Enteritidis being capable of extending its growth (T_{max}) under elevated temperatures.

Understanding and monitoring such responses is essential for managing *Salmonella* risks in LMFs, in the context of climate change.

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S4-P58: Interaction-Driven Persistence of Sensitive and Resistant *Enterococcus* spp. in Aquaculture Environments: Are They Stronger Together?

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Aquaculture environments are increasingly recognized as reservoirs for the emergence and persistence of antibiotic-resistant bacteria, with consequences for animal production, environmental sustainability, and public health. Among the bacterial groups associated with Mediterranean aquaculture, *Enterococcus* spp. are frequently detected throughout the production chain, including in Greek fish farms. Although resistance profiles are commonly reported, the ecological interactions between antibiotic-sensitive and antibiotic-resistant populations under marine conditions remain poorly understood. This raises an important question: are they stronger together?

To address this, two *Enterococcus* strains isolated from Greek aquaculture farms and previously characterized as either sensitive or resistant were studied in sterile seawater under environmentally relevant conditions. Their behavior was evaluated in monoculture and co-culture at 12°C and 30°C, representing the minimum and maximum temperatures relevant to aquaculture environments, under both antibiotic-free and antibiotic-stress conditions. Population dynamics were monitored over time in both planktonic and biofilm phases using culture-based methods. Biofilm formation was also assessed on metal and plastic surfaces relevant to aquaculture infrastructure. In parallel, DMFit modeling was applied to describe survival and growth kinetics, including lag phase and growth rates.

In the absence of antibiotic pressure, both strains persisted in seawater, although their dynamics differed between monoculture and co-culture, indicating that microbial interactions influenced survival. Under antibiotic stress and monoculture conditions, the resistant strain remained viable, whereas the sensitive strain showed a marked reduction. However, in co-culture, the persistence of the sensitive strain was enhanced, suggesting indirect protection or interaction-mediated survival. These findings indicate that resistance in environmental settings may be shaped not only by individual strain characteristics, but also by community context.

Biofilm-associated cells exhibited greater tolerance than planktonic populations, particularly under antibiotic exposure, highlighting the role of surface-associated communities in the environmental persistence of resistance.

Overall, this study supports a more ecological view of antimicrobial resistance in aquaculture systems, showing that persistence is influenced by the combined effects of microbial interactions, biofilm formation, and environmental stress. By clarifying how resistant and sensitive populations survive and coexist under marine conditions, these findings can inform antibiotic resistance risk assessment in aquaculture environments.

S4-P59: Reliable Mutant Construction in Industrial *Listeria monocytogenes* Requires Verification of the Reference pPL2 Insertion Site

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Stable chromosomal reporter insertion is a valuable tool in food microbiology because it enables monitoring of targeted gene expression under process-relevant conditions while avoiding the copy-number bias and instability associated with multicopy plasmids. In *Listeria monocytogenes*, the PSA phage-derived pPL2 system is widely used for this purpose. It carries the PSA integrase and attachment site and mediates site-specific, single-copy insertion into the bacterial attachment site located at the 3' end of a $tRNA^{Arg}$ gene, thereby restoring a complete $tRNA^{Arg}$ sequence after recombination. This feature established pPL2 as a reference system for chromosomal insertion in laboratory strains, and later derivatives further expanded its use for constitutive tagging, promoter monitoring, and fluorescent reporter construction. Our strategy is to use such mutants to monitor stress-responsive biomarker expression in milk and to better relate dairy technological stresses to the physiology and virulence-related traits of industrial *L. monocytogenes* strains. We therefore investigated whether this approach could be transferred to two industrial strains, Lm14 and 07CEB768LM. A pPL2-based fluorescent construct was introduced by both conjugation and electroporation, but no stable chromosomal integrants were recovered.

Two possibilities were considered: either the construct failed to integrate because the target site was not available for insertion, or integration occurred but the resulting integrants were too unstable to be maintained and recovered under antibiotic selection. To explore these hypotheses, the integrity of the canonical insertion site was first assessed by PCR using the diagnostic primer pairs originally designed in the pPL2 study, targeting either the non-lysogenic bacterial configuration or the lysogenic/integrated configuration of the locus. This analysis was further supported by whole-genome sequence analysis of both strains. Together, these data indicated that the chromosomal region corresponding to the canonical PSA/pPL2 target site was not present in the expected vacant configuration and instead appeared associated with prophage-related sequences in both isolates. This point is particularly relevant because pPL2 insertion was previously reported in the reference strain LO28 despite occupation of the $tRNA^{Arg}$ site. In this context, integration most likely occurred through tandem insertion, although the resulting integrants were less stable in the absence of selection. Overall, these results show that accessibility of the reference pPL2 insertion locus is strain-dependent and support systematic verification of insertion-site integrity before engineering industrial food-associated *L. monocytogenes* strains.

S4-P60: Strain-Specific Responses of Industrial *Listeria monocytogenes* to Dairy-Relevant Acidification and Cold Technological Stresses in Milk: Impacts on Survival and Invasion Capacity

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Listeria monocytogenes remains a major concern for dairy food safety due to its ability to withstand technological stresses encountered during processing and refrigerated storage. In predictive microbiology, the impact of such stresses is mainly assessed through growth, survival and cultivability endpoints, with cultivability reflecting only the fraction of cells able to grow on culture media. However, the consequences of these stresses for virulence-related phenotypes remain less documented, particularly in industrial strains. In parallel, recent literature increasingly emphasizes that the food matrix itself can influence the behavior of *L. monocytogenes*, including its response to stress and its virulence-related traits. Altogether, this limits our understanding of how technological stresses affect the biological state of cells beyond cultivability alone.

The aim of this study was therefore to compare the responses of two industrial *L. monocytogenes* strains, Lm14 and 07CEB768LM, to dairy-relevant stress conditions, i.e. acidification and cold storage in semi-skimmed milk using cultivability and invasion readouts. Strains were exposed either to acidification at pH 4.9 for 48 h or to storage at 4°C for 7 days. Loss of cultivability resulting from stress was assessed by plate counting according to ISO 20976. Invasion capacity was evaluated in the Caco-2 cell infection model using bacteria collected before (controls) and after stress exposure.

Under the conditions tested, neither acidification nor cold storage resulted in a significant reduction in cultivability for either strain, suggesting an overall tolerance to the selected stresses when evaluated by conventional plate counts. Preliminary infection experiments in the Caco-2 cell model nevertheless suggested contrasted invasion profiles between the two strains. Lm14 showed very low invasion levels, consistent with the internalin A truncation that we have identified by whole-genome sequencing, whereas 07CEB768LM appeared more invasive. These observations indicate that similar cultivability does not necessarily reflect the same invasion-related behaviour and highlight the importance of genetic background when evaluating a strain's ability to withstand stress. In this context, complementary gene expression analyses would be particularly useful to investigate finer stress-induced changes in virulence-related responses in both strains, and especially in Lm14, for which the interpretation of Caco-2 invasion data is constrained by the altered InlA-mediated entry pathway. Overall, this study supports a strain-specific and matrix-aware view of *L. monocytogenes* behavior in dairy matrices and highlights the need to complement cultivability data in predictive microbiology models.

S4-P61: Induction of *Yersinia enterocolitica* 4/O:3 Dormancy by Lactic Acid in Decontaminated Pork

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Decontamination of pork with lactic acid is considered the last control measure to reduce the microbiological risks for consumers. *Yersinia enterocolitica* 4/O:3 is a priority hazard in pork production, but the effect of decontamination or the survivability of the bacteria is poorly understood. This is especially true for the phenomenon that bacterial cells enter the VBNC (Viable but Non-Culturable) state under unfavourable conditions. The aims of project „Induction and persistence of Viable but Non-Culturable (VBNC) *Yersinia enterocolitica* 4/O:3 in pork" – sleepYE (HRZZ-IP-2025-02-7300) are therefore: *i*) to determine the factors and their interdependence in the induction and persistence of the VBNC state of *Y. enterocolitica* 4/O:3 strains using lactic acid *in vitro*; *ii*) to determine markers (indicators) for the VBNC state of *Y. enterocolitica* 4/O:3 at the proteomic level; *iii*) to investigate the presence of *Y. enterocolitica* 4/O:3 in the VBNC state after decontamination of meat with lactic acid and *iv*) to investigate the persistence of the VBNC state of *Y. enterocolitica* 4/O:3 under altered storage conditions of pork. In acidified broth and meat, the proportion of non-viable and live and non-culturable cells of *Y. enterocolitica* 4/O:3 will be determined by molecular means (PMA-qPCR) as well as the differences in the proteome of these populations. A particular contribution of the research is also expected from the identification of the trigger for the return of dormant cells to a physiological, pathogenic state under conditions of improper storage and handling of meat. The results of this project can make an important contribution to scientific knowledge in the field of food technology, meat hygiene and epidemiology and provide guidelines for hidden risks (VBNC) management in apparently safe meat.

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S4-P62: Survival of STEC During Cheese Ripening: Systematic Review and Meta-Analysis

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Shiga toxin-producing *Escherichia coli* (STEC) are important foodborne pathogens that can cause severe disease, especially in vulnerable populations. Raw milk and dairy products are recognised sources of infection. Although ripening conditions may reduce bacterial counts, STEC have been isolated even from long-aged cheeses. This systematic review collected the available evidence on the effect of cheese ripening on STEC reduction with the aim of answering the following question: 'What is the effect of ripening on the reduction of STEC along the cheese production chain according to experimental studies?' Articles were searched for in the PUBMED and EMBASE databases. The search strategy combined three groups of terms: one relating to the pathogen under investigation, one relating to the type of products of interest, and one relating to the outcome. After study selection data on STEC counts during the ripening of cheeses produced from contaminated milk were collected. The data extracted included product type, study characteristics, serotypes used, virulotype, and count. The methodological quality of the studies was assessed. In order to estimate the effect of ripening, the study results were synthesised via a meta-analysis using regression coefficients obtained from within study linear regression model, as the effect size. In this model, bacterial count was considered the dependent variable and time (days) the independent variable. The meta-analysis was conducted utilising the R metafor package. The literature search identified 4,491 records. 285 were selected based on their title and abstract, whilst following full-text screening, 19 articles (67 studies) were included. Of these, in 57 studies, the STEC count decreased over time with regression coefficients ranging from –0.15 to –0.001 $\log_{10}$ CFU/g/day. The pooled effect size was –0.037 [–0.053; –0.021], thus indicating a reduction of 3.7 $\log_{10}$ CFU/g every 100 days (1 $\log_{10}$ every 27 days). However, the heterogeneity of the studies considered and the presence of studies showing a non-linear trend in bacterial decline over time suggest the need for further investigation. In conclusion, cheese ripening could be one of the factors along the cheese production chain, able to reduce STEC counts. However, due to the extreme variability of cheese making process, raw milk based production systems should be validated also against this risk. Pending the results, these systems could benefit from enhanced analytical monitoring and adequate, comprehensive information for consumers regarding the risks of consuming unpasteurised milk products. This information should be aimed in particular at the most vulnerable sections of the population.

S4-P63: Investigation of Phage EP33 for Control of AMR Dissemination in *Enterococcus faecium*

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Enterococcus spp. is an opportunistic, foodborne pathogen that resides in the intestines of humans and animals, and due to its ubiquity it is often used to study antimicrobial resistance (AMR) transmission. Notably, this bacterium is known for rapid, efficient dissemination of antimicrobial resistance genes (ARGs) via a plasmid-encoded pilus. We hypothesized a novel strategy to block the dissemination of ARGs by using plasmid-specific bacteriophages, viruses of bacteria.

An eight-strain cocktail comprising *Enterococcus faecium* and *Enterococcus faecalis* was used to probe wastewater samples for *Enterococcus*-infecting phages. Host range analysis using the host agar overlay technique was conducted to determine infection patterns of the phages against a panel of *Enterococcus* spp. (n = 32). To identify phages relying on the pilus for infection, *Enterococcus* strains were cured of their plasmid(s) using a novobiocin microdilution assay with subsequent PCR verification. Purified phage lysates were sequentially spotted onto agar overlays of the plasmid-cured and wild-type *Enterococcus* hosts to determine plasmid/pilus reliance. These results were verified in subsequent spectrophotometric phage inhibition analyses which were conducted in $CaCl_2$-supplemented tryptic soy broth over 48 h. Phage-antibiotic synergy (PAS) was evaluated spectrophotometrically to determine synergistic effects between phage and tetracycline on host inactivation. Lastly, phage DNA was extracted with an in-house phenol-chloroform protocol and sequenced on the Illumina MiSeq. Sequence analysis was accomplished with an in-house bioinformatics pipeline.

In total, 20 phages were isolated and showed diverse host ranges. Agar overlay spot tests revealed that EP33 could not infect the plasmid-cured derivative of *E. faecium* Ent-0189, of which the wild-type contained a conjugative plasmid encoding tetracycline resistance and pilus formation. We observed significantly diminished inhibition ($p < 0.05$; Student's t-test) of plasmid-cured Ent-0189 compared to the WT, suggesting that the plasmid-encoded pilus serves as the receptor site. Interestingly, PAS was also observed at 0.5 × MIC of tetracycline (64 µg/mL) combined with varying concentrations of phage ($p < 0.05$; two-way ANOVA). DNA sequencing revealed that EP33 possesses a 34 kb genome with 37% GC and no AMR genes. Our data suggest that because EP33 relies on the plasmid-encoded pilus for attachment, it can potentially limit or block AMR dissemination to other bacteria, thus diminishing the potential for AMR spread. EP33 may also be co-administered with sub-MIC levels of tetracycline (to which the host is innately resistant) for enhanced inactivation, concomitantly preserving the lethality and utility of antimicrobials. Together, our data support unique phage-based approaches to address the global threat of AMR.

S4-P64: Insights into *Listeria monocytogenes* Stress Resistance: Molecular and Phenotypic Characterization of Strains Isolated from a Smoked Salmon Processing Plant

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Listeria monocytogenes (Lm) is a foodborne pathogen that poses a serious risk to food safety, as it can persist in food processing environments (FPEs). This ability may be linked to the acquisition of resistance genes. One example is stress survival islet-1 (SSI-1), a five-gene (*lmo0444*, *lmo0445*, *lmo0446*, *lmo0447*, *lmo0448*) segment known to enhance tolerance to acidic conditions and promote biofilm formation. This study aimed (*i*) to assess the distribution of SSI-1 among 49 Lm strains previously isolated from FPEs and (*ii*) to investigate their acid adaptation capacity and biofilm forming ability. The 49 Lm strains were previously characterized by multilocus sequence typing (MLST) and belonged to different sequence types (STs): ST5 (*n* = 12), ST6 (*n* = 4), ST7 (*n* = 3), ST9 (*n* = 27), ST14 (*n* = 1), and ST155 (*n* = 2). Each gene of SSI-1 islet was assessed by PCR according to Ryan et al., (2010), and sequenced by Sanger. Acid adaptation was evaluated by exposing bacterial cultures to mild acid conditions (pH 5) for 1 h, followed by exposure to more severe acid stress (pH 3) for 30 min (Koutsoumanis et al. 2019). Finally, total viable cells were estimated by plate count. In parallel, biofilm formation was assessed using the microtiter plate assay after 24 h of incubation at 37°C and quantified using the crystal violet method (Stepanović et al., 2007).

The complete SSI-1 segment was detected in 83.7% of strains (41/49), while the remaining 14.3% carried a truncated islet. Following the acid exposure, only 30.6% of candidate strains (15/49) exhibited survival capacity and were classified as acid adapted (AA). Regarding biofilm formation, the majority of strains (57.1%; 28/49) were classified as weak producers, whereas 42.9% (21/49) were moderate producers.

The wide distribution of SSI-1 islet among analyzed strains was expected, except for strains belonging to ST6 which are generally SSI-1 negative (Ryan et al., 2010). The presence of a truncated variant of the SSI-1 is noteworthy and warrants further investigation. Results from genotypic and phenotypic analyses performed in this study showed no association with specific STs, suggesting that these features could be strain dependent.

Future whole genome sequencing (WGS) will allow us to investigate more in depth the truncated form of the SSI-1 and the presence of additional stress resistance determinants. Moreover, transcriptomic analyses will be useful to better understand the mechanism related to *L. monocytogenes* adaptation and persistence in FPEs.

S4-P65: Emetic *Bacillus cereus* – Risks of Outgrowth and Cereulide Production in Dairy Alternative Drinks and Yogurts

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The emetic toxin cereulide, produced by *Bacillus cereus*, is highly stable and resistant to acid and heat, including UHT. As a result, once produced, cereulide can persist in food products even after inactivation or removal of the producing organism. In a previous study, we identified *B. cereus* as a common contaminant in ingredients used for plant-based dairy alternatives, with 13% of isolates carrying the *ces* gene encoding the cereulide synthethase.

The objective of the current study was to assess the growth and cereulide production of *ces*-positive strains in plant-based drinks stored at different temperatures, and to benchmark the findings against existing literature. Additionally, the growth of *ces*-positive strains was evaluated in plant-based yogurt during the fermentation process under both rapid and slow acidification conditions.

The growth and cereulide production of a *ces*-postive *B. cereus* strains were evaluated in coconut water and oat/pea drink, with semi-skimmed milk as a reference matrix. Samples were incubated at 12°C and at 30°C. In addition, growth was assessed during the fermentation of fava- and amaranth-based yogurt alternatives under fast and slow acidification conditions, achieved by using high (10^6 CFU/mL) and low (10^4 CFU/mL) inoculation levels of the starter culture, alongside a non-inoculated control. Cereulide concentrations were quantified using LC/MS-MS analysis.

All tested drinks supported growth and cereulide formation by the *ces*-positive strain, with significantly lower toxin levels at 12°C than at 30°C. At 12°C, the highest cereulide concentrations were found in the oat/pea drink (37 µg/L), whereas at 30°C the concentrations of highest levels were detected in coconut water (94 µg/L).

During yogurt fermentation, *B. cereus* growth in products with the slow acidifying cultures was similar to the product without starter, reaching approximately 10^7 CFU/mL within 10 h with cereulide levels up to 81 µg/kg. Under fast acidification, *B. cereus* reached approximately 10^5 CFU/mL after 10 h, followed by a decline in viable numbers, and no cereulide was detected.

Outgrowth of *B. cereus* and cereulide production in plant-based drinks may occur when time–temperature conditions prior to UHT processing permit growth, or following post-process contamination with this organism. In yogurt alternatives, although the final pH inhibits growth, delayed acidification during fermentation may allow *B. cereus* growth and cereulide production. These results demonstrate the potential risks related to cereulide-producing strains in plant-based dairy alternatives and emphasize the need for effective microbial control throughout production to prevent growth and toxin formation.

S4-P66: Presence of *Listeria monocytogenes* in Ready-to-Eat Meat Products in Portugal

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Listeria monocytogenes remains a major food safety concern due to its ability to persist in food-processing environments and its association with ready-to-eat (RTE) foods. Listeriosis poses a serious risk to vulnerable populations, including pregnant women, infants and the elderly, highlighting the need for continuous surveillance of RTE products to ensure hygienic and safe production conditions. This study evaluated Portuguese RTE meat products with respect to the presence and enumeration of *L. monocytogenes*, together with an assessment of overall microbiological quality.

A total of 92 RTE meat products were collected from supermarkets and analysed for the occurrence and levels of *L. monocytogenes*, as well total aerobic mesophilic counts, lactic acid bacteria, *Enterobacteriaceae*, *Escherichia coli* and coagulase-positive staphylococci. All analyses were conducted according to relevant ISO standards, and results were interpreted using guidelines from the Instituto Nacional de Saúde Doutor Ricardo Jorge.

L. monocytogenes was detected in seven of the 92 products analysed (7.6%), namely turkey bacon, paio de cachaço, alheira de Mirandela and smoked ham in cubes (counts < 100 CFU/g). Of the positive samples, two were unpackaged products sliced in the store (turkey bacon and paio de cachaço) and five were packaged. Only one positive product (alheira de Mirandela) was whole, while the remaining six were sliced. Notably, four of the seven positive samples were smoked ham in cubes, all from the same producer and from four different batches. To investigate whether contamination extended to other products from the same producer, other products were analysed; however, *L. monocytogenes* was not detected in these samples. This pattern suggests contamination at the production line level, most likely associated with cutting equipment. Packaging status did not appear to significantly influence contamination, as both packaged and unpackaged products tested positive. In contrast, general microbiological quality varied considerably between products, particularly in total aerobic mesophilic counts, reflecting differences in handling and processing practices. Lactic acid bacteria were frequently detected in high numbers, especially in fermented products. *E. coli* levels were generally low, although one sample (onion blood sausage) exceeded reference limits (> 100 CFU/g), indicating potential hygiene shortcomings. Counts of coagulase-positive staphylococci were mostly within satisfactory levels, although 12 samples were classified as “Unacceptable” (102– ≤104 CFU/g) and 10 as “Unacceptable/potentially hazardous” (> 104 CFU/g).

Overall, these findings underline the importance of continuous monitoring and targeted hygiene interventions, particularly after processing steps such as slicing, to ensure the microbiological safety of RTE meat products.

S4-P67: Microbiota Matters: Microbial Signature Associated with *Campylobacter* Survival on Broiler Meat During Refrigerated Storage

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Campylobacter is the leading cause of foodborne gastroenteritis in Europe, with poultry recognized as its primary reservoir. Although the physiological responses of *Campylobacter* to abiotic stresses such as cold and oxidative conditions have been extensively described, these mechanisms alone do not fully explain its persistence on broiler meat during storage. This suggests that interactions with the surrounding meat microbiota may play a role. The objective of this study was therefore to investigate whether *Campylobacter* survival during refrigerated storage is associated with specific microbial community taxa.

A total of 310 pooled rinsates from free-range broiler carcasses were collected after chilling (D0) and after 7 days of refrigerated storage (D7). *Campylobacter* levels were quantified using culture-based methods, while microbiota composition was characterized by 16S rRNA gene sequencing (V3-V4 regions). To explore the relationship between microbial communities and *Campylobacter* survival, samples were ranked based on the survival ratio of *Campylobacter*, defined as concentrations (CFU/mL) measured before and after storage in samples from the same broiler carcass. The 25% highest and lowest subsets were then selected. Sparse partial least squares discriminant analysis (sPLS-DA) was then applied to identify bacterial taxa discriminating between high- and low-survival groups after storage. In addition, Poisson log-normal (PLN) network inference was used to investigate co-occurrence patterns between Campylobacter and other members of the microbiota.

Refrigerated storage resulted in a significant reduction of *Campylobacter* levels, decreasing from 1.28 to 0.78 $\log_{10}$ CFU/mL, and a decrease in overall species richness. At the same time, it favored the growth of psychrotrophic aerobic bacteria typically associated with meat spoilage. After storage, the low-survival group showed a mean *Campylobacter* concentration of 0.38 $\log_{10}$ CFU/mL, whereas the high-survival group reached a mean concentration of 1.18 $\log_{10}$ CFU/mL. The low- and high-*Campylobacter* survival groups were clearly separated based on their microbial community composition, with 10 discriminant genera driving this differentiation, including Amplicon Sequence Variants (ASV) associated with either low or high survival levels.

Network analysis focusing on the extreme subsets revealed contrasted association patterns. *Campylobacter* showed positive associations with 25 ASVs, including taxa affiliated with *Pseudomonas*, *Acinetobacter* and *Flavobacterium*, and negative associations with 17 ASVs, notably affiliated with *Brochothrix* and *Myroides*.

Overall, these findings suggest that *Campylobacter* persistence on broiler carcasses is linked to specific microbiota structures and interactions. This work provides new insights into the ecological determinants of *Campylobacter* survival and highlights the potential of microbiota-targeted strategies to reduce contamination and improve poultry product safety.

S4-P68: Nanoemulsified Essential Oils for the Control of Foodborne Pathogens and Biofilm in Cheese

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Biofilm-associated contamination by foodborne pathogens remains a major challenge for the dairy industry, particularly during cheese ripening, where persistent cells may survive on product surfaces and compromise food safety. In this study, nanoemulsions based on *Coridothymus capitatus*, *Thymus vulgaris*, and *Origanum vulgare* subsp. *hirtum* essential oils were characterized and investigated for their antimicrobial and antibiofilm activity against relevant foodborne bacteria, including *Escherichia coli*, *Salmonella enterica* serovar Typhimurium, *Staphylococcus aureus*, *Enterococcus faecium*, and *Listeria monocytogenes*. Their efficacy was also assessed in combination with nisin and validated in a Caciotta cheese challenge test.

The volatile composition of the oils was characterized by GC×GC-MS, revealing phenolic monoterpenes as the dominant compounds. The main compounds associated with known antimicrobial activity were carvacrol, predominantly present in *C. capitatus* and *O. vulgare* subsp. *hirtum*, and thymol, p-cymene, and γ-terpinene in *T. vulgaris*. Nanoemulsions showed mean particle sizes ranging from approximately 160 to 364 nm and negative zeta potential values, indicating good colloidal stability, although nisin addition significantly reduced the absolute zeta potential.

Minimum inhibitory concentration assays showed that essential oil nanoemulsions exerted broad antimicrobial activity against all tested strains. *C. capitatus* nanoemulsions generally exhibited the strongest activity. At sublethal concentrations (0.1 mg/mL), nanoemulsions with all essential oils significantly reduced biofilm formation *in vitro*. *C. capitatus* and *T. vulgaris* were the most effective, whereas *O. vulgare* subsp. *hirtum* showed a weaker but still significant antibiofilm effect. In most cases, the addition of nisin further enhanced biofilm inhibition.

The practical applicability of these formulations was demonstrated in Caciotta cheese artificially contaminated with 4.5 log CFU/cm² on the surface and ripened for 22 days at 8°C and 65% relative humidity. Surface treatments with nanoemulsions containing *C. capitatus* and *T. vulgaris* supplemented with nisin significantly reduced viable counts of all tested pathogens, with reductions generally ranging from about 2 to more than 3 log CFU/g. *O. vulgare* subsp. *hirtum* was less effective overall, although it still significantly reduced several target strains, including *L. monocytogenes*.

These results indicate that essential oil nanoemulsions, particularly those based on *C. capitatus* and *T. vulgaris* combined with nisin, represent promising natural strategies to control foodborne pathogens and biofilms in cheese production. Further studies will focus on formulation optimization and the evaluation of sensory properties and consumer acceptability.

S4-P69: Mapping the Presence and Relationship of Foodborne Pathogens in Horticultural Production Settings

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Due to the nature of their production horticultural crops are at risk of microbiological contamination from a range of sources. It is therefore imperative that growers are aware of where risks may arise on farm so that targeted interventions can be put in place to ensure produce safety by reducing the chance of transference of pathogens onto crops. The objective of this study was to undertake a mapping exercise to identify potential sources of contamination and the areas most likely to harbour foodborne pathogens in commercial horticultural settings in Ireland.

Produce, water and environmental swab samples were collected from commercial production units for four crops with differing risk profiles, namely strawberries, carrots, spinach and lettuce, on four different occasions. Each sample was tested for the presence of *Listeria monocytogenes*, *Salmonella* spp. and Shiga toxin producing *Escherichia coli* (STEC) by standard methodologies. In total 765 samples were collected, including 585 environmental samples, 119 water samples and 61 produce samples. No *Salmonella* was detected in any sample. Shiga toxin genes were detected in 8 samples but it was not possible however to recover any STEC from these samples by cultural isolation. A total of 22 unique *L. monocytogenes* isolates were identified from 15 samples across seven sampling sites, encompassing all target crop production settings and all sample types. Whole genome sequencing was utilised to compare the *L. monocytogenes* isolates, as well as to provide information on their serotype and virulence gene properties.

This study indicated that the horticultural production and processing environment can harbour *L. monocytogenes*, particularly on surfaces, and therefore particular emphasis should be placed on rigorous cleaning and disinfection and minimising cross contamination of the crop.

S4-P70: Seafood Safety at Risk: Unveiling the Temperature-Dependent Resistance of *Listeria monocytogenes* Biofilms

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Foodborne pathogens pose a significant public health risk, particularly because of their persistence in food-processing environments, often linked to biofilm formation and reduced susceptibility to antimicrobial treatments. Among them, *Listeria monocytogenes* is of particular concern due to its frequent occurrence in ready-to-eat and seafood products, as well as its ability to survive under a wide range of conditions. This study investigated the minimum inhibitory concentrations (MICs) of two selected antimicrobials (ampicillin and erythromycin) against *L. monocytogenes* strains with weak- and moderate-biofilm-forming ability under conditions simulating aquatic and seafood-associated environments.

A total of 97 *L. monocytogenes* isolates of food origin were evaluated. Biofilm formation was assessed using polystyrene microtiter plates at 12°C and 30°C, representing low and high temperatures relevant to aquatic environments. Brain heart infusion (BHI), as a nutrient-rich laboratory medium, and fish feed infusion (FFI) as a seawater-based medium, were used. Antimicrobial susceptibility to ampicillin and erythromycin was determined by MIC assays in 29 isolates, and results were interpreted according to EUCAST guidelines. Most isolates formed detectable but generally weak biofilms in BHI at both temperatures. However, a subset shifted towards moderate biofilm formation after prolonged incubation at 12°C, suggesting adaptation to cold conditions. No strong biofilm producers were identified. In contrast, biofilm development was markedly reduced in FFI, particularly at 12°C, where no measurable formation was observed. At 30°C, only limited weak biofilm formation occurred in this medium, highlighting the combined influence of temperature and nutrient availability.

Results of MIC of the studied antibiotics against 29 isolates indicate that most of them remained susceptible to ampicillin and erythromycin at both temperatures (8 and 23 isolates, respectively). Ampicillin MIC values were consistently low, while erythromycin MIC values remained effective, with only a few cases showing higher MIC values at 30°C.

Overall, this study shows that *L. monocytogenes* isolates predominantly exhibit weak to moderate biofilm formation and remain susceptible to two key antibiotics. These findings support the need for efficient cleaning and disinfection strategies and for incorporating such parameters into risk assessment models to better mitigate the transmission of potentially resistant *L. monocytogenes* through the food supply chain.

S4-P71: Effect of Mild Heat Treatment on Lag-Time and Botulinum Neurotoxin Production of *Clostridium botulinum* Group II Strain Cocktail During Cold Storage

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Heat treatment is a central means to control the food safety risk caused by *Clostridium botulinum* Group II and other botulinum neurotoxin (BoNT)-producing clostridia in vacuum−packaged, minimally processed chilled foods with extended shelf life. A reference treatment of 10 min at 90°C is generally considered sufficient to ensure their safety. To reduce the detrimental impact of this heat treatment on food sensory and nutritional quality and the high energy costs, application of less stringent heat treatments in combination with intrinsic factors applied as hurdles to control *C. botulinum* growth is desired. A prerequisite, however, is a rigorous scientific assessment of the treatment's effectiveness.

We tested *C. botulinum* Group II spores for their ability to grow into toxinogenic cultures after a mild heat treatment in an array of different pH and NaCl conditions during cold storage. A spore cocktail of eight *C. botulinum* Group II strains producing BoNT types B, E, and F was exposed to a mild heat treatment and inoculated into the recovery medium, and growth was recorded as a change of optical density during storage at 6 and 10°C for 90 days. BoNT production was determined at the end of the experiment using ELISA.

In the unheated controls, no growth of *C. botulinum* Group II was detected in media with > 3% NaCl (aw < 0.97) or a pH < 5.7 within 90 days of incubation at 6°C. Incubation at 10°C slightly extended the window of growth and distinctly reduced lag-times as compared to 6°C. Mild heat treatment had little impact on these growth boundaries but caused increased lag-times of the cultures. In heat-treated cultures we detected primarily BoNT/B, whereas unheated cultures additionally produced BoNT/E at 6°C and BoNT/E and F at 10°C. Interestingly, during storage at 6°C, we were able to detect BoNT production without significant increase in optical density in some of the cultures.

This work showed that mild heat treatment delayed the growth of *C. botulinum* Group II at chill temperature, however, without major impact on growth boundaries. The applied heat treatment was selective for strains producing BoNT/B, highlighting the importance of considering strain-dependent differences in heat resistance when selecting strains for challenge studies. The detection of BoNT without clear signs of growth in some cultures emphasizes the crucial role of BoNT monitoring when assessing the safety of heat-treated vacuum−packaged, minimally processed chilled foods.

S4-P72: Antimicrobial Resistance in Non-*aureus* Staphylococci and *Mammaliicoccus* sp. Isolated from Dairy Farms in Brazil

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Non-*aureus* staphylococci (NAS) are important opportunistic pathogens and emerging reservoirs of antimicrobial resistance (AMR) in dairy production systems. This study aimed to identify NAS and *Mammaliicoccus* species from dairy farms and characterize their antimicrobial susceptibility profiles. A total of sixty-three NAS and *Mammaliicoccus* sp. isolates, obtained from cow milk, bulk tank raw milk, and farm environment samples collected from two dairy herds in Brazil, were submitted to MALDI-TOF MS for species identification. Among the tested isolates, 5 were *Staphylococcus chromogenes*, 16 *Staphylococcus epidermidis*, 16 *Staphylococcus haemolyticus*, 8 *Staphylococcus hyicus*, 1 *Staphylococcus lugdunensis*, 1 *Mammaliicoccus sciuri* (*Staphylococcus sciuri*), 8 *Staphylococcus saprophyticus*, 3 *Staphylococcus warneri*, and 5 *Staphylococcus xylosus*. Antimicrobial susceptibility was assessed using disk diffusion tests against drugs commonly used in veterinary and human medicine. All isolates were susceptible to sulfamethoxazole-trimethoprim. However, three isolates (*S. xylosus*, *S. saprophyticus*, and *S. haemolyticus*) were resistant to vancomycin, and five isolates (one each of *S. haemolyticus*, *S. lugdunensis*, *S. chromogenes* and two *S. epidermidis*) were resistant to tobramycin. One *S. chromogenes* and one *S. epidermidis* isolates were resistant to chloramphenicol and linezolid, respectively. Two isolates (*S. epidermidis* and *S. xylosus*) were resistant to cefoxitin, and two *S. epidermidis* isolates were resistant to clindamycin. A high frequency of resistance was observed to erythromycin (22/63 isolates, including 12 *S. epidermidis*, 4 *S. haemolyticus*, 2 *S. warneri*, 1 *S. hyicus*, 1 *S. lugdunensis*, 1 *S. xylosus*, and 1 *S. chromogenes*) and penicillin (21/63 isolates, mainly *S. epidermidis*, *S. hyicus*, *S. haemolyticus*, *S. saprophyticus*, *S. warneri*, and *S. chromogenes*). Seven isolates were resistant to gentamicin (one each of *S. epidermidis*, *S. saprophyticus*, *S. haemolyticus*, *S. hyicus*, *S. lugdunensis*, and *S. chromogenes*), and three were resistant to ciprofloxacin (*S. warneri*, *S. chromogenes*, and *S. epidermidis*). Two isolates (*M. sciuri* and *S. warneri*) were resistant to tetracycline. Overall, nine among 63 isolates were classified as multidrug resistant (MDR), including three S*. haemolyticus*, two *S. epidermidis*, and one each of *S. lugdunensis*, *S. warneri*, *S. chromogenes*, and *S. hyicus*. These findings show that NAS and *Mammaliicoccus* sp. from milk and farm environments in Brazilian dairy herds harbor resistance to several classes of antimicrobials that are clinically relevant. Therefore, NAS and *Mammailicoccus* sp. may act as reservoirs and potential disseminators of AMR within the dairy chain, reinforcing the need for improved antimicrobial stewardship and integrated One Health surveillance in food animal production.

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S4-P73: *Listeria monocytogenes* Strain Variability and Its Impact on Sensitivity to UVC Technologies

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Listeria monocytogenes is a significant challenge for food producers globally, particularly those producing 'Ready-To-Eat' (RTE) foods. *L. monocytogenes* strains have previously been demonstrated to be genetically diverse and exhibit phenotypically distinct responses to stress. UVC radiation technologies using traditional low-pressure mercury lamps are approved for use in surface decontamination and the processing of some foods. This study assessed the range of sensitivity of a strain collection of *L. monocytogenes* isolates ($n = 173$) from diverse sources in Ireland to UVC radiation using emerging UVC technologies, UVC-LED (265 nm) and Far-UVC (222 nm). These lamps are of interest as an alternative to mercury-containing lamps due to a global push to reduce mercury levels. Strains were individually cultured in TSB to stationary phase, inoculated at approximately 6 log CFU/mL in PBS and treated with UVC-LED or Far-UVC. UV doses for each lamp were independently selected, targeting 2–4 log reductions. Treated cells were enumerated and log reductions were calculated from three biological replicates per strain-lamp combination. Results revealed significant intra-species differences in UVC sensitivity with both lamps. The mean log reduction across the collection was approximately 2 log CFU/mL, however individual strains ranged from <1 to >3 log reductions. Sensitivities clustered by lineage, clonal complex and isolate source. Lineage II and clonal complex 121 in particular were amongst the most sensitive at both wavelengths. Several pairs of isolates that share the same sequence type displayed clear phenotypic heterogeneity. Whole‑genome sequence comparisons of these strain pairs identified mutations with potential impact. This phenotypic and genomic evidence highlights the need to consider strain‑level variability when implementing UVC‑based control measures for *L. monocytogenes* and emphasizes the importance of using representative isolates when evaluating these technologies.

S4-P74: Characterization of Microbiological Quality Focusing on Spore-forming Bacteria Persisting in Industrially Processed Fish Silage

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Efficient valorization of marine side streams is essential to increase circularity in aquaculture. Fish silage is a nutrient-rich byproduct, but the current EU animal by-product (ABP) regulation restricts the use of ABP Category 2 materials to low-value uses such as biogas and fertilizer. Improved exploitation of the nutrients fish silage is of interest, but requires thorough assessment of microbial risks, including *Clostridium botulinum* which occurs naturally in marine environments and fish. Current processing standards (acidification to pH < 4 for 24 h and heating at 85°C for 25 min) are designed to eliminate pathogens however, spore-forming bacteria may persist.

This study aimed to characterize the microbial community in fish silage to evaluate the overall hygienic status and identify potential pathogens surviving the fish silage processing method.

The level of hygiene indicator microorganisms, foodborne pathogens incl. *Salmonella* and spore-forming bacteria was enumerated in two batches of fish silage at two different time points during storage. The spore-formers were identified using MALDI TOF MS, and selected isolates (*n* = 9) underwent whole-genome sequencing (WGS).

Across all samples, *Staphylococcus aureus*, *Enterococci*, *Enterobacteriaceae*, and *Escherichia coli* were below detection limits (< 1 log CFU/g), as were yeasts and molds (< 2 log CFU/g), and no *Salmonella* was detected. Spore-forming bacteria dominated the microbial population with 3.5–3.9 log CFU/g of aerobic spores consisting primarily of *Bacillus*, *Virgibacillus*, and *Oceanobacillus* (*n* = 120). Sulfite-reducing anaerobic spores (1.1–1.8 log CFU/g) were dominated by *Terrisporobacter*, *Paraclostridium*, and *Clostridium*, accounting for 82–90% of the identified isolates (*n* = 82).

WGS identified isolates with high identity to *C. botulinum* E3 Alaska (98.87–98.96% average nucleotide identity [ANI]), *Clostridium argentinense* strain 89G (97.73–97.82% ANI), *Clostridium aquiflavi* (98.83–98.84% ANI), and *Clostridium cagae* (99.23% ANI). *C. botulinum* and *C. argentinense* isolates were confirmed non-toxigenic by in silico PCR and BLASTn, with absence of the toxin gene cluster (*bont/E*, *bont/G*, and the conserved *ntnh* marker).

The overall microbiological quality of fish silage was dominated by resilient spore-formers. Potentially pathogenic *Clostridium* species persisted despite stringent processing (pH < 4, 85°C), suggesting that standard inactivation methods – often validated using *C. sporogenes* as a surrogate – may not fully capture the intra-species stress resistance within the genus. An on-going study is currently investigating the heat resistance of different *C. botulinum* types.

S4-P75: Effect of a Powered Product Obtained from Blueberry By-Products to Reduce *Listeria* spp. Isolated from the Poultry Food Chain

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The blueberry juice industry is adopting sustainable by-product management in response to consumer demand for healthier and clean-label products. Blueberry (*Vaccinium myrtillus*) by-products have been found to contain high concentrations of phenolic compounds, which are of particular significance due to their antimicrobial and antioxidant properties. The objective of this study is to evaluate the antimicrobial efficacy of a blueberry product against *Listeria* spp..

The minimum inhibitory concentration (MIC) was studied using the broth macrodilution method (4–0.5%) against 3 reference strains of *Listeria monocytogenes*, 5 strains of *L. monocytogenes* isolated from the poultry food chain and one strain of *Listeria innocua*. For that, the blueberry product was obtained through a patented process (ES2424870-2B) and subjected to a heat-treated at 90°C during 90 min to achieve a microbiologically stable product. For each of the strains, the effect of the pH modification (5.3) produced by the blueberry product at 3% was studied, and the results were compared with a positive control after 24 and 48 h of incubation at 37°C and 200 rpm.

The results indicated that for all strains, the MIC was 4%, albeit variable results were observed at 3% between the strains. *L. innocua* showed the highest resistance to the product, with 1.6 log CFU/mL reduction after 48 h of incubation compared with the initial inoculum (6.5 CFU/mL). Three *L. monocytogenes* strains were classified as more resistant to the blueberry product (3 log CFU/mL reduction after 24 h), 2 as intermediate resistant (4 log CFU/mL reduction after 24 and 48 h), and the last 4 strains were the more sensitive, reducing its concentration below the quantification limit (1 log CFU/mL) after 48 h.

The results obtained from the pH control revealed that the strains exhibited divergent responses to the pH reduction caused by the addition of the product, but it can be broadly stated that the bactericidal effect of 4 and 3% was attributable to the phenolic compounds present in the blueberry product.

The results presented in this study offer a promising outlook for the extraction of natural antimicrobials, the promotion of a circular economy within food companies, and the enhancement of food safety in chicken-based products. In this regard, the present study is also significant for the selection of strains with different resistances, which can be used to design a cocktail that can be employed in challenge tests in commercial chicken-based products.

S4-P76: Valorization of Ribera del Duero Wine-Making By-Products as a Natural Antimicrobial Against *Campylobacter jejuni*

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As part of the overall objective of promoting a circular economy and enhancing sustainability, the project aims to explore the well-documented antimicrobial activity of wine-making by-product to produce natural disinfectants that improve food safety within the poultry production chain by eliminating *Campylobacter jejuni* present on food-contact surfaces.

The antimicrobial activity of an extract derived from wine-making by-products from four wineries in three distinct regions of the Ribera del Duero has been examined. The extract was obtained by solid-liquid extraction at a ratio of 1:10, using a mixture of equal parts ethanol and water as the extractant. The extraction process was enhanced by ultrasound under the following conditions: an amplitude of 40%, a temperature of 35°C and a duration of 20 min. The extracts were then evaporated to dryness and the same volume of Mueller Hinton broth (MHB) was added to dissolve them. The minimum inhibitory concentration (MIC) was determined using the microdilution method in MHB, over a 24 h period of incubation under microaerophilic conditions at 37°C against four clinical strains of *C. jejuni*, three strains isolated from a poultry slaughterhouse and the reference strain NCTC11168.

The findings revealed that not all the extracts (from the 4 wineries) exhibited an anti-*Campylobacter* effect, and that there was variable resistance among the strains examined. Despite the grape variety was Tempranillo in all three areas, only the two wineries in the eastern part of the Ribera del Duero region exhibited an antimicrobial effect at lower concentrations. Of these, winery AN exhibited a MIC of 12.5% for a clinical strain, 25% for two slaughterhouse strains, 50% for one clinical strain and the remaining two slaughterhouse strains. While pure extract was required for one clinical strain and the reference strain. In the case of the other winery in the eastern zone (winery DA), a MIC below that of the pure extract (50%) was only observed for one slaughterhouse strain. In both wineries, 75% inhibition was observed at a concentration below the MIC obtained for each strain. Concentrating the extract 5 times derived in reducing the MIC of the resistant strains to 25% for the winery AN and in two strains till 12.5% in the case of winery DA.

These results are preliminary, but are promising for the application of these extracts as natural disinfectants in working environments where *C. jejuni* represents a greater risk to food safety.

S4-P77: Genotype-Phenotype Characterisation Identifies *eslB* Mutations as Drivers of Impaired Biofilm Formation in *Listeria monocytogenes*

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The foodborne pathogen *Listeria monocytogenes* poses a significant concern for food industry stakeholders due to its ability to persist in food processing environments despite stringent hygiene and control measures. The pathogen's remarkable stress tolerance and ability to form biofilms enable it to withstand fluctuating environmental conditions and ultimately reach consumers. Specifically, the ability to form and integrate into biofilms, structured bacterial communities of autoaggregated cells embedded in a self-produced matrix, plays a key role in *L. monocytogenes*' persistence along the food chain. In this study, we examined 25 *L. monocytogenes* strains isolated from clinical cases, food, and food processing environments in Galicia (Northwest Spain). The whole-genome sequences were analysed, and strains were phenotypically characterised for biofilm-forming ability using the crystal violet assay. The phenotype was further characterised by the Congo Red Agar assay and lysozyme tolerance. The phenotypic characterisation revealed notable differences in biofilm formation among three closely related strains isolated from the same location. A SNP (single-nucleotide polymorphism) analysis revealed that one strain carried a defective *eslB* (*lmo2768*), a gene previously associated with lysozyme resistance, cell wall integrity and virulence in *L. monocytogenes*. Functional validation was conducted through gene disruption via plasmid insertion. The *eslB*::pMAD mutants showed impaired biofilm formation on crystal violet and decreased tolerance to lysozyme compared to the wild-type strain. Additionally, confocal laser scanning microscopy revealed that biofilms formed by the *eslB*::pMAD mutants and the wild-type strain with defective *eslB* on stainless steel surfaces exhibited lower biomass and surface area compared to strains with functional *eslB*. This study provides the first evidence linking *eslB* (*lmo2768*) to the biofilm−forming ability of *L. monocytogenes*. Understanding the genetic determinants contributing to persistence in food processing environments enhances our capacity to design more effective control strategies targeting biofilm formation.

S4-P78: Reliable Thermal Process Parameters for Plant−Based Meat Analogues: Insights from *Bacillus cereus* Spore Inactivation Kinetic Studies

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Thermal processing is critical in the food industry to ensure microbial safety and product stability. However, data on bacterial spore inactivation in plant-based solid matrices remain scarce, limiting accurate prediction under industrial processing conditions. The objective of this study was to improve the interpretation of spore inactivation kinetics in plant-based matrices (such as those used in the production of meat analogues) and to provide a practical approach for selecting an appropriate inactivation model to assess inactivation during cooking or extrusion. Crucibles (100 µL volume) for differential scanning calorimetry (DSC) analysis were filled with a soy-protein formulation, inoculated with *Bacillus cereus* spores. Crucibles served as miniaturized containers for measuring spore inactivation and were submerged in an oil bath at 90, 100, 110 and 120°C. After heating, samples were recovered and microbial counts were determined by plating on TSAYE. The two-step model-fitting approach was applied, testing log-linear (estimating log D), log-linear with shoulder (log D and Sl) and Weibull (δ and p) models for each temperature. The secondary model included the classical log-linear Bigelow model (for log D to estimate z) or first-order polynomial for Sl, δ and p (testing ln, square root and inverse transformations). The one-step global fitting was applied to fine-tune model parameter estimation for the same models using the entire dataset. At short treatment times, inactivation curves exhibited a characteristic shoulder, highlighting limitations of the log-linear primary model, which was withdrawn. The log-linear with shoulder model parameters, both shoulder length (1/Sl) and log D, showed a strong linear correlation with temperature. Similarly, for the Weibull model, a well-defined linear dependence with temperature was found for the inverse of the time for the first log reduction, scale parameter (1/δ) and the shape parameter (1/p). The one-step approach reduced the root mean square error (RMSE) by more than 20% in both models. Among all models, the one-step Weibull model provided the best fit, with an RMSE of 0.55. These results demonstrated the potential of microbial modelling to quantitatively describe microbial behavior in solid plant-based matrices. The occurrence of a resistance shoulder is particularly relevant under high-rate thermal conditions, which are characteristic of extrusion processes. The proposed methodology, based on the use of DSC crucibles, enables direct characterization of inactivation phenomena of *B. cereus* spores at high temperatures in a cornerstone ingredient in plant−based foods and provides a new approach that supports the development of more robust thermal process models.

S4-P79: Raw Milk Cheeses as Reservoirs of Multidrug-Resistant Bacteria and Antimicrobial Resistance Genes

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Antimicrobial resistance (AMR) represents a global threat to human health and is addressed within the One Health framework, which recognizes the strong interconnection between environmental, animal, and human health. The dairy production chain is increasingly recognized as a potential reservoir and transmission route for antibiotic-resistant bacteria and antimicrobial resistance genes (ARGs), although this ecosystem remains insufficiently characterized.

This study aimed to isolate and characterize antibiotic-resistant bacteria from raw milk cheeses, detect antimicrobial resistance genes, and develop an experimental model to investigate horizontal gene transfer (HGT). Raw milk cheeses were homogenized in 2% sodium citrate (1:10, w/w) and subjected to bacterial isolation under selective and differential media and selective pressure using ampicillin, erythromycin, tetracycline, and imipenem as a carbapenem antibiotic. Morphologically distinct colonies were isolated, purified, and cryopreserved for further analysis.

High-throughput antibiotic susceptibility testing was performed in liquid culture in 96-well plates using an automated Opentrons platform, enabling the screening of all isolates and the identification of 26 multidrug-resistant (MDR) strains out of 67 total antibiotic-resistant isolates. MDR isolates were further characterized by minimum inhibitory concentration (MIC) assays against a wider range of clinically relevant antibiotics.

Taxonomic identification revealed that MDR isolates mainly belonged to the genera *Staphylococcus*, *Serratia*, *Enterococcus*, and *Lactiplantibacillus*. Whole-genome sequencing was performed using Oxford Nanopore MinION to identify antimicrobial resistance genes and mobile genetic elements associated with multidrug resistance.

Future work will focus on the development of a controlled experimental model combining culture-based and metagenomic approaches, in which microbial communities with defined relative abundances will be assembled to mimic cheese-associated ecosystems. This model will enable the investigation of ARG dissemination dynamics and horizontal gene transfer events under food-relevant conditions, providing insight into the role of dairy matrices in the maintenance and spread of antimicrobial resistance.

S4-P80: Genomic and Phenotypic Characterization of *Salmonella* Mikawasima Strain Isolated from a Foodborne Outbreak in Spain

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In August 2025, a large foodborne outbreak caused by *Salmonella enterica* subsp. *enterica* serovar Mikawasima (ST1815) occurred during a wine festival in Barbastro, Spain, affecting more than 500 individuals. This serovar has shown an increasing presence in recent European zoonosis reports, highlighting its relevance as an emerging Public Health concern. Epidemiological investigations recovered the pathogen from fresh tomatoes, tomato-topped bread slices, and from kitchen utensils used during preparation.

This study provides a genomic and phenotypic characterization of two outbreak-related isolates supplied by Public Health authorities, one recovered from fresh tomato and the other from tomato bread. Whole-genome sequencing and Mash analysis confirmed that both isolates were genomically indistinguishable, and subsequent analyses were therefore focused on fresh tomato isolate SLM250599, pointing to the tomato as the source of the contamination. Bioinformatic analyses identified the strain as *S.* Mikawasima (ST1815), belonging to serogroup C1 with antigenic formula 6,7,14:y:e,n,z15. Phylogenetic reconstruction showed that the outbreak strain clustered with 18 clinical isolates previously reported in the United Kingdom between 2015 and 2018. An analysis using ABRIcate on the ResFinder database identified the *aac(6')-Iaa_1* gene, which is associated with resistance to amikacin and tobramycin.

For the phenotypic characterisation of *S.* Mikawasima, *Salmonella* Typhimurium LT2 (SeT) was selected as the reference strain for comparisons. In growth assays performed at pH values from 4.0 to 5.5 (0.25 intervals) in lysogeny broth medium acidified with a 3:1 mixture of citric and malic acids to mimic tomato acidity, SLM250599 displayed a shorter lag phase than SeT under acidic conditions, suggesting improved adaptation to acidic food matrices. The outbreak strain also showed higher tolerance to biocides, with approximately 3 $\log_{10}$ less inactivation than SeT after exposure to lethal concentrations of 3-(diethylamino)propylamine and sodium hypochlorite. After a heat treatment (30 min at 54°C), SLM250599 showed about 1 $\log_{10}$ greater survival than SeT after. In contrast, the strain was classified as a moderate biofilm producer, whereas SeT was a strong biofilm producer. Antibiotic susceptibility testing by Vitek 2 showed resistance to cephalothin and gentamicin, as in SeT.

Overall, these results indicate that this outbreak-associated *S.* Mikawasima strain combines rapid adaptation to acidic conditions with enhanced tolerance to selected biocides and heat, traits that may have contributed to its persistence and transmission in the implicated food matrix.

S4-P81: Kitchen Sponges as Reservoir of *Bacillus cereus* sensu lato: Prevalence, Genomic Characterization and Persistence

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Kitchen sponges are commonly used in domestic kitchens for cleaning food contact surfaces and utensils. Previous studies demonstrated that they harbor a diversity of microorganisms, including food pathogens such as *Escherichia coli* and *Salmonella enterica*. Interestingly, *Bacillus* spp. were also detected. However, the prevalence of the spore-forming food pathogen *Bacillus cereus* sensu lato remains unknown.

This study reports the isolation and typing of viable *B. cereus* s.l. in used kitchen sponges collected from Belgian households. 100 kitchen sponges were collected from 82 households in Flanders (Belgium). The majority consisting of polyurethane foam. The total aerobic microbial load and *B. cereus* s.l. were enumerated following ISO 4833-1 and ISO 7932, respectively. From 38 positive sponges, 27 presumptive *B. cereus* isolates (from 17 sponges), were characterized by whole-genome sequencing (WGS, Illumina paired-end 2 × 150 bp). Genomic analyses included screening their virulence repertoire and the strain were compared using (core-genome) multi-locus sequence typing ((cg)MLST).

In a second part, the survival and sporulation behavior of *B. cereus* s.l. was examined by artificially contaminating unused polyurethane kitchen sponges (5×3×2 cm). Sponges were inoculated with vegetative cells (3 log CFU per sponge) of isolated obtained in this study. Two independent strain sets (each including two strains isolated from the same sponge) were tested with five technical replicates per set. Vegetative cells and spores were enumerated after 0, 1, 3 and 5 days after inoculation on Mannitol Egg Yolk Polymyxin (MYP) agar. A heat treatment (80°C, 10 min) was performed before enumerating spores. Three independent experiments were performed.

B. cereus s.l. was detected in 38% of the sponges with an average concentration of 3.6 log CFU per sponge. The average total aerobic microbial count of all sponges was 8.9 log CFU per sponge. WGS revealed the presence of multiple (putative) virulence factors, including enterotoxin genes. Twelve distinct genetic lineages (MLST) were identified, of which previously associated with outbreaks. Interestingly, cgMLST demonstrated a clonal strain present in two sponges collected from the same household at different time points. Artificial contamination demonstrated rapid sporulation within 24 h and persistence for at least 5 days in dry sponges.

These findings indicate that presumptive *B. cereus* is prevalent and persistent contaminant in kitchen sponges and capable to survive in domestic households. Kitchen sponges may therefore facilitate cross-contamination in (domestic) kitchens and contribute to the risk of foodborne illness.

S4-P82: Production System Influences Antibiotic Resistance Patterns in *Pseudomonas* and Related Isolates from Broiler Carcasses

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Pseudomonas spp. are among the main causes of spoilage in air-packed poultry meat. In addition, some species are opportunistic pathogens and may act as reservoirs of antimicrobial resistance genes. Because production system may influence the microbiological composition and resistance patterns of poultry products, the objective of this study was to characterize antibiotic-resistant *Pseudomonas* isolates recovered from broilers from conventional, free-range, and organic production systems.

A total of 9 whole broiler carcasses (3 per production system) were sampled by double swabbing with sterile sponges pre-moistened with buffered peptone water. Samples were serially diluted and plated on Pseudomonas agar base supplemented with cetrimide, fusidic acid and cephalothin. To recover resistant isolates, media were additionally supplemented with ciprofloxacin (CIP), colistin (COL), or gentamicin (GEN) at the Clinical and Laboratory Standards Institute (CLSI) breakpoint concentration (1×) and at four times this value (4×).

After incubation, up to 12 colonies were selected per antibiotic and sample, prioritizing growth on 4× plates. Their resistance profiles to the three antibiotics were assessed by agar dilution according to CLSI document M07, using concentrations of 1×, 2×, 4×, and 8×. Isolates were identified by MALDI-TOF, and only *Pseudomonas* and related genera were included in the analysis. A total of 107 isolates were retained, including 28 from conventional, 46 from free-range, and 33 from organic broilers.

Regarding COL resistance, the highest rate was observed in conventional broilers (93% at 1×), followed by free-range (83%) and organic isolates (76%). In case of GEN, however, the highest rate was recorded in free-range broilers (50% at 1×), closely followed by organic broilers (42%), while only a 11% was observed in conventional broilers. For CIP, no resistant isolates were detected among conventional broilers, whereas resistance rates of 46% and 24% at 1× were recorded for free-range and organic isolates, respectively.

The proportion of multidrug-resistant isolates, defined as those simultaneously resistant to CIP, COL and GEN, was also higher in free-range (33%) than in organic broilers (12%), with no multidrug-resistant isolates detected among conventional broilers.

Antibiotic-resistant *Pseudomonas* and related isolates were detected in broilers from all production systems studied. However, resistance rates differed between systems, with free-range samples showing the highest proportion of resistant isolates, particularly of multidrug resistant isolates. The facts underlying these differences require further investigation.

S4-P83: Antimicrobial Resistance and Enterotoxin Gene Prevalence in *Staphylococcus aureus* from Swiss, Italian, and Austrian Artisanal Cheeses

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As a ubiquitous foodborne pathogen capable of producing heat-stable staphylococcal enterotoxins (SEs), *Staphylococcus aureus* represents a significant public health concern. This is particularly relevant in artisanal cheese production, where its persistence poses a dual challenge related to food safety and antimicrobial resistance (AMR).

To assess the genomic diversity and antimicrobial resistance profile of this pathogen, 92 *S. aureus* isolates recovered from artisanal cheeses produced in Switzerland (*n* = 52), Italy (*n* = 20), and Austria (*n* = 20) were analysed. The strains originated from a previous study by Berger et al. (2025). Short-read sequencing was performed on all isolates and WGS data were processed using a Galaxy-based workflow. Genomes were screened for virulence and antimicrobial resistance genes. In addition, isolates were genotyped using multilocus sequence typing (MLST), and clustering analyses based on single nucleotide polymorphisms (SNPs) were performed. Phenotypic antimicrobial resistance was assessed using minimum inhibitory concentration (MIC) testing.

MLST analysis showed that sequence type (ST) 8 was the most prevalent (*n* = 29), followed by ST352 (*n* = 14), a distribution that was also reflected in the main SNP clusters identified. Analysis of antimicrobial resistance genes (ARGs) revealed that *blaZ*, associated with penicillin resistance, was the most frequent accessory gene (*n* = 46), whereas *ermT*, *fosB*, *tet(K)*, *tet(M)*, and *tet(L)* occurred at lower frequencies. Notably, a single isolate was identified as methicillin-resistant *S. aureus* (MRSA). This strain exhibited a unique resistance profile not observed in any other isolate, characterised by the presence of *mecA* together with *ant (4')-Ib*, *ant (6)-Ia*, *dfrG*, and *lsaE*. MIC testing showed that 29 isolates (31%) were resistant to at least one antibiotic, with the highest resistance observed for penicillin (17%). Regarding staphylococcal enterotoxin genes, *sew* and *sex* were nearly ubiquitous, detected in 100% and 97.8% of isolates, respectively. The genes *sej* (*n* = 35) and *ser* (*n* = 34) were also frequently detected. A distinct co-occurrence pattern was observed for the enterotoxin gene cluster (egc) components (*seg*, *sei*, *sem*, *sen*, *seo*, *seu*), which were detected together in 13 isolates belonging to different ST. Additionally, *sey* was identified in eight isolates, seven of which also carried the egc cluster, suggesting a potential genetic linkage or clonal distribution among cheese-associated strains.

Overall, these preliminary results highlight the presence of diverse ST and variable distributions of antimicrobial resistance and enterotoxin genes in *S. aureus* isolates from artisanal Alpine cheeses produced in Central Europe.

S4-P84: Response of *Listeria monocytogenes* Scott A to Peracetic Acid Stress: Implications for Detection and Persistence in Food-Processing Environments

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Listeria monocytogenes is a major foodborne pathogen associated with listeriosis, a severe illness with high mortality rates, particularly among immunocompromised individuals. Peracetic acid (PAA) is widely used in food processing environments as an effective disinfectant due to its broad-spectrum antimicrobial activity. However, sub-lethal exposure to PAA may not fully eliminate *L. monocytogenes*, but instead reduce its culturability, cause sublethal injury, or induce a viable but non-culturable (VBNC) state, with important implications for food safety and disinfection efficacy.

This study aimed to investigate the impact of PAA exposure under both nutrient-rich and starvation conditions on the growth recovery, culturability, and potential induction of VBNC states in *L. monocytogenes* Scott A following both short-term (shock) and prolonged (continuous) stress conditions.

L. monocytogenes Scott A was exposed to PAA at 1×, 2×, and 4× MIC under two conditions: a 20-min shock treatment in Ringer's solution and a 24-h continuous exposure in either nutrient-rich TSB or Ringer's solution (starvation conditions). After exposure, PAA was neutralised using sodium thiosulfate, and the treated cells were transferred into fresh TSB to assess recovery. Culturability was evaluated by plating on non-selective TSA and selective Brilliance agar at 0, 3, 6, and 24 h. In parallel, growth recovery was monitored over 24 h using a Bioscreen C system.

Continuous exposure to PAA under starvation conditions produced the strongest effect, with cells at 2× and 4× MIC remaining below the detection limit on both TSA and Brilliance agar and no detectable growth over 24 h. Similarly, shock exposure at these concentrations caused a loss of culturability between 0 and 6 h, although partial recovery at 24 h indicates that a fraction of cells retained the ability to resuscitate.

In contrast, continuous exposure in nutrient-rich TSB showed that while 4× MIC reduced culturability at early time points, substantial recovery and growth were observed by 24 h, highlighting the role of nutrient availability in recovery. Across all conditions, lower counts on selective media compared to non-selective media indicate the presence of sublethally injured cells and reduced tolerance to selective agents following PAA exposure.

PAA exposure induced a range of stress responses in *L. monocytogenes* Scott A, including sublethal injury, delayed recovery, and loss of culturability, influenced by both disinfectant concentration and environmental conditions. These findings suggest that culture-based detection may underestimate PAA-stressed populations, with implications for assessing disinfectant efficacy in food processing environments.

S4-P85: Metataxonomic Characterization of the Core Microbiota Potentially Associated with *Listeria monocytogenes* Within Polymicrobial Biofilms in Food Processing Environments

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Listeria monocytogenes is widely recognized as one of the most hazardous foodborne pathogens, particularly in ready to eat (RTE) products. It causes listeriosis, a severe disease with a high mortality rate, typically exceeding 15–20 %. A key factor contributing to its persistence in food processing plants is its ability to integrate into polymicrobial biofilms, where it coexists with diverse bacterial communities. These ecological interactions can enhance its resistance against environmental stresses and sanitation procedures.

This study aimed to characterize the succession of the core microbiota potentially cohabiting with *L. monocytogenes* within seafood and meat processing environments (PEs). Being the core microbiota defined as the set of microbial taxa consistently associated with a specific niche, understanding these microbial consortia is critical for designing targeted anti-*Listeria* strategies to enhance food safety systems.

Quantitative microbial community analysis was performed across four food processing plants (two seafood and two meat) by sampling 10–15 strategic surface locations per facility twice over a six month-period to achieve both temporal and spatial coverage. Metataxonomic analysis was conducted using the 16S Barcoding Kit 24 V14. Sequencing and initial data processing – including basecalling, demultiplexing, and read quality assessment – were carried out on a MinION Mk1B device using MinKNOW software. Bioinformatic analysis, featuring genus level alignment against the SILVA_138_1 reference database, was performed using the EPI2ME 16S pipeline, providing interactive taxonomic abundance visualizations at multiple taxonomic levels. MicrobiomeAnalyst was used to generate rarefaction curves, diversity indices, and differential abundance analyses. In addition, *L. monocytogenes* detection was conducted according to the ISO 11290-1 method, and confirmed isolates were genetically characterized by whole genome sequencing (WGS).

Comparative analysis of the core microbiomes provided crucial insights into the dynamic of bacterial populations. While certain genera such as *Pseudomonas* spp. and *Serratia* spp. were prevalent in the seafood sector, *Bacillus* spp. and *Staphylococcus* spp. were common in the meat sector. Additionally, results revealed significant variability in the prevalence of *L. monocytogenes* across processing environments, with no apparent correlation to the type of industry or raw materials processed in each line.

These findings allow the definition of representative microbial consortia for these specific food processing environments. This characterization provides a baseline for developing control strategies against *L. monocytogenes* in polymicrobial biofilms.

S4-P86: Variability in the Thermal Resistance Profile of *Bacillus cereus* Isolated from Different Foodstuffs

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Bacillus cereus is a spore-forming bacterium that produces toxins and causes spoilage by producing extracellular enzymes. The effectiveness of thermal processing in food may be limited by strain variability. Therefore, characterizing the thermal resistance of distinct strains is essential to better understand their behavior under different time–temperature conditions and to support the development of more robust control strategies. Thus, we assessed the thermal resistance profiles of *B. cereus* strains (*n* = 102) isolated from different food matrices. Heat shock treatments ranged from 70 to 100°C, with exposure times of 5 to 20 min. The treatments were carried out in nutrient broth inoculated with 10^3 CFU/mL for each strain. After treatment, the treated tubes were incubated at 30°C for 24 h, and bacterial growth was assessed by turbidity. Survival data were analyzed using hierarchical clustering with the Jaccard distance and Ward's method to identify patterns of thermal resistance among strains. Additionally, the probability of survival was estimated using logistic regression, considering both strain origin and thermal response behavior. At 70°C and 80°C, 15.6% of the isolates (*n* = 16) did not survive the applied heat treatments. Among these, strains originated from cassava-based products (*n* = 16), dairy products (*n* = 9), and ready-to-eat meals (*n* = 9). The surviving strains were grouped into three distinct thermal resistance profiles: a low-resistance group (7.84%; *n* = 8), an intermediate-resistance group (17.65%; *n* = 18), and a high-resistance group (43.13%; *n* = 44), the latter of which showed behavior similar to the reference strain. In particular, most isolates from cassava-based products exhibited thermal resistance profiles comparable to those of *B. cereus* ATCC 14579. For treatments at 90 and 100°C, seven strains survived the heat after 15 min, one strain remained viable after 20 min, and another persisted even after 100°C for 20 min, both were isolated from cassava products. The probability of survival at 90°C for 15 min was estimated at 0.10 for cassava-derived isolates, whereas isolates from dairy products showed a substantially lower probability (0.02). These findings indicate that both intrinsic variability and matrix-related adaptation can affect thermal resistance in *B. cereus*, carrying significant implications for risk assessment and the development of effective thermal processing methods in food systems.

S4-P87: Genomic Insights into *Cronobacter sakazakii* from Industrial Whey Drying Processes

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Cronobacter sakazakii is an opportunistic pathogen frequently associated with dehydrated foods, particularly powdered infant formula. Its ability to survive and persist under harsh conditions makes the investigation of genetic mechanisms related to resistance and environmental adaptation essential. In this study, 15 *C. sakazakii* isolates were obtained from samples collected in an industrial facility during whey concentration and drying processes. Genomic DNA was extracted using a validated in-house magnetic bead-based protocol. Whole-genome sequencing libraries were prepared and quality-checked using Qubit and TapeStation, and sequencing was performed on the Illumina MiSeq platform. Raw reads were processed using the Bactopia pipeline, and resistance genes were identified using AMRFinderPlus. A total of 403 genes were identified and distributed across different functional categories, with a predominance of genes associated with metal resistance (*n* = 296), including systems related to copper (*pco*), silver (*sil*), and arsenic (*ars*). Genes involved in heat stress response (*n* = 52), such as *clpK* and *hsp20*, were also frequently detected, indicating a strong potential for survival under typical industrial processing conditions. Antimicrobial resistance genes (*n* = 35) included determinants associated with β-lactams (bla_{CSA}, bla_{CSA-2}, bla_{EC}), fosfomycin (*fos*), and efflux systems (*acrF*, *emrD*, *mdtM*). Additionally, virulence-associated genes (*n* = 18) were identified, with emphasis on *cpa*, suggesting pathogenic potential of the isolates. Overall, the results indicate that the persistence of *Cronobacter* in this environment may be more strongly associated with adaptation to environmental stressors and metal exposure than with antimicrobial resistance alone. These findings highlight the importance of microbiological surveillance in industrial food production environments, particularly in drying processes, where resistant microorganisms may survive and pose a risk to public health.

S4-P88: The "Nematodes" Catalogue Within the Pathogens-in-Foods (PIF) Database: Global Occurrence Data of Parasites of Public Health Importance in Fishery Products

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The Pathogens-in-Foods (PIF) database is a dynamic repository of occurrence data (prevalence and enumeration) on pathogens in foods sampled across Europe, accessible through a dedicated web application (https://pif.esa.ipb.pt/). Data are periodically extracted from peer-reviewed publications identified through systematic literature searches, following a publicly available protocol describing the search and screening procedures. Eligible primary studies undergo quality assessment, and validated data are integrated into the database using a predefined categorization framework. This framework encompasses general study characteristics, food classification (aligned with EFSA's FoodEx2 system), pathogen information, microbiological methods, and outcome measures (prevalence and/or enumeration).

The "Nematodes" catalogue, launched on December 31, 2024, represents a specialized module within the PIF framework. It compiles occurrence data on nematodes and other parasites in fishery products, covering a broad range of species of public health relevance, including *Anisakis*, *Contracaecum*, *Phocanema* (syn. *Pseudoterranova*), *Opisthorchis*, *Metorchis*, *Pseudamphistomum*, *Dibothriocephalus*, *Bolbosoma*, *Corynosoma*, *Kudoa*, and *Cryptocotyle*.

A key feature of this catalogue is its global scope. The systematic review protocol was adapted to capture worldwide occurrence data, reflecting the international nature of fishery value chains. The data model was refined to ensure a high level of granularity, particularly regarding host taxonomy (family, genus, species), microbiological methods, and fishing zones. When available in the primary studies, geographic information is recorded according to FAO major fishing areas, subareas, and divisions, including precise GPS coordinates.

As of January 2026, the catalogue contains more than 10,000 data entries derived from 551 publications published between 2000 and September 2025, covering both wild and farmed fish produced and marketed within and beyond Europe.

These data supported two EFSA scientific opinions on the risk assessment of parasites in fishery products, published in 2024. Specifically, they were used to evaluate the occurrence of parasites of public health importance in products derived from the most relevant farmed fish species in the EU, as well as to assess evidence on zoonotic parasites in wild-caught fish across global fishing areas.

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S4-P89: Antimicrobial Efficacy of Blackberry By-Products Against Poultry-Derived *Listeria* spp.

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To align with global environmental objectives, this research explores the revalorization of blackberry (*Rubus ulmifolius*) by-products as a source of high-value ingredients. Given their high phenolic content, the objective of this study was to evaluate their antimicrobial capacity against *Listeria* spp. offering a sustainable and clean-label solution for food safety.

The minimum inhibitory concentration (MIC) was studied using the broth macrodilution method (4–0.5%) against 3 reference strains of *Listeria monocytogenes*, 6 strains of *L. monocytogenes* isolated from the poultry food chain and one strain of *Listeria innocua*. For that, the blackberry product was obtained through a patented process (ES2424870-2B) from the by-product derived of juice production. For each of the strains, the effect of the pH modification (pH 5.6) produced by the blackberry product at 2% was studied, and the results were compared with a positive control after 24 and 48 h of incubation at 37°C and 200 rpm.

The results indicated that the MIC was 3% for *L. innocua* and *L. monocytogenes* strain C3_845, while 2% for the rest. In all the strains an immediate effect of the blackberry after inoculation (6.2 CFU/mL) was observed as a reduction ranged from 2.7 to 4.5 log CFU/mL was obtained compared with the inoculum used (6.2 CFU/mL). *L. innocua* survived after 48 h with a reduction of 5 log CFU/mL compared with the positive control while grew 1.8 CFU/mL compared with its counts after the inoculation. Nevertheless, *L. monocytogenes* strain C3_845 did not survive after 48 h, and its counts were reduced in 5.4 log CFU/mL after 24 h compared with the positive control while maintained the initial counts.

To study differences in the rest of the strains the results from the pH control were used. One *L. monocytogenes* reference strain and four form the poultry chain showed a clear resistance as grew after 48 h of incubation. While, ILSI 9 and ILSI 17 (reference strains) and C8_1932 (poultry chain) were more sensitive as the pH had a bacteriostatic effect after 48 h.

These findings support a sustainable a food safety approach by utilizing blackberry by-products as natural antimicrobials. The identification of strains exhibiting diverse resistance levels is a key finding of this research as facilitates the design of specialised bacterial cocktails, which are essential for the validation of the shelf-life and safety of poultry-based products.

S4-P90: The Presence of Multidrug-Resistant Bacteria and Resistance Genes in Raw Milk from Dairy Farms

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Dairy production is an important economic sector in both Slovenia and Serbia. In Slovenia, farms predominately keep 30–50 dairy cows, whereas in Serbia milk production is divided between small farms with up to 10 dairy cows (accounting for approx. 70% of national milk production) and semi-intensive operations with more than 100 cows per farm, with some agricultural holdings housing as many as 800–2,500 animals. More intensive dairy farming increases the risk of disease in dairy cows, especially mastitis. This results in frequent use of antibiotics, which accelerates the development of bacterial resistance and the spread of antimicrobial resistance genes (ARGs) among bacteria in both agricultural environments and human populations. Resistant (and especially multidrug-resistant, MDR) bacteria present in raw milk pose a potential health risk to both consumers and animals. In addition, the presence of their ARGs is concerning, as many of these genes are located on mobile genetic elements that can be horizontally transferred across bacterial strains or species. A bulk tank milk (BTM) sample reflects the health status of the entire dairy herd and the broader farm environment, making it highly suitable for various analyses.

In this study, we analyzed BTM samples from 106 Slovenian and 100 Serbian farms to determine the presence of MDR bacteria. We employed cultivation on selective media, species confirmation by MALDI-TOF mass spectrometry, antimicrobial susceptibility testing to determine the minimum inhibitory concentrations, PCR-based confirmation of selected ARGs and whole-genome sequencing for strain characterization. We obtained 10 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates (five were MDR), as well as 13 *Escherichia coli* and two *Klebsiella pneumoniae* isolates producing extended-spectrum beta-lactamases (ESBL). Moreover, 11/13 ESBL *E. coli* and both ESBL *K. pneumoniae* isolates were MDR. Carbapenemase-producing *Enterobacteriaceae*, vancomycin-resistant enterococci and colistin-resistant *E. coli* were not detected.

The presence of MDR bacteria and ARGs in milk highlights a potential public health risk, particularly with the consumption of raw milk and unpasteurized dairy products – a trend that is becoming increasingly widespread in the developed world.

S4-P91: Retail Meat in Slovenia as a Source of Resistant Bacteria of the ESKAPEE Group

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Food of animal origin plays an important role in the transmission of resistant bacteria or antimicrobial resistance genes (ARGs) to humans through the food chain, especially through milk and meat. In this study, we investigated the occurrence of ARGs and resistant bacteria in retail meat available in Slovenia, focusing on the ESKAPEE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp. and *Escherichia coli*). The meat came from various origins and producers.

A total of 105 poultry, pig and fish retail meat samples underwent non-selective and selective enrichment, followed by cultivation on selective agar plates and species confirmation by MALDI-TOF mass spectrometry. The ESKAPEE isolates (*n* = 70), all originating from poultry or pig meat, were further characterized by whole-genome sequencing (WGS). After removal of epidemiological duplicates, 58 isolates were retained. Of the 45 *E. coli* isolates, 22 were extended-spectrum β-lactamase (ESBL)-producers ($bla_{\text{CTX-M}}$) and one was colistin-resistant (*mcr-1*); 17 were multidrug-resistant (MDR). Of the three *K. pneumoniae* isolates, two were ESBL producers ($bla_{\text{CTX-M-15}}$) and all were MDR. All *P. aeruginosa* (*n* = 9) and *E. faecium* (*n* = 1) isolates were also MDR.

The collected results indicate that raw poultry and pig retail meat available in Slovenia can serve as a source of MDR bacteria from the ESKAPEE group. The relatively high occurrence of such strains, including those resistant to last-resort antibiotics such as higher−generation cephalosporins and colistin, is of particular concern to consumers. To mitigate this risk, a multifaceted approach targeting critical points along the food production chain is required.

S4-P92: Microbial Survival: The Role of Nutrient Enrichment and Storage Temperature

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Food safety is continuously challenged by various intrinsic and extrinsic factors that affect the proliferation and persistence of microorganisms. The present study evaluated whether lactose content and protein supplementation, combined with storage temperature, modulate the growth of *Listeria monocytogenes* FDA LS808, *Staphylococcus aureus* NCTC 6571, and *Escherichia coli* ATCC 25922 in different food matrices. To assess these parameters, controlled inoculation trials were conducted using six food matrices: milk and cottage cheese (with and without lactose), and pudding with two different protein contents. Subsequent microbial counts were monitored via selective culture media over a 120-h period, comparing ambient (22°C) and refrigerated (4°C) storage temperatures. The results indicated that lactose and protein content had distinct effects on microbial growth. At ambient temperature, *L. monocytogenes* (after 48 h) and *S. aureus* (after 24 h) exhibited significantly ($p < 0.05$) higher log (CFU/mL) in lactose-free milk, whereas *E. coli* showed no significant differences in population levels regardless of lactose. Conversely, no significant differences in microbial counts or even inverse trends were observed in lactose-free cottage cheese. Regarding protein supplementation, the only significant effect observed was the enhanced growth of *E. coli* in pudding with lower protein content during refrigerated storage at 24 h and 120 h ($p < 0.05$). Overall, with lactose-containing milk serving as the primary food matrix, microbial counts were notably higher at ambient temperature. Nevertheless, all tested bacterial strains remained detectable in all food matrices throughout the 120-h refrigeration period. Final counts after 120 h at 4°C in lactose-containing milk ranged from 1.22 ± 0.20 for *E. coli* to 5.5 ± 0.21 log (CFU/mL) for *L. monocytogenes*. These data emphasise the necessity of considering both food composition and storage conditions to enhance food safety, particularly regarding psychrotrophic pathogens such as *L. monocytogenes*. Although this study focuses on lactose and protein content, it is essential to acknowledge that additional factors beyond the scope of this study, such as pH and water activity, also significantly influence microbial growth. The persistence of pathogens across all tested matrices highlights a potential vulnerability in the cold chain for dairy alternatives. Therefore, a thorough understanding of these factors helps refine food safety strategies and underscores the necessity for consumers to maintain a strict cold chain as a primary measure to mitigate foodborne risks.

S4-P93: Characterisation of *Listeria monocytogenes* Food Isolates Using Whole Genome Sequencing

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Listeriosis, caused by *Listeria monocytogenes*, is one of the most severe foodborne diseases, characterized by high hospitalization and mortality rates despite its relatively low incidence. Vulnerable populations, including the elderly, pregnant women, newborns, and immunocompromised individuals, are at greatest risk. Due to its ubiquitous presence in soil, water, and food-processing environments, *L. monocytogenes* can contaminate a wide range of foods, including ready-to-eat foods.

In this study, whole-genome sequencing (WGS) was applied to characterize 69 *L. monocytogenes* isolates from food and three isolates from a food-processing environment in Slovenia. All strains were collected between 2020 and 2025. Most food isolates originated from meat and meat products (*n* = 42), followed by vegetables and vegetable products (*n* = 22), with smaller contributions from fish, dairy, and other ready-to-eat foods. Genomic typing was performed using Ridom SeqSphere+, while virulence determinants were identified using BIGSdb and confirmed with AMRFinderPlus.

In silico serogrouping revealed a predominance of IIa (65.3%, 47/72), followed by IIc (20.8%, 15/72), IVb (9.7%, 7/72), and IIb (4.2%, 3/72). Clonal complex analysis showed that CC9 (20.8%, 15/72) and CC121 (13.9%, 10/72) were the most prevalent, followed by CC37 and CC475 (each 11.1%, 8/72), and CC570 (4.2%, 3/72). A total of 21 different sequence types were identified, with ST9 and ST121 being dominant.

All isolates carried LIPI-1 and internalins (*inlA*, *inlB*), while other virulence factors varied. The *inlG* and *inlF* genes were present in 55.6% (40/72) and 75.0% (54/72) of isolates, respectively. The hypervirulence-associated LIPI-3 was detected in 6.9% (5/72) of isolates. Stress adaptation markers were common, with SSI-1 and SSI-2 identified in 37.5% (27/72) and 25.0% (18/72) of isolates, respectively, whereas LGI-2 was rare (4.2%, 3/72). All isolates harbored the regulatory gene *virR*.

A high diversity among the tested *L. monocytogenes* food isolates was observed. The frequency of CC9 and CC121, and the high prevalence of stress survival determinants, suggest their adaptation to environmental persistence rather than enhanced virulence. Whole genome sequencing enables the characterization and comparison of *L. monocytogenes* isolates from food, humans, animals, and the environment, supports surveillance, and contributes to the prevention of human infections.

S4-P94: Characterization of *Staphylococcus aureus* Isolated from Food Products in Kazakhstan

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Staphylococcus aureus in foods frequently carries antibiotic resistance, virulence factors, and enterotoxin genes, which increase the risk of foodborne intoxication, persistence in the food chain, and dissemination of multidrug-resistant strains. Surveillance of these traits informs control and treatment strategies. The aim of this study was to evaluate the phenotypic and genotypic antibiotic resistance of *S. aureus* isolates obtained from food products using whole-genome sequencing (WGS).

Forty-two *S. aureus* isolates were obtained from milk, dairy products, meat, and meat products. Methods included the determination of minimum inhibitory concentrations (MICs) of antibiotics, WGS, multilocus sequence typing (MLST), and analysis of resistance, enterotoxin, and virulence genes.

The sensitivity of *S. aureus* isolates was determined against 12 antimicrobial drugs: penicillin, cefoxitin, erythromycin, clindamycin, linezolid, chloramphenicol, tetracycline, ciprofloxacin, trimethoprim/sulfamethoxazole, gentamicin, vancomycin, and nitrofurantoin. Phenotypic resistance to cefoxitin was detected in 9 strains (21.4%), all of which carried the mecA gene associated with methicillin resistance, indicating the presence of MRSA strains.

Analysis of antimicrobial susceptibility revealed a high level of resistance to penicillin – 35 strains (83.3%). Resistance to other antimicrobial drugs was detected less frequently and included tetracycline (11.9%), chloramphenicol (11.9%), erythromycin (9.5%), clindamycin (7.1%), ciprofloxacin (4.7%), and trimethoprim/sulfamethoxazole (2.4%). Four strains (9.5%) were classified as multidrug-resistant (MDR).

Molecular typing revealed that the predominant ST types among MRSA strains were ST22 (7/9), while two MRSA isolates belonged to a new subtype or variation of ST21. ST22 is a widespread sequence type isolated from various food products; it is known to harbor resistance genes and poses a significant risk of food poisoning. The most common strain overall was ST97, which is often associated with infections in farm animals, especially cattle (causing mastitis). ST5 was also identified, characterized by high adaptability, antibiotic resistance, and the production of staphylococcal enterotoxins.

The staphylococcal enterotoxin genes *sea*, *sec*, *seg*, and *sei* identified in this study indicate the potential of certain strains to cause foodborne toxic infections. The high prevalence of the *tsst* gene in food products, particularly in poultry and milk, suggests a significant level of contamination of the local food chain with potentially hypervirulent strains capable of causing toxic shock syndrome.

Thus, the results of this study highlight the need to strengthen food quality control and antimicrobial resistance monitoring in Kazakhstan.

S4-P95: Ecological and Functional Markers Linked to Persistence of *Listeria monocytogenes* in Agri-Food Industry

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Listeria monocytogenes is a major food pathogen, responsible for listeriosis. It has a strong ability to adapt, allowing it to persist over long periods in food processing facilities, thus constituting a significant food safety issue. The survival of *L. monocytogenes* in processing environments results from a combination of intrinsic genetic factors, external environmental conditions, and interactions with naturally occurring microbial communities. Understanding these mechanisms is essential for developing effective strategies to control this pathogen. Although these issues concern all agri-food sectors, few studies have adopted a comparative approach integrating several industrial sectors. This study will use shotgun metagenomics to analyze the bacterial ecology and functional potential of microbial communities associated with *L. monocytogenes* in three industrial sectors. The objective is to identify ecological or functional markers linked to its persistence and prevalence in agri-food industries.

A sampling campaign was conducted in three sectors of the agri-food industry: seafood, vegetables, and deli meat with three facilities visited per sector. Samples were taken from various environmental surfaces, including equipments, tools and floors. For each sample, the physical characteristics were systematically described, including the nature of the material, temperature, and humidity at the time of sampling. *L. monocytogenes* was then detected by PCR, allowing the samples to be classified as positive or negative. A set of 48 samples, representative of the three sectors and including both *L. monocytogenes* positive and negative samples, was selected for shotgun metagenomic analysis. Finally, bioinformatic analyses were conducted to characterise the microbial ecology of the samples, identify antibiotic (ARG) and biocide resistance genes (BRG), mobile genetic elements, and genes involved in biofilm formation.

Across the different sectors, an ecological gradient is observed in which *Listeria*-negative environments are dominated by *Enterobacter* spp. and *Staphylococcus* spp., whereas samples positive for *L. monocytogenes* are associated with communities enriched in *Acinetobacter* spp., *Yersinia* spp., and *Aeromonas* spp.. *Acinetobacter* spp. and *Aeromonas* spp. act as major ARG carriers, while BRG are mainly linked to *Pseudomonas* spp. in all sectors. Overall, this multi-sector comparative metagenomic approach highlights microbial and functional patterns associated with the presence of *L. monocytogenes* across agri-food sectors. Additional samples are currently being analyzed by shotgun metagenomics to confirm these observations.

S4-P96: Acetic Acid Adaptation in the *Bacillus cereus* Group: Impact on pH Growth Limits, Tolerance, and Resistance

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Whereas the use of acetic acid as a food preservative and disinfectant is increasing, the response of the *Bacillus cereus* group to this organic acid stress remains poorly characterized. In particular, the influence of physiological state or adaptation history on growth limits and survival remains poorly understood, despite its importance for food safety.

This study aims to evaluate the adaptive response of *B. cereus* group strains after acetic acid exposure. For that purpose, three physiological states, meaning non-adapted (pH 6.80, 0 mM acetic acid), low-pH adapted (pH 5.50, 0 mM acetic acid), and acetic-acid adapted (pH 5.50, 14 mM acetic acid), were obtained for 8 representatives strains belonging to different phylogenetic subgroups. Then, the growth limits in terms of pH and acetic acid concentration were determined, leading to a clustering of adaptive behaviours within the group. For one representative from each cluster, acetic acid resistance is being investigated.

Pre-adaptation at pH 5.50 with 14 mM acetic acid significantly increased acid tolerance in all *B. cereus* strains, lowering the pH growth limit by up to 0.30 and doubling acetic acid tolerance from 50 to 100 mM. Strong inter-strain variability, pronounced intra-population heterogeneity, and three distinct adaptive behaviours depending on strain and adaptation history were observed. These findings show that acid tolerance is a dynamic, history-dependent trait that must be considered in predictive microbiology risk assessment. Accordingly, *B. cereus* growth limits cannot be considered fixed intrinsic constants. While technological thresholds such as pH 4.60 remain useful, their interpretation must account for environmental variability, prior exposure to acetic acid, and tolerant subpopulations. This is particularly important for Group III strains, for which growth is associated with cereulide production. Adaptation lowered their pH growth limit to 4.42 ± 0.20 and doubled acetic acid tolerance to 80 mM, providing a more realistic framework for predicting pathogen behaviour in acidified foods.

Preliminary inactivation experiments with *Bacillus weihenstephanensis* KBAB4 (group VI) showed that acid resistance (t4D, time to reach 4 log reduction) increased nearly 3-fold in low-pH-adapted cells and 5-fold in acetic acid-adapted cells compared with non-adapted cells. Adaptation history influences *B. cereus* tolerance and resistance, and therefore must be integrated into risk assessment in the agri-food industry.

S4-P97: Direct Long-Read Sequencing and AI for Rapid Characterisation of *Listeria monocytogenes* in Food Safety

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Food safety testing currently relies on conventional microbiological methods, including culturing, which are time-consuming. These limitations delay decision-making and risk assessment, particularly for pathogens of high public health concern such as *Listeria monocytogenes*.

This project introduces DirectPred, an ongoing effort to develop a workflow for the identification and characterisation of *L. monocytogenes* directly from enriched samples using long-read sequencing and AI. By bypassing traditional culturing and colony-picking steps, DirectPred aims to significantly reduce time to results while providing direct genomic insights that enable prediction of virulence potential and disinfectant resistance.

The approach focuses on Oxford Nanopore long-read sequencing of *L. monocytogenes*-enriched samples, followed by direct genome assembly from sequencing data and the potential to detect multiple *L. monocytogenes* strains within a single sample. In addition, AI-based methods are used to predict virulence potential and resistance to disinfectants, leveraging the existing tool ListPred, while extending predictions to include the disinfectant peracetic acid based on genomic features. These predictions are intended to support better-informed risk assessment.

The project is in active development, with current emphasis on method design and model development. Future work will focus on validating performance across diverse sample types and assessing applicability and implementation potential in routine food safety monitoring. Ultimately, DirectPred is intended to support risk managers by providing faster, higher-resolution insights to enable timely and evidence-based decision-making.

S4-P98: Diverse and Virulent *Listeria monocytogenes* Across Hummus, Fresh Produce, and Food-Processing Environments

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Listeria monocytogenes is ubiquitous, pathogenic, and tolerant to adverse conditions, enabling persistence as a food safety and public health risk. Fresh produce and ready-to-eat (RTE) hummus have been implicated in listeriosis outbreaks causing multiple deaths, yet genetic profiling of isolates from these foods remains limited, particularly in South Africa. Recent hummus-related recalls in South Africa highlight the need for targeted research in this RTE category. This surveillance study (2018–2021) aimed to improve understanding of the genetic diversity, virulence, and resistance of *L. monocytogenes* from RTE hummus, fresh produce, and food-processing environments in the Western Cape, South Africa. Sixty *L. monocytogenes* isolates from different food processing facilities were classified into lineage groups using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Lineage I was prevalent overall (57%; $n = 34$) and associated ($p = 0.04$) with fresh produce (88%; $n = 7$). The proportions of lineage I and II in hummus (lineage I: 48%; lineage II: 52%) and environmental isolates (lineage I: 55%; lineage II: 45%) were not significantly different ($p > 0.05$). Lineage typing guided selection of isolates for whole-genome sequencing (WGS). Analysis of 20 isolates (10 lineage I, 10 lineage II) identified seven sequence types (ST1, ST2, ST3, ST5, ST101, ST121, ST204) across three serotypes (1/2a, 1/2b, 4b). ST204 ($n = 3$) was most common in the processing environment, ST5 ($n = 3$) in fresh produce, and ST5, ST101, and ST121 ($n = 2$ each) in RTE hummus. No subtype was significantly overrepresented ($p > 0.05$). All hummus samples included in this study originated from the same food factory. Phylogenetic analysis showed ST5 isolates from hummus were indistinguishable from those in a separate RTE airline food facility, suggesting a shared ingredient source. Serotype 4b (ST1, ST2) was only found in fresh produce. Antibiotic resistance was assessed using the Kirby-Bauer method. WGS revealed all isolates carried the *fosX* gene (fosfomycin resistance), while two ST5 hummus isolates also harboured *tetM* (tetracycline resistance). Overall, isolates were highly susceptible to key antibiotics used to treat listeriosis. All carried *Listeria* pathogenicity island (LIPI)-1 and LIPI-2 gene clusters, while two isolates (ST1, ST3) also contained LIPI-3, indicating hypervirulence. Some isolates from different sources harboured sanitizer tolerance genes (*emrC* [ST3], *bcrABC* [ST5, ST101, ST204], *qacH* [ST2, ST121]) conferring tolerance to benzalkonium chloride, which may affect sanitation efforts. These findings indicate that RTE hummus, fresh produce, and food-processing environments are susceptible to contamination by diverse, virulent *L. monocytogenes* strains.

S4-P99: Basil Essential Oil as a Food Safety Hurdle Against *Klebsiella variicola*: Proteomic Insights into Impaired Replication and Translation

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Klebsiella variicola is an emerging foodborne pathogen of growing concern due to its ecological versatility and ability to persist under adverse environmental conditions. Its widespread occurrence in plants, soils, insects, aquatic environments, and clinical settings, together with its detection in fresh and minimally processed foods, highlights the need to better understand its response to natural antimicrobial hurdles for improved food safety management.

In this study, the response of *K. variicola* KV7 to sublethal exposure to basil essential oil (BEO) was investigated using label-free quantitative proteomics, with the aim of identifying physiological adaptations associated with survival under food-related stress. A total of 64 proteins were differentially expressed following treatment, indicating a broad cellular response even at low BEO concentrations.

Beyond alterations in central metabolism, oxidative stress response, and membrane-associated functions, a key finding was the impact on informational processes. In particular, proteins involved in DNA replication and repair were affected, including the downregulation of DNA polymerase I, suggesting a reduced capacity to maintain genome integrity under stress conditions relevant to food environments. Moreover, BEO exposure significantly repressed several translation-related proteins, including elongation factor Tu (EF-Tu), an EF-P-like factor, and peptide chain release factor 2, indicating impaired translational efficiency and potential ribosomal dysfunction. Such effects may limit the synthesis of proteins required for growth, stress adaptation, and persistence in food matrices.

Overall, these findings suggest that BEO acts not only as a membrane-active antimicrobial but also interferes with essential cellular processes required for adaptation and survival. This multifaceted mode of action may reduce the ability of the pathogen to persist in food-related environments. In conclusion, BEO exerts a pleiotropic stress on *K. variicola*, targeting DNA replication and protein synthesis. These results support the potential of BEO as a clean-label preservation strategy against emerging foodborne pathogens.

S4-P100: Triplex Real-Time PCR for Rapid Detection of Enterotoxin Genes in *Bacillus cereus* sensu lato from Food, Environmental Surfaces and Human Samples

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Bacteria from the *Bacillus cereus* sensu lato group are widespread spore-forming bacteria and an important cause of foodborne illness worldwide. Food poisoning symptoms associated with this group typically occur as either emetic or diarrhoeal syndrome. The diarrhoeal type of disease is mainly connected to three pore-forming enterotoxins: haemolysin BL (Hbl), non-hemolytic enterotoxin (Nhe) and cytotoxin K (CytK). Due to their ecological adaptability, ability to form spores and resistance to sanitization in food-processing environments, organisms from *B. cereus* s.l. group are frequently detected in a wide range of food samples and represent a continuous risk in the food production chain.

The aim of this study was to develop and validate a real-time PCR assay for simultaneous detection of the enterotoxin genes *hblD*, *nheA* and *cytK2*, and to evaluate their prevalence among *B. cereus* s.l. isolates from different sources. The method was optimised in steps, from singleplex to multiplex format using SYBR Green chemistry. Analytical validation of the method included determination of amplification efficiency, limit of detection, repeatability, reproducibility and the performance of exclusivity and inclusivity test.

Amplification efficiency for multiplex reaction ranged between 90 and 110%. The method shows good repeatability and reproducibility with the estimated limit of detection between 10^2 and 10^3 CFU/µL, depending on the targeted gene. Analytical specificity was confirmed with the absence of amplification in non-target strains and by distinctive melting curve profiles of the amplicons.

The optimised assay was applied to a collection of 179 *B. cereus* s.l. isolates originating from food, surface swabs, and human stool samples. The gene *nheA* was detected in 98% of isolates, confirming the highest prevalence among enterotoxin genes of *B. cereus* group. Approximately half of the isolates (49%) carried all three diarrhoeal toxin genes, indicating the presence of strains with combined cytotoxic potential. No statistically significant differences in gene prevalence were observed between isolates from different sources. The comparison with the BCET-RPLA test for haemolysin BL detection showed high concordance between the two methods (98%).

These findings highlight the widespread enterotoxigenic potential of *B. cereus* s.l. across food, environmental, and human-associated isolates. The developed triplex real-time PCR assay therefore represents a rapid and reliable screening approach for molecular characterisation of isolates and may support improved monitoring of enterotoxigenic strains in food production environments and public health investigations.

S4-P101: Inhibition of *Bacillus cereus* Group and *Listeria monocytogenes* in Ready-to-Eat Seaweed Salads During Refrigerated Storage

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Consumption of ready-to-eat seaweed salads could pose potential health risks due to contamination with foodborne pathogens during seaweed harvesting or processing. The aim of this study was to evaluate the survival of two inoculated pathogens, *Bacillus cereus* group or *Listeria monocytogenes*, in different seaweed salads containing *Alaria esculenta* and/or *Saccharina latissima* during refrigerated storage at 5°C or 10°C, or at ambient temperature. The seaweed salads were made from raw, blanched, or brined seaweed, with or without an oil-vinegar marinade, and were evaluated for their psychochemical and microbiological properties during storage. The *B. cereus* group and *L. monocytogenes* were investigated at inoculum levels of 4 and 6 log(CFU/g), and 3–4 log(CFU/g), respectively. The non-inoculated samples showed that the dry matter of blanched seaweed was halved due to the release of nutrients, NaCl, and iodine during blanching. pH of blanched seaweed increased from pH 5 to neutral 7, while the pH of salads was lowered to 4 due to the acidic marinade. With or without an acidic environment, growth of *L. monocytogenes* was not supported for 7 days at 10°C, except in blanched seaweed, where *L. monocytogenes* grew 2 and 2.5 log in *S. latissima* and *A. esculenta*, respectively. The same was observed when the two seaweed species were mixed at a 2:1 ratio (blanched *S. latissima* and *A. esculenta*). However, lowering the temperature from 10°C to 5°C inhibited *L. monocytogenes*, resulting in levels below the limit of detection (1 log(CFU/g)). Even at an inoculum level of 6 log(CFU/g), the *B. cereus* group did not grow in raw or blanched seaweed under refrigerated storage conditions, compared with ambient temperature, where they grew 2 log in blanched *S. latissima*, while they survived in blanched *A. esculenta*. Regardless of the inoculation culture, other spore-forming bacteria became abundant in the salads, even those marinated, during storage at refrigerated or ambient conditions. Despite the change in the microbial community, the results indicate that the seaweed itself can inhibit the *B. cereus* group and *L. monocytogenes*; however, if the seaweed is blanched, it must be mixed with a marinade or refrigerated (5°C) to control food safety.

S4-P102: Optimization of Self-Assembled Monolayer Formation for an Aptamer-Based Electrochemical Biosensor Targeting *Salmonella* Typhimurium in Complex Food Matrices

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Food safety remains a critical global concern, particularly due to foodborne pathogens such as *Salmonella* spp., which pose significant public health risks and economic losses. Conventional culture-based detection methods are time-consuming (3–7 days) and often incompatible with the rapid production cycles of modern food systems. Although molecular techniques such as PCR and ELISA offer improved sensitivity, they still require enrichment and complex sample preparation steps. In this context, electrochemical biosensors have emerged as promising alternatives for rapid, sensitive, and selective pathogen detection.

In this study, an aptamer-based electrochemical biosensor was developed using gold screen-printed electrodes (Au-SPEs) for the detection of *Salmonella* Typhimurium. A self-assembled monolayer (SAM) was formed on the gold electrode surface using 3-mercaptopropionic acid (3-MPA) to enable covalent immobilization of an amine-modified *Salmonella*-specific aptamer via EDC/NHS chemistry. The stepwise surface modification and bacterial binding processes were characterized using Electrochemical Impedance Spectroscopy (EIS) and Cyclic Voltammetry (CV). To optimize sensor performance, the effect of 3-MPA concentration (0.1%, 0.5%, and 1% v/v) on SAM formation and electrochemical behavior was systematically investigated. The results demonstrated that increasing surface modification complexity led to a progressive increase in charge transfer resistance (Rct), confirming successful layer-by-layer assembly of the biosensing interface. The immobilization of the negatively charged aptamer further increased Rct due to electrostatic repulsion and steric hindrance against the $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ redox probe. Among the tested conditions, 0.5% (v/v) 3-MPA provided the optimal balance between surface coverage and molecular accessibility, resulting in stable and linear Rct responses with respect to bacterial concentration. Lower concentrations (0.1%) resulted in insufficient surface coverage, while higher concentrations (1%) caused excessive packing density and steric hindrance, leading to inconsistent sensor responses.

Overall, this study highlights the critical role of SAM optimization in enhancing the analytical performance of aptamer-based electrochemical biosensors. Ongoing work focuses on multi-parameter optimization, including aptamer concentration and incubation conditions, to further improve sensitivity, selectivity, and reproducibility for applications in complex food matrices.

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S4-P103: Extended Survival of Foodborne Pathogens in Organic Amendments: Influence of Temperature and Amendment Type

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The increasing use of organic amendments in sustainable food systems raises concerns regarding their potential to act as reservoirs of foodborne pathogens, posing a risk to preharvest food safety. Although animal-derived wastes often undergo thermal treatment, pathogens may survive or be reintroduced during storage. This study evaluated the survival of key foodborne pathogens in commercially available organic amendments under conditions representative of Ireland's mean winter (4°C) and summer (14°C) temperatures.

Three amendments were assessed: a farmyard manure-based compost and two pelletised products derived from poultry and horse manure. Samples were inoculated with multi-strain cocktails of *Salmonella*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Escherichia coli* O157:H7, or *Clostridium sporogenes* spores (~6 $\log_{10}$ CFU/g or spores/g). Pathogen survival was monitored over time using selective culture and enrichment methods, alongside measurements of pH and water activity (aw).

Pathogen survival varied significantly across amendment types and temperatures. Survival was prolonged in the farmyard manure-based amendment compared to pelletised amendments and was consistently greater at 4°C than at 14°C. Across all matrices, *L. monocytogenes* survived the longest, followed by *E. coli* O157, with *Salmonella* declining most rapidly. *C. jejuni* exhibited rapid inactivation in pelletised products, whereas prolonged survival (above 400 days) was observed in farmyard manure–based amendments. *C. sporogenes* spores persisted for over one year at both temperature conditions and across all matrices.

Difference in inactivation rates were associated with physiochemical parameters. The farmyard manure amendment exhibited a near-neutral pH (~6.0) and a higher water activity (0.98), conditions favourable for microbial survival. In contrast, pelletised amendments had lower aw (0.66–0.85), likely imposing osmotic stress and accelerating pathogen decline.

These findings highlight that selected organic amendments can support prolonged pathogen survival under typical storage conditions, highlighting the need for targeted risk mitigation strategies in sustainable agricultural systems.

S4-P104: From Milk of a Single Shedding Cow to Cheese: Surface-to-Core Distribution of *Listeria monocytogenes* in Raw Milk Semi-Hard Cheese

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Listeria monocytogenes in raw milk cheese remains a major food safety concern. Shedding from animals is a particularly insidious source of contamination, as it can be undetected for prolonged periods. In semi-hard cheeses, surface-to-core microenvironmental gradients may influence bacterial distribution and complicate risk assessment. This study describes a contamination event traced to a single cow and investigates the spatial distribution of *L. monocytogenes* in raw milk semi-hard cheese.

Following detection of contamination, bulk tank milk, retained milk samples from individual producers, individual cow milk samples, and cheese samples from the affected production batches were collected and subjected to microbiological analyses. Seven cheeses were analyzed qualitatively and quantitatively across defined zones (smear, lower smear, rind, and core). Two cheeses were additionally subjected to high-resolution spatial sampling. Fluorescence microscopy was used to identify *Listeria* within milk leukocytes. Whole genome sequencing (WGS) was performed on isolates from milk and cheese to assess relatedness.

A single cow was identified as the sole source of contamination, with all other animals on the farm returning negative results. *L. monocytogenes* was detected within milk leukocytes of the shedding cow. WGS confirmed a genetic match across multiple isolates recovered from the cow's milk sampled longitudinally over seven months, and the isolates from the corresponding cheese samples.

Quantitative analysis of seven cheeses revealed a consistent spatial pattern. Positive results were predominantly observed in the lower smear and rind, while the smear showed less consistent detection and was not a reliable indicator. The cheese core remained mainly negative; however, this likely reflects limited survival/growth of *L. monocytogenes* during ripening rather than absence of initial contamination.

Detailed spatial analysis of two cheeses confirmed pronounced gradients, again, with high concentrations in lower smear and rind and consistently low or non-detectable levels (< 10 CFU/g) in the core. Retrospective testing of retained milk samples from the cheese factory demonstrated persistent presence of *L. monocytogenes* over several months.

This study demonstrates that a single persistently shedding cow can constitute a long-term source of *L. monocytogenes* contamination in raw milk cheese production, with public health and economic consequences. The marked spatial heterogeneity of bacterial contamination within chesses indicate the critical importance of zone-specific sampling strategies, with rind-associated regions warranting particular attention. Reliance solely on smear and smear water sampling carries a significant risk of underestimating contamination load. Collectively, these findings highlight the need for continuous herd monitoring and rapid source identification to effectively minimize public health risks and economic impact.

S4-P105: Persistence of *Listeria monocytogenes* Under Salt Stress in Nutrient-Rich and Starvation Conditions

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Listeria monocytogenes is a major foodborne pathogen renowned for its remarkable ability to survive harsh environmental conditions. Among its many survival strategies, the viable but non-culturable (VBNC) state is of particular concern, as cells in this state remain alive yet fail to grow on routine culture media, potentially leading to a critical underestimation of contamination during food safety testing. This study aimed to investigate whether osmotic stress, applied under nutrient-rich and starvation conditions at different temperatures, could reduce the culturability of *L. monocytogenes* and induce VBNC state.

Overnight cultures were centrifuged, washed, and standardised to approximately 10^5 CFU/mL. Cells were exposed to NaCl at final concentrations of 6%, 7%, and 8% in either tryptic soy broth (TSB) or Ringer's solution and incubated at 37°C and 4°C for up to 7 days. Sampling was performed on days 1, 3, 5, and 7, after which stressed cells were transferred into fresh TSB to assess their recovery potential. Culturability was evaluated by serial dilution and plating on tryptic soy agar (TSA) and Brilliance agar. In parallel, recovery in liquid medium was monitored using a Bioscreen C system by measuring optical density over 48 hours.

L. monocytogenes maintained high culturability in TSB across all salt concentrations and both temperatures throughout the experimental period, suggesting strong tolerance to osmotic stress under nutrient-rich conditions. In contrast, exposure in Ringer's solution caused a marked and progressive decline in culturability, particularly at 37°C, where colony counts fell to near or below the detection limit by day 3 across all salt concentrations tested. Consistently lower recovery on Brilliance agar compared to TSA indicated the presence of sublethally injured cells across multiple conditions. Bioscreen growth curves further revealed delayed lag phases and reduced overall recovery for Ringer's-stressed samples, particularly at higher salt concentrations and extended exposure durations.

These preliminary findings suggest that the combination of starvation (Ringer solution) and NaCl stress, particularly at 37°C, is a promising approach for inducing loss of culturability and VBNC-like behaviour in *L. monocytogenes*.

S4-P106: Antimicrobial Activity of Limonene Nanoemulsions Against Planktonic Cells and Biofilms of *Staphylococcus aureus* in Food Environments

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Staphylococcus aureus is a major foodborne pathogen responsible for one of the most prevalent forms of food poisoning globally, primarily through the production of heat-stable enterotoxins that remain active even after thermal processing. Its capacity to form robust biofilms on food contact surfaces, including stainless steel, plastic, and glass, poses a critical challenge for the food industry, as biofilm-embedded cells exhibit markedly increased resistance to conventional sanitizers and disinfectants. The development of natural antimicrobial agents with effective antibiofilm activity is therefore of considerable interest for improving food safety and extending shelf life.

Limonene, a monocyclic monoterpene and major constituent of citrus essential oils, has attracted growing attention as a Generally Recognized As Safe (GRAS) antimicrobial compound suitable for food applications. Nevertheless, its practical use is constrained by its hydrophobic nature, volatility, and poor dispersibility in aqueous food matrices. Nanoemulsion technology provides an effective solution to these limitations, enabling the encapsulation of limonene in oil-in-water systems with droplet sizes below 200 nm, resulting in improved stability, solubility, and antimicrobial efficacy.

In this study, limonene nanoemulsions (LNE) were prepared by high-energy emulsification and characterized physicochemically. Their antimicrobial activity against planktonic *S. aureus* was determined by broth microdilution, and antibiofilm efficacy was assessed on stainless steel coupons by crystal violet staining, viable cell counting, and confocal microscopy. LNE showed significantly lower MIC values than free limonene and effectively reduced biofilm formation and pre-established biofilm biomass. These results support the potential of LNE as a natural, food-compatible antimicrobial strategy for controlling *S. aureus* in food processing environments.

S4-P107: Genotypic Characteristics of Foodborne Pathogens Isolated from Organic Meat in Poland

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The consumption of organic food is increasing. In organic farming, animals are free-range, which may increase the risk of infection with various pathogens. The aim of this study was the genotypic characterization of bacterial pathogens isolated from organic meat. A total of 109 isolates of *Listeria monocytogenes* (39), *Staphylococcus aureus* (37), *Campylobacter* (20), *Salmonella* (8), and Shiga toxin–producing *Escherichia coli* (STEC) (4) were subjected to WGS.

The *flaA* and *flaB* genes, encoding flagellar proteins that facilitate cell movement, as well as the *iamB* maker (responsible for adherence and invasiveness), were identified in all *Campylobacter* isolates. The *cadF* gene, encoding a fibronectin-binding protein, was detected in 15 (93.8%) isolates, and the *iamB* gene in 13 (81.3%) isolates. The *cdtA*, *cdtB*, and *cdtC* genes, associated with the CDT toxin production, were found in 10 (62.5%), 13 (81.3%), and 4 (25.0%) *Campylobacter* isolates, respectively.

Most of *L. monocytogenes* isolates harbored the genes located on the *Listeria* pathogenicity island LIPI-1. These genes are essential for intracellular growth, multiplication, and spread to neighboring cells. Most isolates possessed genes encoding internalins, surface proteins that facilitate pathogen entry into target cells. The *inlA*, *inlB*, *inlC*, *inlE*, and *inlJ* genes were identified in all isolates, while *inlH* was found in 38 (97.4%), *inlK* in 36 (92.3%), *inlG* in 29 (74.4%), and *inlF* in 25 (64.1%) *Listera* isolates. All isolates carried the *iap* gene, which is necessary for cell viability, and its product, the p60 protein, has the bactericidal activity. One isolate (2.6%) possessed the gene from LIPI-3, which encodes the bacteriocin listeriolysin S, a hemolytic and cytotoxic factor.

In the genome of *Salmonella* isolates (*S.* Enteritidis, *S.* Indiana, *S.* Infantis), 11 pathogenicity islands were identified, containing the genes encoding numerous proteins involved in the pathogenic process.

All *S. aureus* isolates possessed the *aur* gene, which encodes aureolysin. The *mecA* gene, responsible for methicillin resistance, was identified in four isolates. Genes encoding enterotoxins (A, C, G, H, I, L, M, N, O, P, U) were found in the genome of 27 isolates.

The *stx1* and *stx2* genes were identified in three and one STEC isolates, respectively. The presence of the *ehxA* gene, encoding enterohemolysin, the *scgA* sequence (encoding a major biofilm component), and the *fdeC* and *hra* genes were identified in all isolates.

The prevalence of bacterial pathogens in organic meat and their high genetic diversity suggest a potential public health risk associated with this type of food.

S4-P108: Evaluation of Nitrite Reduction Strategies in Bacon: Implications for Control of *Listeria monocytogenes* and *Clostridium sporogenes*

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The increasing demand for safer and higher-quality pork products has intensified the need to reassess the use of curing agents such as nitrite. Beyond its technological role in developing color, flavor, and texture, nitrite is widely recognized for its antimicrobial activity, particularly in ready-to-eat meat products. In light of recent regulatory efforts within the European Union to reduce permitted nitrite levels, it is essential to evaluate the impact of such reductions on product safety. This study aimed to assess the antimicrobial efficacy of standard (150 ppm) and reduced (80 ppm) nitrite concentrations in bacon formulations, in comparison to a nitrite-free control, against relevant foodborne pathogens.

Three bacon formulations were produced: a conventional formulation containing 150 ppm nitrite, a reduced formulation with 80 ppm, and a control without nitrite. To evaluate spore-forming bacteria, raw bacon was inoculated with spores of *Clostridium sporogenes*, followed by a heat treatment to stimulate germination. In parallel, post-processing contamination was simulated by inoculating sliced bacon with a cocktail of *Listeria monocytogenes*. Samples were stored under both refrigeration (4°C) and temperature abuse conditions (8°C) and analyzed over time, up to 113 days for *C. sporogenes* and 35 days for *L. monocytogenes*.

Nitrite-containing formulations demonstrated a clear inhibitory effect on *L. monocytogenes*, particularly during the early stages of storage. Compared to the nitrite-free control, both nitrite concentrations resulted in significantly lower bacterial counts. The standard formulation (150 ppm) showed the greatest inactivation by the end of storage ($p < 0.05$). Under abused temperature conditions, the antimicrobial effect was diminished, with reductions generally below 0.5 log units. However, at later stages of storage, both nitrite-containing formulations were still able to limit *L. monocytogenes* proliferation compared to the control, which exhibited an increase of approximately 3 log units ($p < 0.05$), although no significant differences were observed between the standard and reduced nitrite treatments ($p > 0.05$). Regarding *C. sporogenes*, nitrite contributed to growth control throughout storage. However, no significant differences were observed between the standard and reduced nitrite formulations, regardless of storage temperature.

S4-P109: Effects of Lactic Acid Fermentation on the Phenolic Architecture and Multifunctional Bioactivity of Murta Juice (*Ugni molinae*)

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Lactic fermentation is an effective strategy to enhance the functional properties of plant-based foods by modifying their phenolic composition. This study investigated how controlled lactic acid fermentation modulates extractable (EP) and hydrolysable (HP) polyphenol fractions in murta (*Ugni molinae*) juice and how these compositional changes translate into antimicrobial, antidiabetic, and intracellular antioxidant bioactivities.

Murta juice was fermented using lactic acid bacteria in monoculture and coculture systems, generating distinct phenolic profiles. The bioactivity of EP and HP fractions was evaluated through *in vitro* antimicrobial assays against foodborne pathogens, inhibition of carbohydrate-digesting enzymes (α-amylase, α-glucosidase, and dipeptidyl peptidase IV), and cell-based assays assessing viability and intracellular reactive oxygen species (ROS) in Caco-2 cells. Multivariate analysis and phenolic-bioactivity correlation networks were used to integrate phenolic composition with functional responses.

Fermentation significantly enhanced the multifunctional bioactivity of murta juice in a fraction- and process-dependent manner. EP fractions obtained from coculture fermentation exhibited strong dose-dependent antimicrobial activity, achieving up to 70% growth inhibition against *Salmonella enterica*, 55% against *Escherichia coli*, and 50% against *Staphylococcus aureus*. These fractions also showed marked inhibition of α-amylase and α-glucosidase, achieving approximately 70–90% inhibition, indicating a rapid functional response associated with readily available phenolics. In contrast, HP fractions displayed moderate antimicrobial effects but contributed more substantially to dipeptidyl peptidase IV inhibition, achieving up to ~50% inhibition, and showed a pronounced capacity to reduce intracellular ROS levels in Caco-2 cells. None of the fermented fractions exhibited cytotoxic effects at the evaluated concentrations.

Correlation network analysis revealed a dual functional architecture underlying the observed bioactivity. Enzyme inhibition and cellular antioxidant protection were primarily associated with specific phenolic compounds, whereas antimicrobial activity emerged from combined phenolic interactions rather than from a single dominant metabolite.

Overall, the results demonstrate that lactic acid fermentation acts as a biochemical modulator, redistributing phenolic functionality between the EP and HP fractions, generating predictable and complementary bioactivity profiles. This integrative approach provides a rational basis for designing targeted fermentation strategies to develop functional beverages and fermented berry-based ingredients with tailored health-related properties.

S4-P110: Microbiological and Antimicrobial Resistance (AMR) Contamination in Salmon and Whole-Head Lettuce Samples on Sale in UK Supermarket Stores

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Monitoring microbiological hazards in the food chain is required to ensure that food reaching consumers is safe. As well as the presence of pathogenic bacteria, it is also important to survey antimicrobial resistance (AMR). While there have been several studies focusing on retail meat, surveillance data for other food commodities is scarce. Here we describe the results of two UK surveys conducted on raw salmon and whole-head lettuce respectively.

Samples were collected following consumer-like purchasing behaviours in top UK supermarkets proportionally selected based on market share data and using a stratified approach to represent all UK regions. Raw salmon (*n* = 307; collected between January and December 2024) was tested for *Listeria monocytogenes* and *Listeria* species and whole-head lettuce (*n* = 316; collected between October 2024 and September 2025) was tested for Shiga-toxin producing *Escherichia coli* (STEC) and *Salmonella*, following in-house methods based on ISO standards. Additionally, all samples were tested for *E. coli* including those with extended-spectrum beta lactamase (ESBL) and/or AmpC-phenotypes or with resistance to carbapenems and colistin using selective media. Minimum inhibitory concentrations (MIC) for a series of antimicrobials in accordance with Assimilated Decision 2020/1729/EU were determined for all *E. coli* isolates. Pathogens isolated were characterised using whole genome sequencing.

L. monocytogenes at low levels (< 20 CFU/g) were detected in 1.6% of salmon fillets tested and were not resistant to common antibiotics. *L. monocytogenes* isolates were characterised as sequence types (ST) 7 (clonal complex CC7), ST8 (CC8), ST18 (CC18), ST121 (CC121) and ST425 (CC90) respectively (all lineage II; serotype 1/2a) with two of them (ST8 and ST121) clustering closely to patient isolates. *Listeria* species other than *L. monocytogenes* were detected in 10.1% of the salmon samples. No STEC or *Salmonella* were isolated from any of the whole-head lettuces. The *stx* gene (encoding for the Shiga toxin) was detected in one lettuce. No AMR *E. coli* were detected using selective media in either survey. Generic *E. coli* were detected in 35.2% of the salmon and in 7.7 % of the whole-head lettuce tested, with all samples having a level of 20 or < 20 CFU/g. Of 102 generic *E. coli* isolates examined from salmon, 3.9% were resistant to ampicillin. None of 24 *E. coli* isolates from lettuce showed any resistance.

Both surveys provide re-assurance of the low microbiological risk to UK consumers from pathogenic bacteria and AMR bacteria in raw salmon and whole-head lettuce available at retail supermarket stores across the UK.

S4-P111: *Listeria monocytogenes* Control and the Microbiome in Different Bacon Formulations

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Nitrites are added to processed meats to inhibit *Clostridium botulinum*, prevent lipid oxidation and to contribute to the colour and flavour. They also preserve the product, inhibiting other pathogenic and spoilage bacteria. The presence of carcinogenic N-nitrosamines, all-be-it at low concentrations, is driving research to reduce the use of nitrites (and nitrates) and to identify alternatives. In this study, seven alternative antimicrobial compounds were screened against *Listeria monocytogenes* in laboratory-based assays. Compounds that inhibited visible growth were subsequently evaluated in a meat slurry system and the most effective candidates (designated compounds A and B) were selected for further testing in bacon formulations. Seven formulations were prepared: [1] 0 ppm sodium nitrite and 0 ppm sodium nitrate; [2] 55 ppm sodium nitrite and 11 ppm sodium nitrate; [3] 120 ppm sodium nitrite and 126 ppm sodium nitrate; [4] 2% (w/w) compound A; [5] 2% (w/w) compound A with 55 ppm sodium nitrite and 11 ppm sodium nitrate; [6] 1% (w/w) compound B; and [7] 1% (w/w) compound B with 55 ppm sodium nitrite and 11 ppm sodium nitrate. All samples were inoculated with *L. monocytogenes* (ATCC 35152) and incubated under aerobic and vacuum-packaged conditions at 12°C. Microbial shelf-life and microbiota studies were also conducted in uninoculated samples under vacuum-packaged conditions at 4°C. Samples were taken periodically and microbial cells enumerated. Under aerobic conditions, the growth of *L. monocytogenes* was significantly higher ($p < 0.05$) in formulations [2] and [3], compared to control [1]. The growth of the pathogen was inhibited in presence of the two alternative compounds ($p < 0.05$) at both packaging systems. In terms of self-life studies, the growth of spoilage microorganisms was significantly lower ($p < 0.05$) in formulations [4]–[7], compared to [1]–[3]. It was concluded that both compounds could serve as nitrites and nitrates replacements to assure microbial safety and extend the self-life in novel bacon formulations. The results will be presented and discussed including the future application of compounds A and B as alternatives to nitrites and nitrates in processed meats.

S4-P112: Assessing microbial quality and Listeria monocytogenes survival in RTE lettuce under MAP using natural antimicrobials and multispectral imaging

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Introduction: Fresh-cut lettuce is a highly perishable product with a limited shelf life due to its high moisture content and susceptibility to microbial growth. Natural antimicrobial agents derived from fruits have gained attention as potential alternatives for extending shelf life and improving food safety. In addition, non-destructive techniques like multispectral imaging can provide valuable information on product quality.

Purpose: This study aimed to evaluate the effectiveness of two natural antimicrobial treatments (20% pomegranate solution and a mixture of 10% pomegranate and 10% lemon solution) on the quality and shelf life of fresh-cut lettuce stored under modified atmosphere packaging (MAP). Additionally, their efficacy under pathogen inoculation conditions was assessed. The study also investigated the potential of multispectral imaging (MSI), combined with predictive modeling, to discriminate between inoculated and non-inoculated samples.

Methods: Fresh-cut Romaine lettuce samples were packaged under MAP conditions (10% CO_2 / 10% O_2 / 80% N_2) and subjected to three treatments: (i) control, (ii) immersion in 20% pomegranate solution, and (iii) immersion in a mixture of 10% pomegranate and 10% lemon extracts. Samples were stored at 4°C and 12°C until spoilage. Microbiological counts and pH were monitored throughout storage. In a second experiment, samples were inoculated with Listeria monocytogenes (3 log CFU/g) and treatments were applied via spraying. MSI data were collected using two systems (Benchtop-MSI and Portable-MSI) and combined with analytical measurements to develop predictive classification models.

Results: Natural antimicrobial treatments resulted in an initial microbial reduction of up to 1 log CFU/g at both storage temperatures and maintained lower microbial growth compared to controls. In the pomegranate–lemon treatment, pH decreased rapidly during the first two days of storage, from 6.4 to 5.5 at both temperatures. In inoculated samples, pomegranate treatment effectively inhibited Listeria monocytogenes, maintaining levels around 3.5 log CFU/g, whereas control samples reached approximately 5 log CFU/g. Predictive modeling based on MSI data successfully distinguished between inoculated and non-inoculated samples, achieving classification accuracy of up to 95%.
Conclusion: The results demonstrate the potential of fruit-derived natural antimicrobials as effective bio-preservation agents for fresh-cut lettuce. Furthermore, MSI combined with predictive modeling represents a promising non-destructive approach for detecting microbial contamination and assessing product quality.

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S4-P113: Kanamycin-Resistant *Salmonella* Maintains Growth Under Antibiotic Pressure with Variable Fitness Costs

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Antimicrobial resistance (AMR) represents a major public health concern worldwide. *Salmonella* spp., one of the leading causes of foodborne disease outbreaks, has developed resistance to antibiotics used in both human and veterinary medicine, including kanamycin (KAN). However, the impact of these resistance determinants on growth fitness and their ability to grow under conditions commonly encountered in food industry settings remains unclear. Therefore, the aim of this study was to evaluate the impact on growth capability associated with KAN resistance in *Salmonella* Typhimurium LT2 under selective and non-selective conditions.

To achieve this, five kanamycin-resistant variants (RVs) were obtained through adaptive laboratory evolution under progressively increasing antibiotic concentrations, with a daily increment of 85%, across 11 sequential passages (SeT_KAN1–5). Growth curves were generated to compare their growth dynamics with those of SeT under both selective and non-selective conditions. Cultures were grown in Mueller Hinton Broth (MHB) at an initial concentration of 10^5 CFU/mL and incubated at 37°C for 14 h in the presence or absence of KAN at 1x the minimum inhibitory concentration. Optical density at 595 nm was recorded every 15 minutes. Growth curves were fitted using a modified Gompertz model to estimate lag time and maximum specific growth rate.

Under antibiotic exposure, the parental strain showed no growth, whereas all RVs retained the ability to grow in the presence of KAN. In the absence of selective pressure, most RVs exhibited growth parameters comparable to the parental strain, although SeT_KAN1 and SeT_KAN2 showed reductions in growth rate of 78% and 41%, respectively. SeT_KAN1 maintained similar growth rates regardless of selective pressure, while the remaining RVs exhibited reductions in growth rate ranging from 27% to 44% in the presence of KAN. No increases in lag time were observed for any RV in the presence of KAN relative to the corresponding condition without antibiotic.

These findings suggest that the acquisition of KAN resistance in *Salmonella* Typhimurium may entail variable fitness costs, affecting growth dynamics under both selective and non-selective conditions. Such alterations in growth behavior could influence competitiveness of resistant strains in environments related to food safety. Further studies are needed to clarify the mechanisms underlying these changes and to assess their potential implications for the survival and transmission of resistant Salmonella in the food chain.

S4-P114: Genome Wide Fitness Profiling of Food-Borne Pathogens on Relevant Food Matrices

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Persistence of pathogens in non-host environments requires rapid adaptation to complex and fluctuating stress conditions. For food-borne pathogens like *Salmonella enterica* and *Listeria monocytogenes*, survival on the food matrix represents a critical, yet understudied phase of the infection cycle. It is characterized by exposure to low temperature, matrix-specific stress factors, changes in nutrient availability, and competition with native microbiota.

Here, we present a comparative, genome-wide analysis of stress adaptation mechanisms that contribute to the fitness of these food-borne pathogens on relevant ready-to-eat foods under conditions that mimic real-world food environments. Identifying highly specific intervention targets may inform highly targeted interventions in high-risk food systems as additional hurdles that complement existing food safety measures.

We used transposon insertion sequencing to identify genetic determinants that contribute to pathogen fitness in several food systems: *S. enterica* on raw melon, raw onions and sprouts, and *L. monocytogenes* on deli meat and cold-smoked salmon.

Our results reveal a core set of stress-response functions under selection across conditions, including pathways involved in cell envelope integrity, global stress regulation, RNA turnover, energy metabolism, resource recycling and motility. However, these responses were highly context-dependent, and we observed distinct metabolic rewiring that is integral to stress resilience in different niches.

These genome-wide findings were validated by competition assays with individual mutants against their wild-type strains to verify phenotypes.

Together, our work highlights how *S. enterica* and *L. monocytogenes* integrate canonical stress-response pathways with niche-specific metabolic adaptations to survive outside animal hosts. This approach offers an opportunity to move toward precision food-safety interventions tailored to individual high-risk food matrices that lack classical kill steps or critical control points in their production process. We present a framework for predictive, mechanism-driven risk mitigation strategies that increase efficacy while minimizing impacts on product quality or beneficial microbiota.

S4-P115: Pathogenic Assessment of Enterobacter Hormachei Isolated from Food of Animal Origin

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The food production chain has been widely recognized as a route of dissemination of pathogenic bacteria posing a heightened risk to public health. Enterobacter hormachei belongs to Enterobacter cloacae species complex, one of the most common Enterobacteriaceae groups leading to nosocomial infections. In a previous study, the virulome of E. hormachei strains isolated from artisanal salami and cheese was investigate. In the present study the pathogenic potential of a selection of foodborne E. hormachei strains was assessed using larvae of Galleria mellonella as in vivo infection model. Results were compared to the respective virulence gene patterns. A total of 120 larvae were infected with 10 ul of bacterial culture suspensions of approx. 10^6 CFU/ml. Thirty larvae per strain were infected for a total of 4 strains. Negative controls as larvae injected with PBS and not-injected larvae were also included. After five days of incubation at 37 °C, the cumulative survival of larvae infected with four strains were 3, 6, 10 and 33% respectively, with 33% value being statistically significantly different in comparison to the other three (p<0,001). Interestingly only the strain with 10% of cumulative survival carried the gene astA previously identified as a genetic marker of pathogenic E. hormachei isolated from nosocomial settings, suggesting the potential pathogenicity for humans of foodborne astaA negative E. hormachei.

S5 Food and Gut Microbiomes for Human Health Benefits

S5-P1: Effect of Oxidative Stress Adaptation on the Survival of *Bifidobacterium* spp. in Yoghurt

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Bifidobacterium spp. are considered the most beneficial probiotic bacterial genera and species, with an extensive range of health benefits linked to their inclusion in foods. However, as strict anaerobes, *Bifidobacterium* spp. are highly sensitive to oxygen, making them susceptible to oxidative stress effects during yoghurt production. This study investigated the impact of oxidative stress adaptation on *Bifidobacterium* spp. viability, fermentation characteristics, and physicochemical properties during yoghurt fermentation and shelf-life at 4°C.

Oxidative (H_2O_2) stress-adapted and unadapted strains of *Bifidobacterium bifidum*, *Bifidobacterium breve* and *Bifidobacterium animalis* subsp. *animalis* were incorporated at the onset of the yoghurt fermentation at 6 to 7 log CFU/mL. Viability (as determined by plate counts and PMAxx-qPCR) and physicochemical properties were monitored over 28 days at 4°C.

Stress-adaptation enhanced *B. bifidum* survival during fermentation by 1.0 log CFU/g compared to unadapted strains ($p < 0.0001$), with *B. breve* showing similar improvement, though this advantage diminished throughout shelf-life in both species. *B. animalis* remained stable regardless of the adaptation treatment. While the plate count method showed a decline in viability of *B. bifidum* and *B. breve*, the PMAxx-qPCR method detected a significantly higher level of viable cells ($p < 0.05$) in the yoghurt. Oxidative stress adaptation may enhance the viability and functional value of *Bifidobacterium* spp. in probiotic yoghurt.

S5-P2: Lactic Acid Bacteria Fermentation as a Tool to Develop an Innovative Lupin-Based Functional Beverage with a Focus on Intestinal Microbiota Modulation

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Lupin has recently gained scientific interest due to its high protein and dietary fiber contents, reduced fat and richness in bioactive compounds. Among the currently available strategies used to remove alkaloids, biological approaches involving fermentation by selected lactic acid bacteria (LAB) strains may offer a promising alternative to conventional methods for enhancing the overall nutritional value of lupin. Within this framework, this present study aims to develop an innovative lupin-based fermented beverage with a focus on its potential impact on gut microbiota modulation.

After optimizing the protocol to obtain the beverage from lupin seeds, 24 LAB strains belonging to *Lactiplantibacillus plantarum*, *Lacticaseibacillus paracasei*, *Leuconostoc mesenteroides* and *Lacticaseibacillus rhamnosus*, were applied to perform single-strain fermentations, monitored by physico-chemical (pH, titratable acidity) and microbiological analysis. In order to assess the effect of fermentation on the alkaloid profile, the main lupin alkaloids (lupanine, sparteine, multiflorine, albine, angustifoline, hydroxylupanine) were quantified by HPLC-MS/MS. All tested strains demonstrated the ability to reduce the total alkaloid content in a strain-specific manner and, for some of them, the residual levels were below the maximum legal limit (200 mg/kg). In particular, *L. plantarum* O13 showed the most marked reduction (111.55 ± 1.1 mg/kg) and was therefore selected for the production of the lupin-based beverage. In order to investigate its potential beneficial properties, the beverage was then subjected to a simulated colonic fecal fermentation in a small-scale bioreactor system (Gut Model System), inoculated with human feces from healthy donors. Microbiomics analysis by quantitative real-time PCR (qPCR) were performed to evaluate the effects of the fermented product on specific gut microbial taxa.

Overall, the results indicated that the lupin-based beverage did not negatively affect the composition of a healthy human microbiota, while stimulating a significant increase in *Bifidobacterium longum*, commonly associated with a beneficial microbiota profile. Collectively, the variations observed in specific bacterial populations suggest that the beverage may influence the microbial composition, without disturbing its homeostatic overall balance.

S5-P3: Probiotics and the Microbiome–Brain Axis: Implications for Pain Modulation and Neurological Recovery

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The microbiome–brain axis is a complex communication network that includes bidirectional interactions between the gut microbiota and the central nervous system through immune, neural, and metabolic pathways. Probiotics may assist in modulating this axis, influencing both neurological recovery and pain perception, especially in neurosurgical and potential neurosurgical patients.

This study integrates findings from literature reviews examining probiotic effects in (*i*) patients with low back pain and (*ii*) patients following brain surgery. Literature searches were conducted in ScienceDirect, CINAHL, PubMed, MEDLINE, and Wiley databases, following PRISMA principles. A total of 11 studies were included, five for low back pain and 6 for post-neurosurgical patients. The included studies comprised randomized controlled trials, systematic reviews, and meta-analyses.

In low back pain, probiotic supplementation showed minimal and statistically non-significant effects on pain reduction despite evidence of immunomodulatory activity. For example, long-term supplementation with *Lacticaseibacillus rhamnosus* GG resulted in only a small reduction in pain scores, without meaningful changes in disability or overall clinical outcomes.

In neurological patients, probiotics were associated with clinically relevant improvements. These included reduced inflammatory markers (e.g., CRP, IL-6, TNF-α), decreased infection rates, lower mortality risk, shorter hospitalisation, and faster recovery of gastrointestinal function. In patients with traumatic brain injury, probiotic supplementation combined with enteral nutrition significantly reduced inflammatory markers and improved clinical scores, while meta-analyses confirmed reductions in infection rates, gastrointestinal complications, and length of stay in intensive care units.

These effects are linked to modulation of the microbiome–brain axis via regulation of cytokine production, enhancement of gut barrier integrity, and production of neuroactive metabolites (e.g., short-chain fatty acids, tryptophan derivatives). Strain-specific effects were evident. The strains used in the clinical trials included: *L. rhamnosus* GG, *Bifidobacterium animalis* subsp. *lactis* BB-12, *B. animalis* subsp. *lactis* HN019, *Limosilactobacillus reuteri* HA188, *Saccharomyces cerevisiae* var. *boulardii*, as well as several multistrain probiotic formulations. These were mainly associated with anti-inflammatory effects, improved gut motility, and better gastrointestinal outcomes after surgery.

Although the clinical effects remained limited in pain-related conditions, stronger benefits were observed in the neurological recovery; these findings suggest that the microbiome–brain axis represents an important target for probiotic interventions. However, heterogeneity in study design, strain selection, and dosage and duration limits comparability. Future research should focus on strain-specific, well-designed clinical trials that combine microbiome and neuroimmune biomarkers to better define the potential therapeutic potential of probiotics.

S5-P4: Growth of the Probiotic Strain *Lacticaseibacillus rhamnosus* GG in a Rice-Based Beverage

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Lactic acid bacteria (LAB) represent a diverse group of microorganisms associated with plants (cabbage, corn, barley), meat, and dairy products. LAB also play an important role in the food industry in the processing of meat, alcoholic beverages, and vegetables. *Lacticaseibacillus rhamnosus* GG (LGG) was the first strain of the genus *Lactobacillus* (before reclassification) to be patented in 1989 due to its ability to survive and multiply at the acidic pH of the stomach, in the presence of bile, and its ability to adhere to enterocytes. The aim of this study was to evaluate the growth behavior of the probiotic strain *L. rhamnosus* GG in a commercially available rice-based beverage under varying temperatures ranging from 12 to 44°C, and to analyze the experimental results using principles of predictive microbiology. A traditional three-step approach was used for the kinetic analysis of the experimental data. In the first step, the measured growth data of LGG were described using the Baranyi-Roberts model for the mean value of h_0. In the second step, the effect of temperature on the key kinetic growth parameters, the maximum specific growth rate (μ_{max}) and the lag phase (λ), was described using the cardinal model. By linking the primary and secondary models, a tertiary model was developed in the third step, suitable for direct prediction of LGG growth. In the rice-based beverage, *L. rhamnosus* GG reached a maximum average population of 7.65 ± 0.25 log CFU/mL (CV = 3.3%) in the stationary phase (the initial average count was 2.77 ± 0.11 log CFU/mL (CV = 4.0%)). The μ_{max} at the lowest cultivation temperature was 0.042 h^{-1}, while at 37°C the highest specific growth rate of 0.755 h^{-1} was observed. The following cardinal values: T_{min} = 5.4°C, T_{opt} = 40.0°C, T_{max} = 46.8°C, and μ_{opt} = 0.757 h^{-1} resulted from secondary modelling.

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S5-P5: Postbiotics from Fermented Foods: Biological Effects and Potential for Health Promotion

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Fermented foods have long been part of the human diet, traditionally valued for their improved shelf life and sensory properties. In recent years, scientific interest has increasingly focused on their health-promoting components, particularly postbiotics. According to the International Scientific Association for Probiotics and Prebiotics (ISAPP), postbiotics are defined as preparations of inanimate microorganisms and/or their components that confer a health benefit on the host. These bioactive compounds, formed during fermentation or released after microbial inactivation, represent a promising yet still emerging area in food microbiology. A systematic literature review was conducted using PubMed, Scopus, Web of Science, and Wiley Online Library. The search strategy included terms related to postbiotics and fermented foods, without time restriction, and was based on predefined inclusion and exclusion criteria. A total of 26 studies (including *in vitro*, *in vivo*, and clinical studies) met the inclusion criteria and were analyzed using descriptive and comparative methods to identify common patterns, biological effects, and research gaps. The analyzed studies demonstrate that postbiotics derived from fermented foods exhibit multiple beneficial biological effects. Antimicrobial activity was consistently observed, mainly due to organic acids, bacteriocins, and other metabolites that inhibit pathogenic microorganisms. A key finding across studies is their immunomodulatory potential, including the stimulation of both innate and adaptive immune responses, such as enhanced cytokine production and improved immune signaling pathways. Postbiotics are also important for maintaining intestinal barrier integrity, as evidenced by reduced intestinal permeability and increased transepithelial resistance in both experimental and clinical settings. Additionally, they contribute to the maintenance of a balanced gut microbiota (eubiosis) and the reduction of inflammation. Notably, their effects extend beyond the gastrointestinal tract. Anti-inflammatory, antioxidant, antihypertensive, and metabolic benefits have been reported, suggesting broader systemic health impacts. Compared to probiotics, postbiotics may offer advantages in terms of safety, stability, and shelf life, as they do not contain viable microorganisms, which makes them particularly suitable for vulnerable populations. Postbiotics from fermented foods represent a promising functional component with multi-target effects on host health. While current evidence supports their antimicrobial, immunomodulatory, and gut-protective roles further well-designed randomized controlled trials are needed to confirm clinical efficacy, elucidate mechanisms of action, and establish optimal application strategies in functional foods. Addressing current limitations will be essential for translating experimental findings into evidence-based dietary recommendations and practical applications in health promotion.

S5-P6: Evaluation of the Antimicrobial Potency and Storage Stability of Anthocyanin-Rich Extracts from Cypriot Flora Against Foodborne Pathogens

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Anthocyanins, a class of water-soluble polyphenolic pigments responsible for the diverse red, purple, and blue hues in various flora, have emerged as potent natural antimicrobial agents with significant potential for enhancing food safety and extending shelf stability. Research indicates that these compounds exhibit broad-spectrum activity against both Gram-positive and Gram-negative bacteria. Their antimicrobial efficacy is primarily attributed to a multi-targeted mechanism that disrupts the integrity of microbial cell walls and plasma membranes, resulting in the leakage of intracellular components and subsequent cell lysis. The objective of the present study is to evaluate the antimicrobial potency of anthocyanin-rich plant extracts against a panel of foodborne microorganisms, specifically: *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enterica*, *Saccharomyces cerevisiae*, and *Candida* sp.. The extracts were derived from plant materials of ecological or economic significance to Cyprus, including lentisk fruits (*Pistacia lentiscus*), mulberry fruits (*Morus nigra*), grape skins (*Vitis vinifer*a), and Damask rose buds (*Rosa* × *damascena*).

Results demonstrated robust antimicrobial activity across the extracts, although responses varied significantly between plant materials. As hypothesized, the anthocyanin extracts exhibited greater efficacy against bacteria than fungal species. Furthermore, a definitive ranking of the extracts based on universal potency proved elusive, as distinct inhibitory profiles were observed for each microorganism. Additionally, the stability of the antimicrobial potency was assessed under realistic food storage conditions. Specifically, 25% (w/w) aqueous solutions were stored at 4°C and 25°C under both dark and light-exposed (470 lumens) environments. The findings indicate that antimicrobial efficacy is highly sensitive to storage conditions, with observed reductions in activity ranging from 10.1% to 50.8%. Subsequently, the anthocyanin profiles were analyzed via High-Performance Liquid Chromatography (HPLC) to characterize compositional shifts and correlate these changes with fluctuations in antimicrobial potency. Overall, this work underscores the potential of anthocyanin extracts as natural preservatives and highlights the necessity of selecting specific extracts based on target spoilage organisms and intended storage parameters.

S5-P7: From By-Product to Bioactivity: Chicken Bone Peptides Shape the Gut Microbiome in SHIME®

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Poultry slaughterhouse by-products, particularly chicken bones, represent an underexploited source of bioactive peptides with potential applications as functional food ingredients. The present study evaluated the impact of chicken bone–derived peptides on the human gut microbiome using the validated Simulator of the Human Intestinal Microbial Ecosystem (SHIME®) model. Chicken bones (1:2 w/v, bone : water) were subjected to thermal treatment (121°C for 2 h) followed by enzymatic hydrolysis with Alcalase® (EC 3.4.21.62), under controlled conditions at pH 8.0 and 50°C for 180 min to obtain peptide fractions. After hydrolysis, the enzyme was inactivated by heating at 90°C for 15 min. Their effects were investigated through a long-term twin-stage colonic L-SHIME® experiment inoculated with faecal microbiota from one healthy adult donor. The test product was supplemented directly to the artificial diet at the stomach compartment and compared with a negative control receiving only the basal diet. The treatment simulated a dietary supplementation of 3 g/day of the studied peptides. Samples were collected before treatment (day 0), during the treatment phase for 16 days, and during a 9-day washout period (every three days). Microbial activity was evaluated through short-chain fatty acid (SCFA) production, while microbial community composition was assessed using a metataxonomic approach. During peptide supplementation, concentrations of acetic and propionic acids (mM) significantly increased compared with the control condition, in which SCFA levels remained relatively stable. This trend was consistent across the three simulated colon regions. SCFA concentrations returned to levels comparable to the control during the washout phase, suggesting a reversible microbial response. Changes in microbial community composition were also observed following peptide supplementation. In particular, the relative abundance of *Prevotella* spp. and *Phocaeicola* spp. differed significantly between treatment and control conditions. *Prevotella* spp. increased during peptide supplementation, whereas *Phocaeicola* spp. were more abundant in the control samples.

Overall, these results indicate that chicken bone–derived peptides can modulate gut microbial metabolism and composition. This study highlights the potential of poultry processing by-products as sustainable sources of functional ingredients for gut microbiome modulation.

S5-P8: The Dogma Project: Exploring the Potential of Traditional Fermented Foods to Restore Gut Microbiome Diversity

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Over recent decades, the progressive Westernization of lifestyle, together with the decrease in overall microbial exposure linked to hygiene practices typical of industrialized societies, has profoundly reshaped the human gut microbiome. A consistent body of evidence indicates a marked loss of microbial diversity in industrialized populations compared to rural or traditional communities, associated with the global rise of diet-associated chronic diseases such as obesity, type 2 diabetes, and inflammatory disorders. Fermented foods (FFs) represent one of the most relevant and complex sources of live microorganisms in the diet. FFs harbour diverse microbial communities capable of transiently or permanently interacting with the host gut microbiome. The DOGMA project aims to elucidate the role of foodborne microorganisms in modulating the gut microbiome structure and functionality, exploring whether the consumption of FFs with high microbial diversity can restore gut microbial diversity and improve host health status. A multidisciplinary strategy combining metagenomics, metabolomics, and a nutritional intervention will be adopted. Three main activities are foreseen: (*i*) mapping the microbiome of traditional non-Westernized FFs and of their habitual consumers, to understand if the consumption of foods with higher microbial diversity is associated with a positive gut microbiome balance; (*ii*) *in vitro* gastrointestinal simulations will be employed to discriminate between the direct effects of viable microbial cells and the indirect, postbiotic effects of fermentation-derived metabolites, and to define the threshold dose of live microorganisms required to induce measurable changes in gut microbiome composition and metabolome; (*iii*) a randomized controlled trials in obese/type-2 diabetic subjects will be carried out to assess the impact of the consumption of a diet rich in FFs. DOGMA will clarify the extent to which dietary microbes contribute to the resilience and functional recovery of the gut microbiome and will provide a mechanistic framework to design evidence-based nutritional strategies aimed at counteracting the loss of microbial diversity.

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S5-P9: Characterization and Regional Variation of the Gut Microbiome in African Populations Consuming Traditional Fermented Food

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Fermented foods (FFs) intake might modulate the gut microbiome, thus promoting human health. However, despite their growing popularity worldwide, there is still much to learn about how different FFs matrices and habitual intake patterns influence the composition and diversity of the human gut microbiome. In this study, we investigated the relationship between FFs consumption and gut microbiome composition in non-Westernized communities. A total of 498 Nigerian participants living in rural areas provided information on their consumption of local traditional FF, as well as on their health status. Whole metagenome sequencing was performed on fecal samples donated by the participants. Species-level taxonomic composition and functional potential were assessed through bioinformatics pipeline.

Participants regularly consumed various traditional FFs, such as fufu (a starchy staple), akamu (a fermented cereal pudding) and palm wine (a naturally fermented alcoholic beverage), which were prepared using artisanal methods. Importantly, the distribution and prevalence of these foods varied considerably between villages, suggesting distinct local dietary patterns and heterogeneous exposures to microbial communities of FF that may contribute to microbiome variability. Indeed, species-level profiling of the gut microbiomes revealed marked differences in microbial composition across the samples, with clear clustering patterns associated with distinct FF consumption profiles. Interestingly, this was also reflected on the metabolic potential of the microbiomes.

Collectively, our findings suggest that habitual consumption of different traditional FFs might shape the gut microbiome by promoting distinct taxonomic and functional profiles associated with the habitual intake of different fermented food matrices. Further studies integrating strain-level taxonomic profiling, dietary and functional data will be essential to elucidating these relationships more deeply.

S5-P10: Exploring the Food-Gut Axis: Traditional Fermented Foods as a Source of a Beneficial Microbiome

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In Africa, particularly in tropical regions such as Nigeria, high temperatures accelerate food spoilage, necessitating effective preservation methods. Fermentation serves as a natural and accessible solution to extend the shelf life of raw materials. As a result, fermented foods have gradually become a primary source of nutrition in daily diets. In Nigeria, a wide variety of fermented foods are derived from local staples, including cassava (Garri, Fufu, Lafun), yam (Amala), cereals such as maize and millet (Ogi, Agidi, Kunu, Fura), as well as legumes and seeds used for condiments like Dawadawa, Ogiri, and Ugba, which are consumed regularly across different populations. Many traditional fermented foods are a vehicle for delivering live microorganisms to the human gut, where they may produce beneficial metabolites and interact with the human gut microbiome. However, research on the relationship between fermented foods and the human gut microbiome, as well as their impacts on human health, remains fragmented. To address this gap, the DOGMA project aims to explore the impact of traditionally fermented foods produced in non-Westernized countries (e.g., Africa and South America) on the gut microbiome of the populations habitually consuming them. The project aims to analyze > 2,000 fecal and fermented foods samples. As a preliminary analysis, we focused on 496 samples, including fermented foods produced in Nigeria and fecal samples from subjects with different patterns of consumption, using shotgun metagenomic sequencing. We highlighted higher microbial diversity in subjects consuming > 2 portions/day of fermented foods. In addition, specific strains of lactic acid bacteria were shared between the food and gut microbiome, suggesting a potential transfer of microorganisms along the food-gut axis. Next steps will focus on exploring the genomic potential of these strains, to highlight their possible role in improving human health.

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S5-P11: Next-Generation Probiotic-Based Synbiotic Dietary Supplement to Modify the Gut Microbiome and Metabolome

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The term Next Generation Probiotics (NGPs) refers to microbial strains that have the potential to confer health benefits to humans but do not fall within the traditional categories of probiotics commonly used in food or supplements. In the present study, we performed genomic and phenotypic characterization of 14 bacterial strains isolated from the gut microbiota of healthy individuals. The primary goal was to evaluate their capacity to produce beneficial metabolites, including urolithins, equol, and short-chain fatty acids (SCFAs), which are known to play key roles in gut health and systemic metabolism. Among the 14 strains tested, the four most promising candidates were identified as *Bacteroides uniformis* A4, *Bacteroides thetaiotaomicron* A14, unclassified *Bacteroidaceae* A26 and unclassified *Lachnospiraceae* A49. These strains were selected for the development of a synbiotic formulation, which included both the bacterial strains and specific dietary precursors to support the production of health-promoting metabolites (fibers, ellagitannins, daidzein). The daily administration of the synbiotic formulation in the mucosal Simulator of the Human Intestinal Microbial Ecosystem (mSHIME) was evaluated. The system was inoculated with a fecal sample obtained from a donor characterized by a low dietary fiber intake. The synbiotic supplement was administered for a period of 2 weeks, followed by 2 weeks of washout to assess the persistence of the introduced strains. A total of 204 samples were collected from both luminal and mucosal compartments and shotgun metagenomic sequencing was performed to monitor microbial composition. In addition, concentrations of SCFAs, equol, and urolithins were measured to assess metabolic activity. Statistical analysis revealed that the introduced NGP strains were able to persist within the gut ecosystem even after the washout period. Furthermore, treatment with the synbiotic formulation promoted an enrichment in beneficial taxa and a significant increase in SCFA. Overall, these results demonstrate that supplementation with NGPs in combination with appropriate dietary precursors can effectively modulate both the composition and metabolic output of the gut microbiota, highlighting the potential of this approach to support human health. While these findings are promising, further *in vivo* studies are required to confirm the efficacy and safety of the synbiotic formulation in human populations.

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S5-P12: Facility-Specific Microbial Signatures Shape Table Olive Fermentation Dynamics

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The fermentation of table olives is a spontaneous process driven by complex microbial interactions, strongly influenced by the production environment. In this study, we investigated the microbial ecology of olive fermentation in two processing facilities to determine how specific conditions shape community composition and dynamics. Comparative analysis of olive and environmental samples revealed clear segregation of microbial communities. Principal coordinates analysis (PCoA) distinguished the microbiome of olives and environmental samples and highlighted sub-clustering specific to each facility. These results suggest that both the substrate and the processing environment contribute to the structuring of microbial assemblages. Taxonomic profiling at the species level revealed marked differences in olive fermentations between the two facilities. One facility was consistently dominated by lactic acid bacteria (LAB), particularly *Lactiplantibacillus plantarum*, *Lactiplantibacillus pentosus* and *Lactiplantibacillus parafarraginis*. By contrast, the second facility exhibited higher abundance of *Candida boidinii*, besides LAB of the genera *Leuconostoc*, *Weissella* and *Lactococcus*. Furthermore, strain-level analysis revealed facility-specific divergence within key taxa, including *Leuconostoc mesenteroides*, as supported by average nucleotide identity-based dissimilarity. Overall, this study demonstrates that the production environment influences the dynamics of olive fermentation at species and strain levels, potentially affecting the sensorial properties of fermented olives. These findings pave the way for the rational design of starter cultures and improved standardisation strategies in table olive fermentation.

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S5-P13: A Plant-Based Postbiotic Fermented with *Lacticaseibacillus paracasei* CBA L74 Modulates the Gut Microbiome *in vitro*

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Legume-based matrices, such as navy beans, are promising substrates due to nutrient density and support for beneficial gut microbes. Although their consumption is encouraged, legumes contain antinutritional factors and may cause gastrointestinal discomfort. However, it has been reported that fermentation might improve the nutritional properties of legumes. In this study, we provided a Simulator of the Human Gastrointestinal Ecosystem (SHIME) inoculated with fecal samples from a healthy donor with either a postbiotic navy bean flour fermented with *Lacticaseibacillus paracasei* CBA L74 (a patented strain), or with non-fermented navy bean flour. Two doses of each treatment were tested, equivalent to an intake of 2.4 g/day and 24 g/day in adults. Each treatment was administered over a two-week period, and was followed by two weeks of washout. We performed whole metagenome sequencing on a total of 272 samples collected from lumen and mucosa compartments of vessels simulating the proximal and distal colon.

While both the fermented and non-fermented formulations induced a shift in the microbiome composition, the postbiotic formulation was associated with selection of *Faecalibacterium prausnitzii*, a species known for its health-promoting properties. Consistently, we observed a drop *Ruminococcus gnavus* and in other species reportedly prevalent in dysbiotic microbiome upon treatment with postbiotic. At functional level, a higher abundance of genes involved in polyphenols metabolism and fiber degradation was observed in samples receiving the higher dose of postbiotic, suggesting that fermentation might improve the bioaccessibility of health-promoting compounds. Notably, the health-promoting effects were enhanced by the higher dose of postbiotic. These findings support the potential of legume-based postbiotics as functional ingredients that modulate the gut microbiome in a health promoting, dose-dependent manner, warranting further investigation in microbiome-targeted intervention studies.

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S5-P14: Lesvos Breed Sheep Milk: An Unexplored Source of Lactic Acid Bacteria with Probiotic Potential

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In Greece, one of the most common agricultural practices is livestock farming, mainly of small ruminants, particularly sheep and goats. There are more than 20 indigenous Greek sheep breeds reared for meat, but mainly for milk, with most of it being used to produce Protected Designation of Origin (PDO), and Protected Geographical Indication (PGI) cheeses. Sheep milk not only possesses high nutritional value but also considerable potential as a functional food, as it is a rich source of bioactive compounds such as proteins, peptides, and fatty acids with well-established health-promoting properties. Therefore, it also serves as an excellent medium for the growth of various microorganisms, which may facilitate fermentation and potentially confer health benefits to humans. Raw sheep milk from indigenous Greek breeds represents a rich and largely unexplored reservoir of autochthonous lactic acid bacteria (LAB) with potential technological and health-promoting properties. The Lesvos sheep breed is an autochthonous Greek breed well adapted to the xerothermic conditions of Lesvos Island and is mainly exploited for milk production. Therefore, the objective of the present study was to isolate, identify and characterize LAB from raw milk of that breed, with a focus on evaluating their probiotic potential and identifying promising candidate strains for future application in functional products. More precisely, 119 bacterial isolates were identified from eight geographically diverse raw milk samples collected from Lesvos breed sheep. A diverse LAB population was isolated and identified, including representatives of *Limosilactobacillus fermentum*, *Lactobacillus delbrueckii*, *Lacticaseibacillus casei*/*paracasei*/*rhamnosus*, *Streptococcus macedonicus*, *Streptococcus lutetiensis* and *Enterococcus* spp., highlighting the microbial complexity of this traditional dairy ecosystem. Based on rep-PCR genotypic profiling, 47 LAB isolates were selected and subsequently evaluated for their probiotic potential, with several demonstrating multifunctional probiotic-related traits. Notably, a substantial proportion of the isolates exhibited medium to high tolerance to simulated gastrointestinal (GIT) conditions, supporting their potential to survive passage through the human GIT tract. Outstandingly, *L. fermentum* 503 exhibited angiotensin-converting enzyme inhibitory (ACE-I), antimicrobial and γ-aminobutyric acid (GABA) producing activities. Among the *L. casei*/*paracasei*/*rhamnosus* isolates, strains 103 and 708 exhibited ACE-I activity and adhesion to collagen-coated plates, whereas strains 702 and 712 demonstrated ACE-I, antimicrobial, and bile salt hydrolase (BSH) activities. *S. macedonicus* 311 demonstrated ACE-I, antimicrobial and adhesion properties. Overall, raw milk from Lesvos breed sheep constitutes a rich source of promising probiotic candidates for the development of functional foods.

S5-P15: Translational Potential of Microbiome-Based Biomarkers: Current Status and Limitations

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The human gut microbiome produces a wide range of metabolites, structural components, and functional signals that are increasingly proposed as biomarkers of health and disease. However, despite strong biological plausibility and major advances in sequencing and metabolic profiling technologies, only a limited number of microbiome-based markers have shown real translational potential. This work critically examines the current status of microbiome-derived biomarkers and the main factors limiting their clinical application.

Representative classes of candidate biomarkers were assessed, including saccharolytic and proteolytic fermentation products, host–microbe co-metabolites, neuroactive compounds, microbial structural components, extracellular vesicles, and functional gene markers. Translational relevance was considered in relation to biological specificity, analytical feasibility, host–microbe attribution, and interpretability in connection with health outcomes.

It was found that many proposed biomarkers are mechanistically relevant, but only a few appear suitable as standalone indicators. A major limitation is that most microbiome-related signals represent dynamic ecological outputs rather than fixed biological traits. Their abundance or concentration is shaped by microbial composition, functional redundancy, substrate availability, cross-feeding, host absorption and metabolism, clearance, diet, medication use, and disease state. In addition, many candidate markers are chemically unstable, compartment-specific, or strongly influenced by pre-analytical and analytical variability. In several cases, circulating or excreted levels reflect composite host–microbe signals, making the microbial contribution difficult to isolate and universal healthy reference ranges difficult to define.

Overall, the main challenge is not that microbiome-based biomarkers lack biological relevance, but that their meaning often depends strongly on context. Many signals are influenced by diet, host metabolism, microbial interactions, and sampling conditions, which limits their specificity and makes single-marker interpretation difficult. Their greatest potential may therefore lie in combined frameworks that integrate microbial, functional, and host-related data. Progress will depend on the harmonization and standardization of these approaches, as well as stronger longitudinal and mechanistic evidence.

S5-P16: Targeting Hidradenitis suppurativa Inflammation with Dairy-Derived Lactic Acid Bacteria: Potential Immunomodulatory Effects

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Hidradenitis suppurativa (HS), also known as acne inversa, is a chronic inflammatory skin disorder marked by recurrent abscesses, painful nodules and scarring in various areas of the body, often rich in apocrine glands. Recent studies have implicated keratinocytes (KCs) as active participants in HS lesions indicating that dysfunction in these cells may contribute to the production of pro-inflammatory cytokines within and around the affected tissue, along with increased immune cell infiltration. The present study aimed to evaluate the production of pro-inflammatory cytokines in HaCaT cells, an immortalized human keratinocyte line, following stimulation with either lipopolysaccharide (LPS), a commonly used model for general inflammatory responses, or tumor necrosis factor alpha (TNF-α), a cytokine implicated in HS, leading to further induction of other mediators, such as IL-6 and IL-1β. Using both stimulation models, 32 lactic acid bacteria (LAB), isolated from dairy products and previously characterized for their promising probiotic properties, were assessed for their ability to reduce pro-inflammatory cytokine levels induced by HaCaT with the goal of identifying candidates with potential anti-inflammatory effects against HS or other immune-mediated skin disorders. Generally, stimulation with either LPS or TNF-α increased IL-6 and IL-8 production in HaCaT cells, with TNF-α demonstrating a stronger effect. Still, 11 out of the 32 tested LAB strains managed to decrease IL-6 and IL-8 induction, by more than 50% relative to the control, when pre-incubated with HaCaT for 6 h and maintained in the medium with the HaCaT cells throughout the subsequent 18-h stimulation period with either LPS or TNF-α. The most promising ones included *Lacticaseibacillus plantarum* and *Leuconostoc mesenteroides* strains; these results highlight their potential as modulators of keratinocyte-driven inflammation that warrants further investigation in more complex *in vitro* and *in vivo* models associated with HS and possibly other inflammatory skin disorders.

S5-P18: (Poly)phenol–Gut Microbiota Interactions and Human Health: Evidence from Human Intervention Studies

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(Poly)phenols are naturally occurring bioactive compounds predominantly found in plant-based foods and beverages. Their intake has been associated with a range of health benefits, although the precise underlying mechanisms are not fully understood. As many dietary (poly)phenols reach the lower intestinal tract, the gut microbiota is likely to play a role in biotransformation of these compounds into bioactive metabolites and reciprocally, polyphenols modify intestinal microbial composition and function. To evaluate the relationship between (poly)phenol intake, gut microbiota and human health, we conducted a systematic review of human intervention studies assessing clinical outcomes related to cardiometabolic health, inflammation, neuroprotection or immune function together with microbial (poly)phenol metabolites, gut microbiota composition and/or microbiota-derived metabolites. Overall, the most consistent evidence was observed for cardiometabolic health outcomes, with several studies reporting associations between (poly)phenol intake, increased microbial-derived metabolites, microbiota modulation and improvements in vascular function, blood pressure, lipid metabolism, glucose regulation or adiposity. Findings related to inflammation and immune function were more limited and inconsistent. The review highlights the likely contribution of the gut microbiota explaining individual differences in response to dietary (poly)phenols while also highlighting the substantial variability in current human evidence. Further well-designed studies integrating microbiome data, metabolite profiling and host-response measures are needed to establish causal relationships and identify responder phenotypes.

S5-P19: Fermented and Fruit-Based Interventions for Constipation Relief: The Nutriplant Randomized Trial

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Constipation is highly prevalent health condition that can notably impact individual's health related quality of life. In European dietary traditions, fermented foods (e. g. kefir, sauerkraut) and other plant-based products such as dried plums, apples and flaxseeds have been traditionally used for their potential beneficial effects on bowel function; however, research data supporting their efficacy in constipation management remain limited.

The aim of NutriPlant study (ARIS L7-50154) was to assess and compare the effects of sauerkraut, apples and dried apples on constipation symptoms, using psyllium as an active control. Adult participants (18–70 years, 120 participants enrolled) with functional constipation (FC, *n* = 77) and irritable bowel syndrome with constipation (IBS-C, *n* = 43) were randomized into a controlled parallel, four-arm intervention trial (sauerkraut, *n* = 30; apples, *n* = 30; dried apples, *n* = 30; psyllium, *n* = 30). Participants received daily portions of the investigated foods, standardized to contain 3 g of dietary fibre, over a four-week period.

As a part of larger study, changes in constipation-related during the intervention were assessed weekly using the Patient Assessment of Constipation Symptoms (PAC-SYM) questionnaire. The study was successfully completed by 112 participants (FC, *n* = 71; IBS-C, *n* = 41) with following distribution through arms: sauerkraut, *n* = 27; apples, *n* = 27; dried apples, *n* = 29; psyllium, *n* = 29. All interventions resulted in reductions in PAC-SYM scores compared with baseline values, for sauerkraut: –6.6, dried apples: –9.0, apples: –9.2; compared to psyllium with a reduction of –7.0 points.

These findings suggest that a portion of sauerkraut, large apple or 12 pieces of dried apples may be as effective as a fibre-matched dose of psyllium (3 g dietary fibre) in alleviating constipation symptoms in adults.

S5-P20: Low-Fiber Western Diet Promotes Enterohemorrhagic *Escherichia coli* Colonization in the Human *in vitro* Mucosal Artificial Colon

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Enterohemorrhagic *Escherichia coli*, particularly O157:H7 serotype, is a major food-borne pathogen responsible for human diseases ranging from mild diarrhea to life-threatening complications such as hemolytic and uremic syndrome. Evidence from murine models suggests that low-fiber diets increases susceptibility to enteric infection, but their impact on EHEC colonization and the role of the human gut microbiota remain unknown. This study investigates how a Standard versus a low-fiber Western diet influences EHEC colonization using the human *in vitro* Mucosal ARtificial COLon (M-ARCOL) model, together with examining associated shifts in microbiota composition and metabolic activity. The M-ARCOL model reproduces key nutritional, physicochemical, and microbial (both luminal and mucus-associated) characteristics of the human colonic environment.

Donor selection was first performed using simplified fecal batch experiments (*n* = 9 donors). Four donor fecal samples were used to inoculate M-ARCOL bioreactors. The bioreactors were operated in parallel under two dietary conditions: a Standard diet and a low-fiber Western diet. Both reactors were then infected with EHEC O157:H7 reference strain EDL933. EHEC survival was monitored by qPCR, gut microbiota composition was analyzed by 16S metabarcoding and gut microbial activities were evaluated through gas and short chain fatty acid (SCFA) analysis.

The low-fiber Western diet significantly altered the gut microbiota composition, particularly in the mucosal compartment, with notable shifts in beta-diversity. These effects were donor-dependent, as reflected by variations in the relative abundances of major bacterial families. EHEC colonization was dependent on both donor and diet in the luminal samples of M-ARCOL. Interestingly, the presence of mucus allows a long-term maintenance of EHEC colonization in the colonic environment, indicating that it may serve as a protective niche and reservoir for the pathogen. Additionally, the low-fiber Western diet influenced bacterial SCFA and bile acid profiles, with increased butyrate concentrations potentially supporting prolonged EHEC colonization.

The sustained EHEC colonization under low-fiber Western diet suggests that such dietary patterns may increase susceptibility to EHEC infection in humans. This study underscores the value of a sophisticated *in vitro* colon model such as M-ARCOL for investigating the interplay between diet, microbiota, and pathogen interactions in the human gut and highlights the critical role played of mucus in EHEC pathophysiology.

S5-P21: Phenotypic and Proteomic Responses of *Campylobacter jejuni* to *Lactococcus cremoris* in Dual-Species Biofilms

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Campylobacter jejuni remains one of the most important foodborne pathogens and its persistence in food-processing environments is strongly linked to its ability to form biofilms under stress conditions. This persistence increases the likelihood of contamination spreading across contact surfaces and food products, ultimately raising the risk of human exposure and infection. In this study, we investigated whether *Lactococcus cremoris*, a beneficial transient bacterium associated with dairy fermentations, can interfere with these traits using phenotypic assays and label-free quantitative proteomics. In adhesion and invasion assays with Caco-2 cells, *L. cremoris* reduced *C. jejuni* adhesion by up to 50% and invasion by up to 92%, with the strongest effect observed when *L. cremoris* was present before pathogen exposure. We also examined dual-species biofilm formation on both abiotic and biotic model surfaces. In 48 h dual-species biofilms on polystyrene, *C. jejuni* culturability decreased by 88%, while *L. cremoris* remained unaffected. In contrast, no clear effect was observed on mucin after 48 h, even when *L. cremoris* was allowed to establish for 1 h before the addition of *C. jejuni*, indicating that the antagonistic effect is surface dependent. We further tested whether extracellular polymeric substances (EPS) or cell lysate derived from *L. cremoris* are sufficient to affect *C. jejuni* adhesion and biofilm formation on polystyrene and mucin. The strongest effect was observed with the cell lysate treatment, while EPS produced weaker and less consistent effects. To understand how *C. jejuni* responds to competitive biofilm conditions, we compared the proteomes of single- and dual-species biofilms using label-free quantitative mass spectrometry. Co-cultivation with *L. cremoris* induced broad proteomic reprogramming in *C. jejuni*, including increased abundance of proteins linked to central carbon metabolism, amino acid and nucleotide metabolism, chemotaxis, oxidative stress defence, and energy conservation. The most strongly upregulated proteins included the tungstate-binding transporter TupA, S-ribosylhomocysteine lyase LuxS, the DNA protection protein Dps, and hydrogenase subunits HydA and HydB. Overall, *L. cremoris* reduced virulence-associated properties and culturability of *C. jejuni* under selected conditions, while triggering an adaptive proteomic response consistent with nutritional and oxidative stress in dual-species biofilms. These findings highlight *L. cremoris* as a biologically relevant antagonist of *C. jejuni*, although its effects appear to depend on the interaction surface and experimental setting.

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S5-P22: *Lactiplantibacillus plantarum* as a Dual-Function Postbiotic: Extracellular Vesicles for Skin Health and Pomegranate Peel Fermentation Driving Polyphenol Biotransformation and Gut Microbiome Modulation

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The skin microbiome plays a key role in maintaining cutaneous homeostasis through continuous interaction with host cells, partly mediated by bacterial extracellular vesicles (EVs). In this study, we investigated the bioactivity of EVs derived from *Lactiplantibacillus plantarum* (LpEVs) on keratinocytes, alongside the metabolic potential of the same strain in a biotechnological context. LpEVs were isolated and tested on HaCaT cells under oxidative and inflammatory stress conditions, showing antioxidant, anti-inflammatory, wound healing, and barrier-enhancing effects, supporting their role in modulating epithelial responses. In parallel, *L. plantarum* was evaluated for its fermentative capacity on pomegranate peel, a polyphenol-rich agro-industrial byproduct. Preliminary genomic screening revealed the presence of genes involved in polyphenol metabolism, including tannase-encoding genes *tanA* and *tanB*. Consistently, experimental fermentation demonstrated the release of bioactive compounds such as gallic and ellagic acids. The resulting fermented extract will be further investigated using *in vitro* batch fermentation models of the gut microbiome, employing fecal inocula from three healthy donors and incubation times of 24 and 48 h, to assess its impact on microbial metabolism, particularly the production of short-chain fatty acids (SCFAs) and urolithins. Overall, this study highlights the dual potential of *L. plantarum* as both a source of bioactive EVs for skin applications and a functional fermentative agent capable of valorizing plant waste into health-promoting compounds, supporting the development of innovative postbiotic and microbiome-targeted strategies.

S5-P23: Risks and Benefits of Exposing the Gut Microbiota to Innovative Bakery Ingredients: A Proposed *in vitro* New Approach Methodology (NAM) to Assess Their Impact on Gut Health Using Microbial and Metabolite Markers

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The development of innovative bakery foods incorporating alternative protein sources and bioactive plant-based ingredients requires the need of a risk/benefit assessment tool to evaluate impact on gut microbiota functionality and safety. In this context, New Approach Methodologies (NAMs) are increasingly promoted by Regulatory frameworks to support evidence generation while reducing reliance on animal experimentation. The present study applied the MICODE platform, combining simulation of oro-gastro-duodenal digestion, gut microbiota fermentation and omics technologies, to investigate microbiota-mediated effects of novel baked products designed for specific nutritional conditions.

Five experimental case studies are presented using the MICODE platform, which reproduces key ecological and metabolic features of the human colonic environment under controlled conditions, including fresh and live human gut microbiota. The collection includes breads enriched with polyphenol-rich dietary fibers, insect powder, Spirulina biomass, hydrolyzed hemp bran, and antioxidant plant by-products. Following gastrointestinal digestion with the INFOGEST protocol, substrates were fermented using gut microbiota from healthy donors or subjects affected by metabolic syndrome. Changes in microbial composition were evaluated by 16S rRNA sequencing and qPCR, while microbiota-derived metabolites by SPME–GC/MS.

Breads enriched with polyphenol fibers promoted a favorable modulation of the gut microbiota, including increased abundance of health-associated taxa such as *Faecalibacterium prausnitsii* and enhanced production of short-chain fatty acids compared to reference products. Insect-enriched breads showed improved microbiota responses when processed by sourdough fermentation, with increased levels of *Bifidobacterium* spp. and short-chain fatty acids, although with limited overall prebiotic capacity. Hydrolyzed hemp bran protein breads exhibited the most pronounced functional effects, significantly increasing short- and medium-chain fatty acid production, particularly hexanoic acid, and reducing opportunistic taxa such as *Bilophila wadsworthia*. Spirulina-enriched breads induced moderate microbiota shifts, especially when made with sourdough, and were associated with the release of bioactives. Products containing antioxidant vegetal by-products improved gut microbiota profiles in fermentation systems inoculated with microbiota from metabolically compromised subjects.

Overall, the MICODE platform proved to be a robust NAM for the comparative assessment of innovative bakery foods, enabling mechanistic insight into diet–microbiota interactions under standardized and reproducible conditions. By supporting the Replacement and Reduction principles of the 3Rs, this approach aligns with EFSA's vision for human-relevant, ethically sustainable risk–benefit assessment. The integration of such *in vitro* microbiome models represents a key step toward future modular systems capable of simulating increasing levels of human physiological complexity, contributing to the long-term ambition of reconstructing whole-human responses without the use of animal models.

S5-P24: Anti-Obesity Potential of Lactic Acid Bacteria Isolated from Kefir

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The increasing prevalence of obesity represents a major global health concern, closely associated with metabolic disorders and cardiovascular complications. Recently, lactic acid bacteria (LAB) have attracted considerable interest due to their potential role in regulating host metabolism and modulating gut microbiota, thereby contributing to anti-obesity effects. This study aimed to isolate and identify LAB strains from kefir with potential anti-obesity properties. A total of 30 LAB strains obtained from kefir were initially screened using the 3T3-L1 adipocyte model. Cell-free extracts (CFEs) prepared from these strains were applied to differentiated adipocytes to evaluate intracellular lipid accumulation and the expression of adipogenesis-related genes. Strains showing significant *in vitro* activity were further assessed *in vivo* using a high-fat diet (HFD)-induced obese rat model. Among the isolates, strains YPK11 and YPK12 exhibited pronounced anti-obesity effects. Treatment with these strains significantly reduced lipid accumulation in adipocytes and suppressed the expression of key adipogenic regulators, including C/EBPα, PPARγ, SREBP-1c, and FAS. In the animal study, administration of a high dose of YPK11 (10^{10} CFU in 0.2 mL PBS/kg body weight/day) led to a reduction in body weight gain and adipose tissue mass. This effect was accompanied by decreases in serum and hepatic cholesterol and triglyceride levels. Furthermore, YPK11 supplementation down-regulated hepatic SREBP-1c expression while up-regulating CPT1, suggesting enhanced fatty acid β-oxidation. Gut microbiota analysis indicated that HFD increased the relative abundance of *Firmicutes* and the *Firmicutes*-to-*Bacteroidetes* ratio, and this trend was further elevated following YPK11 treatment. Overall, these findings demonstrate that YPK11 and YPK12 effectively inhibit lipid accumulation in adipocytes through modulation of adipogenesis-related gene expression. In addition, YPK11 shows potential in reducing obesity-related parameters in vivo and altering gut microbiota composition. These results suggest that YPK11 may serve as a promising functional food component for obesity management.

S6 Safe Water and Biofilms Control

S6-P1: Nanoparticle-Based Antimicrobial Strategies Against Biofilm-Forming *Staphylococcus* ssp. from Slaughterhouse Surfaces

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The persistence of bacterial contamination on food processing surfaces represents a significant challenge for food safety, particularly in slaughterhouse environments where cross-contamination events are frequent. Among the microorganisms commonly detected in these settings, *Staphylococcus* spp. are of particular concern due to their ability to form biofilms and persist under disinfection treatments. Therefore, improving antimicrobial strategies targeting surface-associated bacteria remains a priority.

In the present study, we evaluated the antimicrobial activity of different metal-based nanoparticles (Au NPs, Ag NPs, ZnO NPs and CuO NPs), alone and in combination with EDTA or a disinfectant formulation (HLE), against *Staphylococcus* spp. strains isolated from slaughterhouse environmental surfaces. Particular attention was given to differences in response between strains and treatments, in order to better assess their applicability under different contamination scenarios.

The antimicrobial response varied depending on both the nanoparticle type and the strain. While individual nanoparticles showed variable activity, their combination with EDTA or HLE significantly enhanced antimicrobial activity, leading to a greater reduction of both developing and preformed biofilms. Notably, consistent strain-dependent differences were observed across treatments, highlighting the importance of considering the microbial composition when selecting antimicrobial strategies for surface decontamination, and suggesting that no single antimicrobial approach is universally effective.

Overall, these findings highlight the potential of nanoparticle-based combinations to improve current disinfection approaches in slaughterhouse environments. This strategy may contribute to reducing bacterial persistence on food processing surfaces and to optimizing antimicrobial treatments according to specific contamination scenarios.

Keywords: antimicrobial resistance; *Staphylococcus*; metal-based nanoparticles; biofilms; slaughterhouse surfaces; food processing environments

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S6-P2: Survival Dynamics of Mono- and Multispecies Dry Surface Biofilms of *Listeria monocytogenes* and *Enterococcus faecium*

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Dry surface biofilms (DSB) represent a challenge for microbial control in food processing plants, since they show high desiccation resistance. This study evaluated the formation, viability, and composition of *Listeria monocytogenes* DSB in mono- and cocultivation with *Enterococcus faecium*. DSB were formed on polypropylene (PP) and stainless steel (SS) coupons using two hydrated–dry cycling protocols: P1 (48/48 h) and P2 (24/120 h), followed by storage at 25°C for 42 days. At the forming endpoint, *L. monocytogenes* population in DSB monospecies was ca. 2 log CFU/cm² higher on PP than on SS ($p < 0.05$). In DSB multispecies, the pathogen achieved 1.6 and 1.4 log CFU/cm² on PP and SS, respectively. *L. monocytogenes* remained viable for up to 42 days, with reductions of ≥ 1.7 log CFU/cm². Cocultivation with *E. faecium* inhibited *L. monocytogenes* in DSB regardless of surface or protocol, maintaining pathogen levels at or below the detection limit (1.4 log CFU/cm²) throughout storage. Meanwhile, *E. faecium* achieved 5 log CFU/cm² by the end of DSB formation and 3.4 log CFU/cm² after 42 days. Catalase treatment resuscitated *L. monocytogenes* VBNC cells on day 0 (endpoint) and within 14 days of storage, increasing culturability by up to 1.0 log CFU/cm². In general, DSB matrices contained higher protein levels than carbohydrates and eDNA, and *L. monocytogenes* exhibited morphological alterations, including elongation and L-form-like cells. Overall, the findings highlight the need for strict hygiene measures to control DSB and suggest that *E. faecium* may be an effective strategy to inhibit the *L. monocytogenes* DSB formation.

S6-P3: Inactivation of *Bacillus subtilis* Spores in Slaughterhouse Process Water by UV-C and Sodium Hypochlorite Highlights the Potential for Water Reuse

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Water reuse is gaining increasing attention as a strategy to reduce drinking water consumption in the meat industry. However, concerns about microbiological safety remain a major barrier to its implementation, particularly in cleaning applications. The use of untreated process water for cleaning livestock transport vehicles after the delivery of animals for slaughter at pig slaughterhouses may contribute to the cross-contamination of animal pathogens between farms via slaughterhouses. A key challenge in the inactivation of microorganisms in slaughterhouse process water is the high organic matter content. In this study, we evaluated the efficacy of UV-C and sodium hypochlorite for the inactivation of *Bacillus subtilis* spores in a slaughterhouse process water model. *B. subtilis* was selected as a safe surrogate for *Bacillus anthracis*, the causative agent of anthrax.

Model process water was prepared from pork loin and porcine blood diluted in tap water and filtered through filter cloth. Spores from four *B. subtilis* strains were combined into a cocktail used to inoculate the model water to approximately 106 spores/mL. Three treatment strategies were tested: UV-C alone, sodium hypochlorite alone, and combined UV-C followed by sodium hypochlorite. UV-C treatment was performed at pilot scale at irradiation doses of 188, 244 and 318 mJ/cm^2. Chlorination was tested at laboratory scale using 0.5%, 1.0% and 2.0% sodium hypochlorite for 10, 20 and 30 min. Surviving bacteria were quantified as total colony-forming units and as total spore count.

UV-C treatment caused dose-dependent spore inactivation, with mean reductions of 4.3, 4.7, and 5.4 log(spores/mL) at 188, 244, and 318 mJ/cm^2, respectively. Sodium hypochlorite efficacy was both concentration- and time-dependent. At 0.5%, spore reductions were 1.7, 3.1, and >5.6 log(spores/mL) after 10, 20, and 30 min., respectively, while at 1.0% and 2.0%, reductions of 3.3– >5.6 log(spores/mL) were achieved within 10–30 min. Combined treatment using UV-C at 252 mJ/cm^2 followed by 0.5% sodium hypochlorite reduced surviving bacterial cells and spores to below the detection limit after 30 min.

These results show that substantial spore inactivation can be achieved in a highly challenging slaughterhouse water matrix containing protein and blood. The findings indicate that physical and/or chemical water treatment may support the safe reuse of process water in slaughterhouses; however, further studies are needed to validate efficacy against a broader range of relevant animal pathogens.

S6-P4: Difference in Biofilm Formation Between Persistent and Transient *Listeria monocytogenes* Isolates and Intra-Pulsotype Variability at Temperatures Relevant to Food Processing Environments

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Listeria monocytogenes can persist for longer periods in the food production environment. Studies show that certain *L. monocytogenes* strains are more persistent and others more transient. One of the persistent strategies mentioned in literature is biofilm protection. We studied the difference in biofilm formation of persistent and transient *L. monocytogenes* isolates at lower temperatures, which better reflects realistic conditions in food production environments. First, the dynamic changes in biofilm biomass and planktonic cell numbers during nine days at 10°C and 18°C were studied for nine isolates randomly selected from 40 persistent isolates. Results showed that biofilm production was highest on day three at 10°C and 18°C, with an optical density ranging from 0.18 to 0.62 and from 0.29 to 0.94 for the nine *L. monocytogenes* isolates respectively. Next, the biofilm formation capacity of 40 persistent and 36 transient isolates was studied using the crystal violet straining method after 3 days of incubation at both temperatures. Temperature proved to be an influential factor, with the higher temperature supporting increased biofilm production. Additionally, persistent *L. monocytogenes* isolates produced significantly more biofilm than transient isolates at 10°C and 18°C, with a more pronounced difference at 18°C. Finally, the effect of intra-pulsotype variation in biofilm-forming potential was analyzed. Seventeen pairs of isolates exhibited significant differences at least at one temperature ($p < 0.05$). These findings improve further insights into the factors contributing to *L. monocytogenes* persistence and offer valuable information for controlling contamination in the food industry.

S6-P5: Inter-Species Interactions in a Four-Species Biofilm Formed by *Listeria monocytogenes* and Background Bacteria Isolated from Meat and Sauce Industry

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Persistent *Listeria monocytogenes* strains usually form stronger mono-species biofilms than transient ones, yet their behavior within multispecies communities remains less understood. Interspecies interactions in such biofilms play a critical role in shaping bacterial persistence. In this study, two strong and two weak biofilm-forming *L. monocytogenes* strains, either a persistent or a transient one, were each combined with 11 background bacterial species isolated from meat and sauce production environments after cleaning and disinfection to construct four-species consortia. A total of 120 four-species combinations were evaluated for biofilm interaction at 10°C and 18°C using crystal violet staining. Results showed that antagonistic interactions dominated (97.1%) across the four-species combinations. All seven observed synergistic interactions occurred at 10°C, indicating that lower temperature may favor cooperative biofilm interaction compared to 18°C. Notably, also all seven involved weak biofilm-forming *L. monocytogenes* strains suggesting a greater tendency for synergistic behavior for the weak biofilm formers. Experiments with additional *L. monocytogenes* strains confirmed this finding. Four key combinations involving *Brochothrix thermosphacta* (D1), *Pseudomonas fluorescens* (D6), and *Psychrobacter pulmonis* (D7) and the four *L. monocytogenes* strains were further assessed for dual, triple, and four species biofilm interactions. Results revealed that the *B. thermosphacta* and *P. fluorescens* pairing serves as the core driver of synergy within the D1-D6-D7-L43 (*L. monocytogenes*) four species biofilm. These findings provide new ecological insights into how persistent and transient *L. monocytogenes* strains integrate into complex communities, informing targeted strategies for biofilm control in food processing environments.

S6-P6: Combining Enzymatic Cleaning and Nisin Improves the Control of *Listeria monocytogenes* in Multispecies Biofilms Under Food Processing Conditions

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Listeria monocytogenes biofilms represent a persistent challenge in food processing environments due to their resilience and increased tolerance to sanitation procedures, particularly within multispecies communities. This study investigated a combined enzymatic–bacteriocin approach aimed at destabilizing the biofilm matrix and enhancing targeted inactivation of *Listeria*.

The efficacy of an enzymatic cleaning formulation, nisin, and their combination was evaluated using complementary experimental models. These included microplate-based biofilm assays (monospecies and multispecies), confocal laser scanning microscopy (CLSM) analysis, and a surface contamination model simulating salmon processing conditions with lipid-rich organic matter on stainless steel.

In monospecies biofilms, both enzymatic treatment and nisin alone achieved significant reductions (~3.5 log), while their combination resulted in > 5 log reductions, significantly outperforming individual treatments. In multispecies biofilms containing *Pseudomonas* spp., overall efficacy decreased; however, the combined treatment consistently achieved higher reductions (~4 log) than either treatment alone. CLSM analysis confirmed that only the combined strategy induced a marked reduction in viable *L. monocytogenes* biovolume within multispecies biofilms.

In the food soil contamination model, neither enzymatic cleaning nor nisin alone significantly reduced *L. monocytogenes*. In contrast, their combination achieved approximately 4.7 log reductions, comparable to conventional alkaline and oxidative sanitation procedures, even under challenging conditions involving lipid-rich residues.

These findings demonstrate that enzyme–bacteriocin combinations can significantly enhance *L. monocytogenes* control across biofilm models of increasing complexity. This strategy represents a promising complementary approach to conventional sanitation, particularly for targeted pathogen control in high-risk food processing environments, while supporting efforts to reduce reliance on harsh chemical sanitation.

S6-P7: Chitosan-Enhanced Graphitic Carbon Nitride for Visible-Light-Driven Water Disinfection: Mechanistic Insights into Reactive Oxygen Species Mediated Bacterial Inactivation

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Safe water disinfection is critical for public health and food-related sanitation systems. Graphitic carbon nitride (g-C_3N_4), a visible-light-responsive metal-free photocatalyst, has attracted increasing attention for microbial inactivation, yet its practical application is limited by relatively low bactericidal efficiency. In this study, we developed a chitosan (CS)/g-C_3N_4 composite through hydrogen bonding and electrostatic interactions without the use of crosslinking agents, thereby preserving the intrinsic antibacterial activity of CS. The as-prepared composite exhibited outstanding photocatalytic bactericidal performance, achieving up to an 8-log reduction of both Gram-negative and Gram-positive bacteria within 60 min under visible light irradiation, representing a 6–7 log enhancement compared to CS or g-C_3N_4 alone. This superior activity is attributed to a synergistic mechanism involving enhanced bacterial adhesion and increased generation of reactive oxygen species (ROS). Electron microscopy and zeta potential analysis confirmed that CS facilitated close contact between bacterial cells and the photocatalyst surface, thereby improving photocatalytic efficiency. Mechanistic investigations identified hydrogen peroxide (H_2O_2), superoxide radicals ($\bullet O_2^-$), and photogenerated electrons (e^-) as the dominant bactericidal species. At the cellular level, the composite induced membrane disruption, metabolic inhibition, oxidative stress imbalance, and DNA damage. Lipidomic analysis of *Salmonella* Typhimurium further revealed that bacterial membrane integrity was compromised through perturbation of fatty acid biosynthesis and glycerophospholipid metabolism pathways. Overall, this study provides a facile strategy for constructing metal-free photocatalytic materials with enhanced antimicrobial performance and elucidates the critical role of chitosan in promoting contact-dependent photocatalysis. The findings highlight the strong potential of CS/g-C_3N_4 for safe water treatment in environmental and food-related sanitation applications.

S6-P8: Exploring Microbial Interactions and Biofilm Formation Between *Salmonella* and *Janthinobacterium*

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Microbial interactions can influence the colonization of microorganisms on fresh produce. Investigating these interactions under realistic environmental conditions, particularly in the case of pathogenic bacteria, remains a major challenge in food science. Laboratory media may not accurately represent natural habitats, potentially leading to less reliable data. Therefore, data derived from food-related environments are of particular importance. In addition, the inclusion of microorganisms relevant to the food or food environment under study is essential.

In this context, a multifactorial experiment was designed to evaluate the effects of growth medium and inoculum level on the growth of *Salmonella* and *Janthinobacterium* (isolated from leafy vegetable), their interactions, and biofilm formation. Four inoculum levels (2 to 5 log CFU/mL) and three media (TSB, rocket extract, and spinach extract) were tested. Microbial growth in mono- and co-culture was assessed by measuring absorbance at 600 nm. Biofilm formation was qualitatively evaluated using the microtiter plate method by measuring the absorbance at 575 nm following crystal violet staining and ethanol solubilization.

The results indicate that the growth medium significantly influenced both microbial growth and biofilm formation ($p < 0.05$). Moreover, higher inoculum levels of *Janthinobacterium* significantly influenced *Salmonella* growth and biofilm formation ($p < 0.05$). These findings demonstrate that microbial interactions can influence the colonization of *Salmonella* on fresh produce and, consequently, may impact the associated risk for consumers.

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S6-P9: Temperature–Driven Microbial and Chemical Stability of Dairy Condensate, Tap Water and Rainwater During Storage

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Safe reuse of alternative water streams in circular dairy processing requires understanding how storage affects both microbiological and chemical quality. Within the CircSyst EU project, we evaluated microbial growth, total cell counts and compliance with the Swedish dairy industry guidelines for hygienic water quality. Five types of waters were studied. Two different product/process condensates, one steam condensate, roof-harvested rainwater and tap water. All waters were stored at 10°C and 22°C for up to 14 days.

Temperature had a strong impact on microbial development. At 22°C, product and process condensates showed rapid microbial growth, increasing from undetectable levels to high counts within a few days. At 10°C, they remained low or near detection limits. Tap water also increased at both temperatures, but more noticeably at 22°C. Rainwater consistently showed high microbial levels regardless of temperature, while steam condensate displayed the lowest growth potential and stayed close to detection limits at 10°C.

Flow cytometry confirmed these trends. Product and process condensates increased substantially at 22°C but stayed low at 10°C. Tap water rose at both temperatures, though more at the higher one. Rainwater had the highest total cell numbers across conditions, while steam condensate remained the lowest.

Assessment against the Swedish dairy industry guidelines showed that microbiological quality in the product/process condensates no longer fulfilled the stricter water classes after 14 days at 22°C, whereas they met the criteria throughout storage at 10°C. Steam condensate met microbiological requirements under both temperature conditions. Chemical parameters including copper, manganese, sodium, calcium, magnesium and turbidity remained within acceptable limits across all samples. Minor deviations were observed for iron, pH and COD-Mn, predominantly in product/process condensates stored at 22°C.

Overall, three characteristic behaviours emerged: (1) temperature-sensitive product/process condensates, which lose microbiological stability at 22°C but remain stable at 10°C; (2) moderately temperature-responsive tap water; and (3) temperature-robust rainwater and steam condensate.

Ongoing work includes microbiota characterisation by sequencing to identify taxa driving these temperature-dependent shifts, as well as advanced analysis of flow cytometry data to resolve microbial population structures and early indicators of instability.

S6-P10: Transitioning from Plate-Based to Digital Microbial Water Quality Monitoring to Support Safe Water Use in the Food Industry

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Safe drinking water management relies on rapid detection and interpretation of microbial water quality changes. However, many water treatment and distribution systems still depend on cultivation-based microbiological analyses with response times ranging from 24 h to several days. Such delays limit process control, postpone corrective actions, and increase vulnerability to microbial contamination. These limitations affect not only municipal drinking water supply but also water dependent sectors such as the food industry, where timely microbial information is critical for hygienic processing, equipment cleaning, and overall food safety management.

From Plate to Pixel is a transition lab funded by the Swedish Impact Innovation program Water Wise Societies (2025–2028), designed to accelerate the shift from plate-based methods toward rapid, digital, and molecular tools for microbial water quality monitoring. The initiative develops and evaluates technologies such as flow cytometry, qPCR, rapid analysis workflows, and continuous online monitoring. These methods enable near real-time insights and support the development of early warning systems that can strengthen operational decision making across the entire water chain, from source to tap and into industrial processes.

A core feature of the transition lab is its integration of technical, organizational, and regulatory innovation. The project explores how continuous digital data streams can be interpreted using AI-based analytics and incorporated into automated process control and decision-support tools. By linking microbial data to operational parameters, the project aims to create faster and more proactive strategies for identifying deviations, managing risks, and optimizing treatment processes.

Coordinated by Sweden Water Research, the consortium includes municipal water utilities, authorities, academia, research institutes, technology providers, and actors from the food industry. For food producers, the project provides an opportunity to evaluate how rapid microbial water quality data can support improved hygiene management, reduce production downtime due to water-related contamination events, and strengthen risk-based control strategies. Cross-sector collaboration enables knowledge transfer between drinking water systems and industrial water users, increasing scalability and supporting future implementation beyond the initial project scope.

Expected outcomes include shorter response times for detecting microbial deviations, more proactive risk management enabled by digital and rapid methods, and improved integration between microbial data and operational decisions. The results are anticipated to support safer drinking water, more efficient resource use, and enhanced food safety in water-dependent industrial processes.

S6-P11: Molecular and Structural Characterization of *Salmonella* spp. Biofilms in Hydroponic Leafy Green Nutrient Solutions

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Salmonella spp. persists in leafy green hydroponic production systems in controlled environments. Understanding the role of *Salmonella* biofilms is essential to reduce food safety risks. This study assessed the ability of *Salmonella* spp. to form biofilms in hydroponic leafy green nutrient solution and characterized the biofilms at different temperatures and carbohydrate concentrations relevant to hydroponic leafy green production.

Biofilms of 13 *Salmonella* serovars were grown in hydroponic leafy green nutrient solution (LG), LG with lettuce exudates (LGE), and LG amended with glucose, fructose, sucrose, or inositol (0.1–0.4%). Biofilms were assessed *in vitro* (25°C/28°C/37°C) and under controlled environment conditions. Total biofilm production was quantified by crystal violet (OD_{570}) and categorized into weak/moderate/strong. Viable cells were enumerated, and RDAR (red-dry-and-rough) morphotype analyzed. Genetic analysis was performed to establish the presence of biofilm- and virulence associates genes (*gcpA*, *csgD*, *adrA*, and *invA*).

Salmonella Anatum and Newport formed weak biofilms at 25°C. In the presence of lettuce exudates, all strains formed moderate biofilms at 25°C. At 28°C, 6/13 strains formed moderate biofilms, while 5/13 formed weak biofilms. *S.* Enteritidis and *S.* Heidelberg formed strong biofilms at 37°C. In the presence of sucrose, glucose, and inositol, > 70% of the strains formed moderate-strong biofilms under all tested conditions. Five strains exhibited RDAR morphology in LG, while nine had RDAR morphology in LGE at 28°C. Molecular profile of each strain was generated.

Salmonella spp. can form biofilms in leafy green nutrient solutions, especially in the presence of plant exudates at temperatures specific to leafy green production. While the characteristics of biofilms varied, growth at 28°C and the presence of sucrose were the most favorable conditions for biofilm production among the tested strains. Future studies will be conducted to test the biofilm characteristics in near-commercial scale and behavior of the pathogen in multi-species biofilms.

S6-P12: New Insights into the Resistome-Mobilome–Biofilm Relationship in *Vibrio parahaemolyticus*

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Vibrio parahaemolyticus is a major foodborne pathogen frequently isolated from seafood, notable for its biofilm-forming capacity, which enhances its persistence in environments and increases resistance to disinfection. This study investigated the associations between the virulome, resistome, and mobilome of *V. parahaemolyticus* and its biofilm forming ability to better understand the genomic determinants of persistence. Among 845 isolates from imported seafood (mainly shrimp), 20.6% showed acquired resistance to at least one clinically important antibiotic class, most commonly tetracycline, sulfamethoxazole-trimethoprim, and streptomycin. Thirty-nine representative strains (resistant and susceptible) were selected for confocal laser scanning microscopy (CLSM) observation of biofilms and whole-genome sequencing (WGS). CLSM combined with image analysis revealed significant variability in biofilm formation, with no relation with specific phylogenetic lineage. Hybrid WGS (short and long reads) identified 49 distinct antimicrobial resistance genes (ARGs) and 44 highly conserved virulence genes. ARGs were found on both chromosomes and plasmids and were strongly associated with mobile genetic elements (MGEs). *dfrA8* and $bla_{\text{CARB-3}}$ and several novel ARG–MGE associations were detected for the first time in *V. parahaemolyticus* genome, including $bla_{\text{CARB-3}}$-IS6 or *dfrA8*-IS6. Interestingly, several resistome–mobilome associations, notably *dfrA23*, $bla_{\text{CTX-M-15}}$-IS6, and *floR*-IS91, were significantly correlated with variations in biofilm height, roughness, substrate coverage, and volume. Moreover, a particularly strong association was observed for *tet(A)*, whose presence was closely linked to reduced biofilm roughness. However, no association was found between phenotypic antibiotic resistance and biofilm formation metrics, except for a significant link between quinolone resistance and increased biofilm height. These findings provide new insights into the genomic factors underlying biofilm-associated persistence in *V. parahaemolyticus*.

S6-P13: Impact of Temperature and Oxygen on Biofilm Development and Aerotolerance in *Campylobacter jejuni* Strains from Clinical and Food Processing Environment

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Campylobacter jejuni is a foodborne pathogen capable of developing biofilms under *in vitro* conditions which enhances its persistence and survival in food processing environments. The aim of this study was to evaluate biofilm-forming ability and aerotolerance of *C. jejuni* strains isolated from human infections and food processing environments.

Four clinical strains of *C. jejuni* (H249, H378, H518 and H660) and three environmental strains (M327, M418 and M604) were assessed for biofilm formation on polystyrene plates. Inoculum concentration were adjusted to an OD590 of 0.7 (corresponding to account of 109 CFU/mL). Negative control (Muller Hinton Broth without inoculum) and positive control (reference strain NTC11168) were included. All plates were incubated at 25°C, 30°C and 37°C for 96 h under aerobic and microaerobic conditions. Aerotolerance was evaluated by sampling at the three temperatures followed by serial dilutions and enumeration on blood agar. Biofilm biomass was quantified using Bouin Solution to fix the cells attached and 0.1% crystal violet to stain layer cells. Finally, cells suspension with ethanol and acetone was transferred into 96 wells-plate for reading at OD_{590}.

Results and statistical analysis demonstrated that biofilm development was significantly influenced by strain characteristics, temperature and incubation time (p-value < 0.009). The relation between strains and temperatures shown that biomass on biofilm was increased at higher temperatures (30°C and 37°C) and aerobic condition, in where H378, H660 and H518 were moderate and strong biofilm former respectively, while weak biofilm was observed at 25°C. Otherwise, H249 and M604 only developed biofilm at 37°C. However, H249 was moderate biofilm former under microaerophilic conditions, while M604 formed a weak biofilm in aerobic atmosphere. In any of the analysed conditions, strains M327 and M418 were incapable of forming a biofilm.

Aerotolerance results were alignment with biofilm formation. The most significant cell damage was observed after 72 h at 37°C (p-value < 0.001). Nevertheless, H378, H660, NTC11168, H518 and M327 were strains more resistant to aerobiosis and high temperature, whereas M604, H249 and M418 were more susceptible to non-optimal conditions.

In conclusion, some strains of *C. jejuni* are able to survive and form biofilm at high temperatures and aerobic conditions, commonly found in the initial phases of poultry slaughtering. Thus, these results highlight the need for effective cleaning and disinfection procedures to prevent the persistence of this pathogen in the food chain and to ensure the safety of poultry products.

S6-P14: Microbiological Hygiene Assessment of Public Fountains and Fountain Fields

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Public fountains and fountain fields have been increasingly integrated into the design of public spaces and urban centers in recent years and represent popular urban water features with high human contact, especially children. Driven by urbanization, reduced green spaces, and increasing heat island effects, such systems contribute to local microclimate regulation through evaporative cooling. However, their open design and intensive use also introduce l hygiene challenges, as unintended activities (bathing, laundry) and external inputs (e.g., litter, fecal contamination, runoff, and elevated temperatures) promote microbial contamination and thrive of pathogens. In addition, the interpretation of microbiological data is complicated by residual disinfectants (chlorine, hydrogen peroxide), which may lead to systematic underestimation of microbial loads if not properly neutralized during sampling. Moreover, fountain systems vary widely in design and water sources (e.g., tap water, groundwater, rainwater) and, despite continuous technical treatment (circulation, filtration, UV, disinfection), remain highly susceptible to external influences that compromise microbial water quality.

A pilot study aimed to assess the influence of technical, mechanical, and chemical treatment measures on the microbiological quality of fountain water and to establish a baseline for future risk assessment and management strategies. Therefore, five selected fountains (different types, locations and frequencies of usage) were investigated, and a total of 116 water samples were analyzed for microbiological parameters, including indicator organisms and total plate counts. The results demonstrate that inadequate maintenance and suboptimal technical conditions can lead to elevated microbial loads. At least 79% of the samples did not meet the requirements of the German Ordinance on the Quality of Water intended for Human Consumption (TrinkwV) and DIN SPEC 31062 for disinfected fountain water. Frequent contamination with fecal indicator organisms *Enterococcus* spp. and *Escherichia coli* was observed. Pseudomonas was identified as a dominant biofilm-forming organism and effective reduction of microbial loads was only achieved through combined mechanical and chemical treatment, while UV treatment alone showed limited effect, particularly against *Pseudomonas*, and did not ensure compliance with drinking water standards. *Legionella* was not detected.

These findings highlight the need for improved monitoring approaches independent of costly and time-consuming cultivation methods. The new cooperation-project “SpriBak” focusses on the development and validation of resazurin-based assay which shall be integrated as an on-site detection method, to enable real-time assessment of microbial activity and support adaptive management of fountain systems without exclusive reliance on laboratory analyses.

S6-P15: Human-Associated Fecal Contamination Identified in Irrigation Water by Microbial Source Tracking

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Irrigation water can serve as a vehicle for fecal contamination of fresh produce, making it directly relevant to food safety. Routine monitoring based on fecal indicator bacteria such as *Escherichia coli* confirms the presence of fecal contamination but cannot identify its origin. Source attribution is nonetheless important for interpreting contamination events and supporting risk-based management of irrigation water quality. Microbial source tracking (MST) using host-associated genetic markers addresses this gap, enabling more informed risk assessment along the farm-to-fork continuum. Aim of the study was to determine the likely host source(s) of fecal contamination in irrigation water samples previously identified as contaminated based on *E. coli* results, using MST with host-associated genetic markers.

Four irrigation water samples collected during routine monitoring were subjected to MST analysis. *E. coli* concentrations (range: 50–150 CFU/100 mL) had been determined according to ISO 9308-1. For MST, 500 mL per sample was filtered and analyzed by multiplex digital PCR targeting a panel of seven host-associated fecal markers: human-associated (BacH, BacHum, crAssphage), ruminant (Rum2Bac), cattle (CowM3), pig (Pig2Bac), and avian (AV4143), alongside the general fecal marker GenBac3. A result was considered positive at ≥ 3 positive partitions.

GenBac3 was detected in all four samples, confirming fecal contamination. At least one human-associated marker was detected in each sample: crAssphage in three samples (480–960 copies/100 mL), BacHum in two samples (330–660 copies/100 mL), and BacH in one sample (660 copies/100 mL). No animal-associated markers (Rum2Bac, CowM3, Pig2Bac, AV4143) were detected in any sample. Collectively, the MST results indicate a predominantly human fecal contamination pattern across all sampling sites.

MST strengthened the interpretation of routine microbiological monitoring by identifying the likely origin of fecal contamination – an insight not obtainable from indicator bacteria alone. This is particularly relevant in the context of fresh produce safety, as irrigation water in direct contact with crops represents a plausible route of pathogen transfer. Human-associated fecal markers are epidemiologically linked to enteric pathogens of public health concern, and their consistent detection in the absence of animal markers points to a wastewater-related source. These findings support the integration of MST into irrigation water monitoring frameworks as a tool for evidence-based food safety risk management.

S6-P16: Surface Microbiota and the Presence of Disinfectant Residues Influence Biofilm Formation by *Listeria monocytogenes*

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Listeria monocytogenes (Lm) is a foodborne pathogen responsible for many outbreaks worldwide. Most of these foodborne illnesses are caused by the consumption of food contaminated during production. It is therefore essential to better understand how Lm can survive in food processing facilities despite the repeated application of disinfectants. The aim of this study, part of the ANR SUBLIM project, was to determine whether and how surface microbiota, disinfectants and food residues could influence the ability of Lm to form biofilms.

Three environmental sampling campaigns were conducted in nine food plants from the seafood, vegetable and pork sectors. A total of 1,097 samples were analyzed for the presence of Lm, the enumeration of total mesophilic counts and lactic acid bacteria, residues of quaternary ammonium (QAs) and triamine (TA) compounds. Dominant mesophilic aerobic bacteria and lactic acid bacteria were isolated on Plate Count agar and MRS agar, respectively, and identified by MALDI-TOF. Most represented species were used to prepare synthetic consortia, using nutrient-rich inoculum representative of seafood, vegetable, and pork sectors, co-inoculated with Lm to form biofilms on stainless steel coupons. QAs and TA residues were deposited onto surfaces prior to biofilm formation. Biofilm formation kinetics over 96 h were assessed by plate counts on TSAYe agar (total biofilm) and ALOA agar (Lm).

Lm was detected in 114 out of 1,097 samples (10.8%). Exploratory analysis of data from the sampling campaigns revealed a positive correlation between Lm detection on surfaces, high levels of lactic acid bacteria and mesophilic aerobic counts, and the use of QAs and TA. QA residues were detected in 438 samples (39.9%) at concentrations ranging from 0.5 to 32,200 ng/cm², while TA residues were detected in 253 samples (23.1%) between 0.5 and 11,300 ng/cm². On stainless steel coupons, Lm showed limited growth in biofilms cultivated with vegetable residues but strong growth with pork and seafood residues. In mixed biofilms, lactic acid bacteria inhibited Lm growth and limited total biofilm development, whereas mesophilic aerobic flora allowed low Lm growth but strong total biofilm growth. Adhesion of QAs and TA residues on coupons inactivated or inhibited Lm and suppressed total biofilm growth with lactic acid bacteria but not with mesophilic aerobic flora.

These findings highlight that the nature and composition of organic soiling, together with surface microbiota and residual biocides, influence Lm biofilm formation and underscore the importance of microbial interactions in Lm mitigation strategies.

S6-P17: Biofilm Formation of *Pseudomonas aeruginosa* in Raw Milk Environments: Implications for Dairy Hygiene and Milk Quality

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Raw milk production systems harbor complex microbial communities that directly influence the quality and shelf life of dairy products. Among microorganisms associated with dairy environments, *Pseudomonas aeruginosa* is of particular concern due to its high ecological adaptability, persistence on equipment surfaces and strong biofilm-forming capacity.

Biofilm formation represents a common microbial survival strategy that enables bacteria to persist in food production environments. In dairy systems, *P. aeruginosa* can colonize materials used in milking and milk storage, including stainless steel surfaces, where biofilms may develop during refrigerated storage and act as persistent sources of contamination. The presence of *P. aeruginosa* in raw milk environments is associated with several technological and hygiene challenges. This bacterium produces extracellular proteolytic and lipolytic enzymes that may remain active even after heat treatment, thereby contributing to milk deterioration, flavor defects, and reduced shelf life of dairy products. Biofilm formation further enhances bacterial persistence in milking systems by protecting cells from mechanical removal and routine cleaning procedures, allowing biofilms to function as long-term reservoirs of contamination within dairy equipment.

In this study, 18 *P. aeruginosa* isolates originating from bulk tank raw milk samples were evaluated for their biofilm-forming capacity using phenotypic screening methods. The analysis revealed substantial variability among isolates: 72,2 % were classified as strong biofilm producers and 27,8 % did not form detectable biofilms. Interestingly, no isolates exhibited moderate or weak biofilm forming capacity.

These findings demonstrate the considerable biofilm-forming potential of *P. aeruginosa* associated with raw milk environments and highlight its role in the persistence of contamination in dairy production systems. A better understanding of the ecology and persistence of *P. aeruginosa* biofilms may support the development of more effective sanitation strategies, ultimately contributing to improved milk quality and enhanced safety of raw milk production.

S6-P18: *Bacillus subtilis* PS-216 Disrupts Mature *Campylobacter jejuni* Biofilms: Role of Surfactin and the Protease AprE

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Campylobacter jejuni is the leading cause of bacterial gastroenteritis in the EU and US. Although a fastidious pathogen sensitive to oxygen, low temperatures, and nutrient limitation, its environmental persistence suggests resilience mechanisms, likely including biofilm formation. Biofilms confer substantial resistance to antimicrobials and disinfectants, yet the interaction between *C. jejuni* biofilms and probiotic bacteria remains poorly understood.

Bacillus subtilis PS-216 (Bs) exhibits strong anti-*Campylobacter* activity in planktonic co-culture, reduces *C. jejuni* colonization in the chicken cecum, and prevents *C. jejuni* adhesion to polystyrene and early biofilm establishment. Here, we investigated whether Bs can also disrupt a mature *C. jejuni* biofilm and the mechanisms underlying this interaction.

We established a mature 48-h *C. jejuni* biofilm in 24-well microtiter plates, forming characteristic finger-like floating structures adhered to polystyrene walls and a network-like condensation in the well interior. Mature biofilms were challenged with Bs wild-type (wt) and mutant strains lacking the global regulators ComA (Δ*comA*) and DegU (Δ*degU*), surfactin (Δ*srfAA*), EPS (Δ*epsA-O*), and the two most abundant proteases AprE (Δ*aprE*) and NprE (Δ*nprE*) for 24–48h at 42°C in microaerobic conditions. *C. jejuni* and Bs populations were quantified by CFU counting and visualized by confocal laser scanning microscopy.

Bs wt reduced *C. jejuni* within the biofilm (1 log reduction compared to t0 and 2 log reduction compared to untreated control), but the effect was substantially weaker than in planktonic co-culture (> 4 log reduction), highlighting the protective role of the *C. jejuni* biofilm matrix. Mutants lacking the global regulators ComA and DegU permitted biofilm persistence and enabled the emergence and outgrowth of planktonic *C. jejuni* cells, suggesting these regulators are essential for effective biofilm disruption and *C. jejuni* growth control. In contrast, mutants lacking surfactin and the protease AprE exhibited stronger *C. jejuni* reduction within the biofilm than Bs wt, implying that these factors may paradoxically limit Bs efficacy in the biofilm context, possibly by modulating Bs's own biofilm behavior, resource allocation, and/or channeling the resources toward synthesis of *C. jejuni* antagonists.

These findings reveal that the anti-*Campylobacter* activity of *B. subtilis* PS-216 is mechanistically distinct in mature biofilm versus planktonic settings. Understanding these interactions opens new avenues for the rational design of probiotic interventions aimed at reducing *C. jejuni* biofilm reservoirs in poultry production environments and, ultimately, improving food safety.

S6-P19: Tracing Microplastics on Packaged Chicken Meat Surface and Assessing Co-Occurring Foodborne Bacteria

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Plastic waste breaks down into increasingly smaller particles, ranging from 1 µm to 5 mm in size, which enter human and animal food chains through air, soil, and water. These particles pose health risks, from physical irritation of tissues as foreign objects to chemical risks, including the migration of their components into food. Additionally, growing evidence suggests that microplastics can serve as mobile surfaces facilitating the transfer of organic and inorganic substances as well as microorganisms.

In our study, we optimised a method for isolating microplastics from chicken meat and skin and applied it in a pilot study to assess the degree of microplastic contamination on the surface of packaged chicken meat and skin. Based on visual characterisation of the isolated particles under a stereomicroscope and their chemical characterisation using Fourier Transform Infrared Spectroscopy (FTIR), we identified their potential origin as either packaging or other sources. Furthermore, standard microbiological analysis and MALDI-TOF mass spectrometry were performed for additional identification of the isolates to assess the presence of pathogenic and spoilage-associated taxa, in order to evaluate microbiological safety and quality of chicken meat samples.

This pilot study examined 10 samples of packaged chicken meat (5 with skin and 5 without skin) and identified 22 microplastic particles ranging from 119 to 2605 µm in size, of which 12 chemically matched the packaging material in which the meat was packaged. Interestingly, contamination of the chicken skin originated entirely from sources other than the packaging, whereas particles from the packaging material contaminated chicken meat packaged without skin. Microbiological analysis of the same chicken meat samples revealed co-occurring contamination with foodborne pathogenic and spoilage-associated bacteria, highlighting the potential for microplastics to support the survival and transmission of foodborne pathogens and spoilage bacteria under food-relevant conditions.

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S7 Hygiene and Human Interaction in the Food Environment

S7-P1: Investigation of *Clostridium* spp. Along the Farm-to-Fork Chain Using the ISO Reference Medium ISA and a New Chromogenic Medium

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Bacteria of the genus *Clostridium* are obligate anaerobes widely distributed in nature. They colonize a diverse array of environmental niches and constitute a significant component of the gastrointestinal microbiota of livestock species. Their ubiquity, spore forming ability, and persistence under harsh conditions can facilitate cross-contamination across multiple food categories. Within this genus, *Clostridium perfringens* is the principal foodborne pathogen, requiring sensitive and reliable methods for detection and quantification to support effective surveillance and outbreak management. According to the standardized culture-based methodology defined in NF EN ISO 15212, the procedure requires the use of two distinct culture media, Iron Sulfite Agar (ISA) and Tryptose Sulfite Cycloserine agar (TSC), to be internationally validated for *Clostridium* spp. and *C. perfringens* enumeration, respectively. An innovative chromogenic medium provides a single-plate alternative, enabling simultaneous enumeration of sulfite-reducing *Clostridium* spp. by differentiating *C. perfringens*. This study compares the performance of the chromogenic medium against the ISO reference method with different food and environmental samples.

Firstly, *Clostridium* strains from collection were spread onto both the ISA medium and the chromogenic medium. Growth assessment using pure cultures demonstrated that all *Clostridium* strains developed equally well on both media. Complete alignment was observed in terms of qualitative recovery, confirming that the chromogenic formulation supports the growth of *Clostridium* spp. (black colonies) and *C. perfringens* (red colonies) as effectively as the ISA medium.

Secondly, different samples from environmental sources (cultivated soil, compost), food-processing surfaces (hand swabs, cutting boards, etc), raw materials (raw milk, meat, vegetables) to processed products (ready to eat meals, meat dishes, cheese, etc) with a cold chain break were analyzed. After standardized sample preparation and serial dilution, aliquots were plated into the two-culture media, followed by anaerobic incubation. Contamination levels were generally higher in matrices collected at the beginning of the food chain. In contrast, processed food products showed lower contamination frequencies and concentrations, reflecting the effect of processing steps and hygienic controls. For contaminated samples, enumeration results obtained on the chromogenic medium were comparable to those obtained on the ISA medium.

Overall, the novel medium yields quantitative and qualitative performances that are at least equivalent to the existing methods, while offering practical advantages in terms of selectivity, time to result, reagent consumption, hands on time, and readability. Its implementation could contribute to improved routine monitoring and quality control workflows in food microbiology laboratories, as well as in laboratories involved in the analysis of environmental samples.

S7-P3: Weekly Temperature Dynamics in Household Refrigerators: Seasonal Variation, Hygienic Conditions, and Consumer Behaviour

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Domestic refrigerators represent a critical and often overlooked link in the cold supply chain. Previous studies consistently show that many household refrigerators operate above the recommended temperature, supporting the growth of foodborne pathogens.

Fourteen household refrigerators in Slovenia were monitored continuously for seven days during both winter and summer period, yielding 28 measurement cycles. Air temperature was recorded at four positions (upper and lower shelf, near the back wall, and near the front door), and the core temperature of a standardised tyloses gel test product was measured at 30-min intervals using Thermotrack 21G data loggers (± 1°C accuracy). Hygienic and technical conditions were assessed using a structured evaluation form, and door-opening frequency was recorded by participants throughout each monitoring week. A parallel online survey (n = 108) investigated consumer attitudes towards refrigeration practices.

The overall weekly mean air temperature across all monitoring cycles was 6.12°C. The mean core temperature of the test product was 5.05°C. Only four out of 28 measurement cycles yielded mean air temperatures compliant with the ≤ 5°C recommendation. Summer temperatures were higher in ten out of fourteen households, with a mean seasonal difference of 1.2°C (maximum 3.7°C). Refrigerators without a fan showed more pronounced temperature gradients between the upper and lower compartments. Overfilled refrigerators had core product temperatures on average 2.8°C above the recommended value. Door-opening frequency averaged 15 times per day and was higher in summer in nine of fourteen households; however, no statistically significant association between opening frequency and core product temperature was observed. Expired products were found in 43% of refrigerators in winter and 58% in summer. Hygienic conditions were assessed as fully satisfactory in 64% of refrigerators at the first visit, declining to 36% at the second; moreover, 64% of participants stored food solely according to available space, without applying cross-contamination prevention principles. Although 71% of participants correctly identified the recommended refrigerator temperature, this knowledge was not consistently applied.

Domestic refrigerators in this study frequently operated above safe storage temperatures, particularly when overfilled. Consumer knowledge about correct refrigeration temperatures does not reliably translate into compliant practice. Seasonal thermostat adjustment and adequate loading were identified as key modifiable behaviours. These findings highlight the need to strengthen the domestic segment of the cold chain and reduce the risk of foodborne illness.

S7-P4: Uneven Implementation of *Listeria monocytogenes* Hygiene Monitoring in Slovenian Kindergartens and Primary Schools: A Cross-Sectional Analysis Across Two School Years

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Listeria monocytogenes remains one of the most serious challenges in food safety, and listeriosis incidence is rising across the EU. EU regulations require food business operators to verify the effectiveness of their hygiene regimes through environmental and food testing, with frequency determined by individual risk assessment. This flexibility may produce uneven implementation in practice. Kindergartens and primary schools represent an ideal setting for evaluating regulatory adherence: they serve a vulnerable population, operate under legal obligations to provide up to four meals daily, and deliver both ready-to-eat meals, raw produce and dairy items. We assessed the intensity and regional distribution of hygiene verification testing in Slovenia across two school years.

We conducted a retrospective cross-sectional analysis of hygiene verification sampling – commissioned by the schools and independently executed by an accredited laboratory – conducted in Slovenian kindergartens and primary schools in two school years (2018/19 and 2024/25). The analysis included 518 and 510 institutions respectively, covering approximately 87–89% of all students registered in the country. Sampling intensity was normalised per 100 children for surface swabs and per 1,000 children for food samples. Regional variation and temporal trends were assessed by comparing medians and interquartile ranges using non-parametric tests.

Nationally, overall surface swabbing for hygiene indicator bacteria intensity remained stable across both periods (median ~3.1 swabs/100 children/year). *Listeria*-specific surface swabbing was virtually absent (median = 0 in both periods), with significant activity confined to a single region (1.0–1.3 swabs/100 children/year). Food testing intensity was also stable (3.6 vs. 4.1 samples/1,000 children/ year) but *L. monocytogenes*-specific food testing increased markedly between periods (median 0 vs. 1.9 samples/1,000 children/year). Striking regional and inter-institutional heterogeneity persisted across all testing activity measures. Some regions performed no *Listeria*-specific food testing whatsoever, while others exceeded the national median by nearly fivefold.

These findings suggest that relying on self-assessed risk for hygiene verification, despite EU legislation, results in highly fragmented and inconsistent environmental and food testing, even within highly regulated, standardised institutional catering serving young children across Slovenia. While overall testing activity was stable and *Listeria* food testing has improved in the observed period, robust environmental *Listeria* monitoring remains concentrated in a single region. The relative shift from environmental *Listeria* monitoring toward end-product testing suggests a potential misapplication of risk-based strategies. These results highlight the need for clearer guidance on testing frequencies to ensure harmonized food-safety outcomes and the consistent protection of vulnerable populations nationwide.

S7-P5: Evaluation of Easy Plate EB for *Enterobacteriaceae* Enumeration Using ISO 16140-2, and AOAC Certification Standards

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In food and food processing environments, the enumeration of *Enterobacteriaceae* is performed to monitor the level of hygiene in the factory environment and to check process efficacy. Enumeration is conventionally performed using Violet Red Bile Glucose (VRBG) agar. Easy Plate EB (E-EB), a newly developed ready-to use (RTU) medium, serves as an alternative method for *Enterobacteriaceae* enumeration. Compared with VRBG, the RTU medium offers practical advantages, including reduced preparation time, ease of use, and improved sustainability. In addition, an automated colony-counting system can be integrated into the platform, enabling faster and more standardized workflows in routine testing.

This study evaluated the performance of E-EB according to the ISO 21528-2:2017 reference method (VRBG) for the enumeration of *Enterobacteriaceae* following a joint ISO 16140-2 and AOAC validation study design.

This study evaluated the inclusivity, exclusivity, repeatability, accuracy and relative trueness of E-EB as required by ISO 16140-2. All samples were tested following the manufacturer's instructions for E-EB and ISO 21528-2:2017 for the reference method. In the inclusivity and exclusivity study, 50 *Enterobacteriaceae* strains and 30 non-target strains were analysed. For relative trueness study, 105 samples were analysed across 7 categories; heat processed milk and dairy products, multicomponent foods, fresh produce, fishery products, raw meat and poultry, pet food and animal feed and environmental samples. In addition to the method comparison, a colony-counting system was assessed against manual visual counting to determine enumeration accuracy.

Forty-nine out of 50 *Enterobacteriaceae* strains showed positive results with both the RTU medium and reference method and 29 of the 30 non-target strains were negative with both methods. The results of inclusivity and exclusivity showed that E-EB is comparable to the conventional VRBG method. The relative trueness study revealed good agreement between the RTU medium and the reference method, with a correlation coefficient of 0.964. In the accuracy profile study, all but one sample satisfied the 0.5-log acceptability limit or the recalculated limits. Additionally, the automated counting system demonstrated high concordance with manual counting, highlighting its potential to improve laboratory productivity.

E-EB demonstrated equivalent selectivity for the *Enterobacteriaceae* and gave good agreement with the ISO 21528-2:2017 reference method across a broad range of foods after 24 ± 2 h of incubation at 37 ± 1°C. This RTU device is a convenient alternative for the enumeration of *Enterobacteriaceae*.

S7-P7: Shelf-Life Extension of Fresh Fish Through Washing Treatments: Identification of Specific Spoilage Organisms and Microbial Control

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Fresh fish deteriorates rapidly compared to meat and vegetables, resulting in a limited shelf life. Control of specific spoilage organisms (SSOs), which dominate microbial communities during storage, is essential for shelf-life extension. This study aimed to identify SSOs in fresh fish and evaluate the effectiveness of washing treatments using food additives and electrolyzed water.

To identify SSOs, horse mackerel (*Trachurus japonicus*) was stored on ice for up to 14 days, and microbial dynamics were analyzed by plate counting and 16S rRNA gene sequencing. Initial bacterial counts were 4.4 log CFU/fish (total viable counts) and 5.1 log CFU/fish (marine bacteria), exceeding 7 log CFU/fish by day 4. Microbial community analysis revealed a shift toward *Moraxellaceae* during storage. *Psychrobacter*, a psychrotolerant genus, was found to dominate under the storage conditions used in this study, suggesting that it may act as a potential SSO.

Washing treatments using sodium hypochlorite, slightly acidic electrolyzed water, acidified sodium chlorite, and calcium hydroxide solution (derived from calcined shells) were evaluated. Sodium hypochlorite and slightly acidic electrolyzed water showed limited bactericidal effects, whereas acidified sodium chlorite and calcium hydroxide treatment resulted in substantial reductions in bacterial counts. In particular, calcium hydroxide treatment reduced total viable counts by approximately 1.3 log CFU/fish and marine bacteria by 1.6 log CFU/fish. During subsequent storage, bacterial growth remained consistently lower in treated samples than in untreated controls, and the time required to reach spoilage levels was extended by approximately 6 days. The presence of injured bacteria during storage suggested sublethal effects of the treatment. However, microbial community analysis indicated that none of the treatments selectively eliminated *Moraxellaceae*, suggesting limited efficacy against SSOs despite reductions in total bacterial load.

These findings demonstrate that *Psychrobacter* may act as a potential SSO under the storage conditions used in this study, and that calcium hydroxide washing can effectively reduce initial bacterial loads and extend shelf life. Further strategies targeting SSOs are required for more effective spoilage control.

S7-P8: Consumer Food Safety During Shopping and Transport - A Mixed Methods Investigation of Knowledge, Attitudes and Practices (KAP) in Slovenia

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Food safety is a shared responsibility and critical for public health. Consumer responsibility begins during grocery shopping, where food placement, temperature control, and minimizing time out of refrigeration are essential to prevent cross-contamination and pathogen growth. The aim of this study was to investigate consumer knowledge, attitudes, and practices related to food shopping and transport in Slovenia using a mixed-methods approach combining a structured questionnaire (n = 1621) and focus group discussions (n = 40).

The questionnaire covered different aspects of food safety during food shopping and transport. Most participants agreed that raw meat/poultry/fish should be separated from other foods during shopping or transport and that wet foods should be separated from dry foods. The use of plastic bags (without or with disposable gloves) was most common for buying unpackaged fruits and vegetables. The aspect of shopping order in the store shows room for improvement. Participants were more concerned about the quick transport of frozen and perishable foods to their home than about adding these foods to the shopping basket last. About two thirds of participants added frozen and perishable foods to their shopping basket last. The use of cooler bags/insulated bags to transport perishable foods needs to be increased as only around a third of participants used them. The transport time from store to home was up to 30 min for the majority of the participants.

Focus group discussions provided further insight into food shopping order and what participants are considering during food shopping. The most common food shopping order was: unperishable, fresh vegetables, refrigerated perishable and frozen foods. Participants revealed that shopping order was influenced by the store layout, shopping lists, item placement within the shopping cart, etc.

The mixed methods approach revealed diverse aspects of consumer food safety during food shopping and transport. The findings highlight the gaps between knowledge and practices as well as attitudes and practices. Based on these findings practical solutions can be created to improve consumer food safety during food shopping and transport.

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S7-P9: *Bacillus cytotoxicus* Dynamics in the Food Processing Environment

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Bacillus cytotoxicus is a recent member of the *Bacillus cereus* group that has been linked to severe foodborne outbreaks, including fatal cases, and is frequently detected in dried potato products, raising food safety concerns. This study aimed to identify characteristics that confer *B. cytotoxicus* a competitive advantage in this food environment by investigating nutrient utilisation, phenotypic traits, and spore survival during simulated potato flake processing. Nutrient utilisation profiles were assessed using API systems and growth assays, while phenotypic traits including starch hydrolysis, hemolysis, swarming, and biofilm formation were evaluated. In addition, a laboratory-scale potato flake processing model was developed to examine spore survival under representative thermal and dehydration conditions, with comparison to *B. cereus*. The results demonstrate that *B. cytotoxicus* spores exhibit higher heat resistance in the potato matrix and maintain viability during long-term storage, unlike *B. cereus* spores. Furthermore, strong biofilm formation capacity observed in several strains supports their potential to colonise industrial processing equipment and contribute to recurrent contamination. Overall, these findings highlight the role of phenotypic heterogeneity in the persistence of *B. cytotoxicus* and underscore the need for targeted mitigation strategies and further research into its survival mechanisms and ecological niche.

S7-P10: Bridging the Knowledge-Behaviour Gap in Food Safety Through Artificial Intelligence

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A persistent gap remains between food handlers' knowledge and their actual behaviour, with conventional training approaches often failing to achieve lasting behavioural change. This review synthesises current literature on the integration of artificial intelligence (AI) into food safety training and behavioural research, examining how AI technologies can address the limitations of traditional methods.

A review was conducted of peer-reviewed literature, regulatory documents, and industry reports on AI applications in food safety training, behavioural monitoring, and research methodologies. Sources were analysed to identify current challenges in conventional training, emerging AI-powered solutions, and future research priorities.

Conventional food safety training faces several challenges: low engagement with passive delivery methods, language and literacy barriers in diverse workforces, poor knowledge retention without reinforcement, weak feedback loops reliant on infrequent audits, and organisational barriers such as inconsistent management support. AI technologies offer promising solutions across multiple domains. Immersive training using AI-enhanced virtual and augmented reality enables realistic simulation of food safety scenarios with real-time feedback. Intelligent assistants provide multilingual, role-specific guidance available continuously, reducing dependence on human trainers. Computer vision systems can monitor hygiene behaviours continuously and objectively, overcoming the limitations of manual observation, including reactivity bias. AI-enabled nudging delivers timely, context-specific prompts to reinforce safe practices during routine work. Beyond training, AI expands the methodological toolkit for behavioural food safety research by enabling scalable, unobtrusive observation and large-scale behavioural analytics. However, implementation challenges include data privacy concerns, algorithmic bias, digital divide constraints, the need for human oversight, and regulatory alignment.

AI has considerable potential to transform food safety training from episodic, generic instruction to continuous, personalised, and behaviour-focused learning systems. When implemented responsibly with appropriate governance, bias controls, and human-in-the-loop oversight, AI can strengthen learning transfer, improve accessibility for diverse workforces, and support evidence-informed intervention design. Future research should evaluate the effectiveness of AI-assisted training across multicultural food industry contexts, examine impacts on observable behaviours rather than knowledge alone, and develop privacy-preserving approaches that balance monitoring capabilities with worker trust.

S7-P11: Understanding Household Food Waste Reduction Through Behavioural Interventions, Communication and Safe Reuse

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Household food waste remains one of the most persistent barriers to sustainable food systems, despite growing awareness of its environmental, economic and social consequences. Current research indicates that food waste in households is driven not only by material constraints, but also by everyday behavioural routines, insufficient food management skills, poor meal planning, confusion about date labels, low confidence in leftover reuse, and a frequent gap between awareness and actual behaviour. This poster presents a literature-review-based research framework focused on sustainable food waste management, with particular emphasis on behavioural factors, consumer awareness, and the safe reuse of edible food fractions. The framework is based on a structured literature review of scientific studies and selected policy-oriented resources addressing household food waste, communication strategies, behavioural interventions, nudges, digital tools, and food safety aspects relevant to reuse practices. The reviewed literature suggests that information-based approaches alone are rarely sufficient to achieve lasting behavioural change. More effective approaches tend to combine practical food-management support with behaviourally informed interventions such as prompts, reminders, comparative feedback, social norm messaging, implementation planning, and tailored communication adapted to specific consumer groups. The literature also highlights the importance of psychographic, demographic and socio-economic differences, indicating that food waste reduction measures should be targeted rather than generic. In addition, food waste prevention strategies that promote reuse must more explicitly address hygiene, storage conditions, date-label interpretation, cold-chain maintenance, and consumer risk perception, since food safety concerns often shape disposal decisions. By integrating behavioural science, communication, and food safety perspectives within a circular economy framework, this literature review provides a foundation for ongoing research on more effective guidelines, support tools, and policy recommendations to reduce household food waste and promote the safe and responsible use of edible food resources.

Keywords: household food waste; consumer behaviour; behavioural interventions; communication strategies; safe reuse; food safety; circular economy

S7-P12: Occurrence of Foodborne Bacteria and Quantitative Microbial Risk Assessment of *Salmonella* in Street-Vended Sliced Papaya in Urban Benin (West Africa)

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Ready-to-eat fresh fruits sold in informal urban environments are particularly vulnerable to microbial contamination due to inadequate hygiene and handling practices. This study assessed the microbiological quality of street-vended sliced papaya (*Carica papaya*) in urban Benin and evaluated how hygiene conditions influence contamination and consumer exposure to foodborne pathogens. A total of 135 sliced papaya samples were randomly collected from street vendors in three major cities (Abomey-Calavi, Cotonou and Porto-Novo) at different time points along the selling period (freshly prepared, mid-sale and end of sale) to capture realistic exposure scenarios. Microbiological analyses targeted *Escherichia coli*, fecal coliforms, *Staphylococcus aureus* and *Salmonella* spp. using standardized culture-based methods, including chromogenic media for selective enumeration. *E. coli* and coliforms were enumerated on Rapid'*E. coli* 2 agar, and *S. aureus* on Baird-Parker agar supplemented with rabbit plasma fibrinogen. *Salmonella* spp. detection was performed using a validated chromogenic method, and quantification was carried out using the Most Probable Number (MPN) approach. Presumptive isolates were confirmed by 16S rRNA gene sequencing, and *Salmonella* isolates were further characterized by PCR targeting *invA* and *iroB* genes. Hygiene and handling practices, as well as time-temperature profiles, were documented for each vendor (*n* = 135) through structured questionnaires combined with direct observations. Sliced papaya consumption data were collected from 437 respondents to support exposure assessment. High levels of contamination were observed. *E. coli* was detected in 75.6% of samples, with loads ranging from 3.4 to 3.6 log CFU/g, while fecal coliforms reached approximately 6 log CFU/g. *S. aureus* was detected in 100% of samples, with loads between 5.3 and 6 log CFU/g. *Salmonella* spp. was detected in 4.4% of samples. The observed contamination patterns were consistent with critical hygiene deficiencies, including the use of non-potable water, inadequate utensil cleaning, bare-hand handling, and prolonged storage at ambient temperature (~30°C) for up to 12 h. These conditions, combined with time–temperature abuse, create favorable environments for microbial growth and cross-contamination. A quantitative microbial risk assessment (QMRA) specifically targeting *Salmonella* spp. is currently being developed using a probabilistic Monte Carlo approach. This pathogen was selected due to its epidemiological relevance. Overall, this study highlights the central role of hygiene in shaping the microbiological safety of ready-to-eat foods in informal settings and provides evidence to support risk-based interventions aimed at improving food handling practices and reducing foodborne disease risks in urban West Africa.

Keywords: street food, microbiological quality, *Salmonella*, pathogenic bacteria, food hygiene, QMRA

S8 Microbiological Risk Assessment: Tools and Data Integration

S8-P1: A Farm-to-Fork Qualitative Risk Assessment Tool for Microbial Hazards in Ready-to-Eat Fresh Produce (RTEFP)

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Ready-to-eat fresh produce (RTEFP), such as salads, leafy greens, and fruits, are widely consumed as part of a healthy diet. These foods are often eaten raw and undergo minimal processing, and can therefore pose a food safety risk. Managing these risks is challenging due to the many actors and practices involved in the farm-to-fork production chain. This research developed an easy-to-use decision support tool that helps in identifying where microbial contamination is most likely to occur in fresh produce production systems. The tool brings together key risk factors from both pre-harvest and post-harvest stages and converts common farming and handling practices into a clear risk rating. It enables users to compare practices, highlight high-risk areas, and prioritise practical improvements. The findings show that water quality, soil and manure management, worker hygiene and sanitation of equipment have the greatest influence on food safety. By clearly identifying these critical points, the tool supports growers, processors and regulators in targeting risk reduction measures more effectively. The significance of this research lies in its practical impact. It supports evidence-based decision-making, aligns with existing food safety guidance and can inform training, regulatory oversight and policy development. Ultimately, its use can help reduce the risk of foodborne illness, improve consumer confidence and strengthen the safety of fresh produce supply chains.

S8-P2: Evaluating Microbiological Compliance of Packaged Pasteurised Milk in South Africa: A Risk-Ranking Approach

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Milk is highly consumed; therefore, its safety and quality are of concern to prevent foodborne illnesses. Regulations exist to ensure that all milk produced complies with specific standards. In a South African context, milk and dairy fall within the regulatory scheme of R. 1555 of 21 November 1997, published under the Foodstuffs, Cosmetics and Disinfectants Act, Act 54 of 1972. Storage conditions and the time of consumption play an important role in milk quality. For this reason, quantitative microbial risk assessment (QMRA) and comparative risk ranking will assist in predicting the probability of consumers becoming ill after consuming milk and in identifying the most implicated pathogens. Using a secondary dataset of microbial counts for indicator organisms (*Escherichia coli*, coliforms, and *Enterobacteriaceae*), this study will assess and rank potential pathogens. The estimated probability will be determined for areas where consumers are exposed to high-risk pathogens. This will be achieved by using various applicable simulations across the milk production supply chain. This, in turn, could possibly lead to improvements in regulations and benefit the industry.

This study aims to evaluate the safety of packaged pasteurised milk through risk ranking of indicator organisms to improve consumer safety.

A secondary dataset of indicator organisms will be standardised and analysed, which can identify benchmark contamination levels, profiles, and assist in compliance checking. FDA-iRisk will be used to conduct the risk ranking comparison.

Expectations are that a majority of samples will comply with regulatory standards, and that, toward the end of their shelf-life, a greater percentage will show contamination. During simulations where temperature abuse and extended shelf-life will be chosen, the chances of illness might increase.

S8-P3: Microbial Quality and Safety of Plant-Based Cheese Alternatives on Belgium Market

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Plant-based cheese alternatives have become increasingly popular among consumers following vegan or environmentally sustainable diets. These products are often highly processed and contain diverse plant-based ingredients with variable microbial loads. There are some concerns about vegan cheese alternatives considering the risk of pathogen contamination. This is underscored by two *Salmonella*-related outbreaks linked to fermented vegan cheeses in the United States and Canada, and one *Listeria monocytogenes* outbreak in France. *Bacillus cereus*, commonly found in plant-based foods, is another pathogen of concern. Studies on the microbiological quality and safety of vegan cheese alternatives are scarce. This study aims to analyze a variety of vegan cheese products available on the Belgian market, assess their characteristics and microbial loads.

From January to September 2025 the wide variety of vegan cheese alternatives products were collected from supermarkets and small retail shops across Flanders, Belgium. The product range includes ca. 82 product items. Each product was purchased in duplicate from the same batch, one sample was analyzed on the day of purchase, and the other after storage at 9°C until the end of shelf life. Product characteristics such as water activity (aw), pH, and headspace composition ($\%O_2/\%CO_2$) were measured. These analyses were performed: *i*) for microbial safety: detection of *L. monocytogenes* and *Salmonella* per 25 g and enumeration of *B. cereus*; *ii*) for microbial quality: enumeration of total aerobic count (TAC) and spores, lactic acid bacteria (LAB), yeasts and molds, *Listeria* spp., *Escherichia coli* and coliforms.

Products were categorized as fermented (n = 34) or non-fermented (n = 48). The mean pH values ranged from 4.26 to 4.66, while mean aw was approximately 0.975. Most products (60%) were packaged under modified atmosphere. No *Listeria* spp., *L. monocytogenes*, or *Salmonella* were detected in any samples. Presumptive *B. cereus* was found in three samples (100–200 CFU/g), and *E. coli* was detected in three samples (10–250 CFU/g). Aerobic spores were present in most fermented products (67%, 1–3 log CFU/g), whereas the majority of non-fermented products (84%) had levels below 1 log CFU/g. TAC were high in most fermented products (68%, > 6 log CFU/g), while nearly half of non-fermented products (57%) remained below 1 log CFU/g. LAB dominated in fermented products but not in non-fermented ones. Yeasts and molds were frequently observed in fermented products, likely as part of starter cultures, but were rarely found in non-fermented products.

S8-P4: From Sea to Plate: Quantitative Risk Assessment (QRA) of *Anisakis* Through Hake Consumption

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Anisakiasis is a foodborne illness caused by consuming viable *Anisakis* larvae in fish, resulting in gastrointestinal and allergic symptoms. Due to high prevalence in hake (*Merluccius*), mitigation measures such as freezing and thermal processing are essential. This study conducted a Quantitative Risk Assessment (QRA) where freezing scenarios included 0–1 day, 1–5 days, and > 5 days at temperatures ranging from –5°C to –25°C, aligned with Regulation (EC) No 853/2004 (≥ 24 h) and AESAN guidance (≥ 5 days). Thermal scenarios considered < 60°C and ≥ 60°C for ≥ 1 min. Data were derived from systematic reviews of larval concentrations (45 eligible studies out of 356 studies), freezing efficacy (17 out of 434), thermal treatments (7 out of 668), and consumption (g/day) from the EFSA consumption database. An exponential dose-response model was considered from the literature-based ID50 value (mean 17000, SD 14000) to calculate the daily probability of illness, and later it was extrapolated to calculate the annual risk, which was comparable to the WHO risk category. A probabilistic model in MS Excel incorporated variability using cumulative distributions, with 50,000 Monte Carlo simulations using @Risk add-in (Lumivero LLC).

The simulated Anisakis concentration in hake muscle was 4.6×10^{-2} numbers/g of wet weight (90% Confidence Interval, CI: 2.3×10^{-6} – 1.5×10^{-1}). Freezing-only scenarios yielded annual risk probabilities (simulated mean Pannual) 3.6×10^{-4} to 2.9×10^{-3} per person per year (pppy), while thermal-only scenarios yielded 4.5×10^{-3} and $4.6 \times 10^{-}4$ pppy. Combined treatments reduced risks to a range of 6.4×10^{-5} to 1.1×10^{-3} pppy. The worst-case, untreated raw fish consumption, scenario showed Pannual = 1.2×10^{-2} pppy or 1 in 84 posing high risk, extrapolated from (*i*) human exposure to *Anisakis* as 4.14 numbers/day, (*ii*) corresponding daily risk of 2.5×10^{-4} per person per day (*iii*) the assumption of 1–2 times hake consumption per week. Moderate risks persisted under inadequate freezing (< 5 days) or suboptimal heating (< 60°C). Sensitivity analysis identified key contributors: larval concentration (Spearman's rank-order correlation coefficient, typ. = 0.64), muscle ratio (0.38), freezing effect (0.36), and heating effect (0.32). Cooking at ≥ 60°C and proper freezing markedly reduce the risk of anisakiasis, highlighting QRA as a valuable seafood safety tool. Future work should assess Pulsed Electric Field (PEF) technology as an alternative or complementary control method.

S8-P5: Growth Variability of *Listeria monocytogenes* on Semi-Soft and Soft Cheeses

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Listeria monocytogenes is a gram positive, facultative, non-spore former, psychrotrophic pathogen that poses a critical food safety risk, particularly in ready-to-eat products like soft cheeses. This study evaluates the growth behaviour of five distinct strains of *L. monocytogenes* on two types of cheese: semi-soft cheese and soft cheese. Challenge tests were performed in order to assess strain variability in growth rates at three different temperatures (4°C, 10°C, and 20°C). Strains were selected based on their tolerance to organic acids in laboratory media, CC type and source. Growth rates were calculated using the Robert and Baranyi model in DMFit of ComBase. The results showed higher variability in strain growth rates at refrigeration temperature (4°C) compared to abuse temperature (10°C). While at room temperature (20°C) no variability was observed. It was also seen that organic acid tolerance in laboratory media was not found to be a reliable indicator of growth on the actual food products. For semi-soft cheese, the observed growth rates were significantly slower than those predicted by ComBase at all temperatures. In contrast, for soft cheese, growth rates at abuse temperature were in close alignment with the ComBase predictions, while at 20°C, the growth rate was again significantly slower than predicted. This study provides detailed strain-specific data of *L. monocytogenes* growth rates on different cheese types and valuable insights into growth rate variability. It highlights the limitations of using laboratory broth data as a sole predictor. These data are scarce in publicly accessible databases and are essential for designing more effective challenge studies.

S8-P6: Enhanced Rapid Detection of Pathogenic *Bacillus cereus* s.l. Using MALDI-TOF MS Integrated with Machine Learning

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The *Bacillus cereus* group (*B. cereus* sensu lato) comprises closely related species, including foodborne bacteria associated with both emetic and diarrheal syndrome. The high genetic and phenotypic similarity within this group complicates rapid identification and species differentiation, which is critical in food production environments. Current phenotypic and genetic methods are either unable to reliably distinguish species within the *B. cereus* group or are too time-consuming for routine use. Strains capable of producing the highly toxic cereulide are restricted to specific phylogenetic groups, and *panC* classification can help identify isolates with emetic potential.

Therefore, the aim of this study is to investigate whether MALDI-TOF mass spectrometry spectra, facilitated with machine-learning MSclassifR, can be used to directly identify *panC* phylogenetic group of *B. cereus* s.l. isolates and rapidly predict their cereulide production potential.

100 *B. cereus* group dairy isolates were assessed for toxigenic potential using PCR/qPCR and spectra were obtained by using MALDI-TOF MS and combined with supervised machine learning classification. This classifies isolates by *panC* groups (III and IV) and to infer their likelihood of carrying toxin genes relevant to emetic (*ces*) syndromes. 75 *B. cereus* s.l. strains were used to train and test 16 machine learning pipelines. Sanger sequencing of the *panC* gene was used to characterize these strains, identifying 47 strains as group III and 28 strains as group IV.

The performance of the models was evaluated on the test dataset with a Cohen's Kappa coefficient indicating almost perfect agreement between predictions and actual labels. Further, the ML-based classifiers showed high accuracy for *panC* group identification with sensitivity in correctly identifying group III and IV ranging from 0.95 to 1.

In conclusion, MALDI-TOF MS combined with supervised machine learning provides a robust and effective approach for rapid identification and pathotype differentiation within the *B. cereus* group.

S8-P7: Predictive Modelling of the *Bacillus cereus* Group in Thermally Processed Ready-to-Eat Deli Meats

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Food business operators producing ready-to-eat deli meats that are heat-treated in the final consumer package must manage the risk of germination and growth of surviving bacterial spores. For *Clostridium botulinum* in meat products, growth can be predicted using DMRI Predict; however, no equivalent predictive tool is currently available for the *Bacillus cereus* group in meat products. This study aimed to develop and validate a growth model for the *B. cereus* group in meat products, accounting for relevant intrinsic and extrinsic factors. The objective was to describe growth not only of *B. cereus* sensu stricto, but of species across the *B. cereus* group.

Based on 87 challenge tests, members of the *B. cereus* group were shown to grow in a bologna-style deli meat. When growth was predicted using an existing psychrotolerant *B. cereus* model developed for dairy and starch products, the model systematically underpredicted growth in meat products, with a bias factor Bf of 0.31. Literature data indicate that the optimal growth rate in a meat substrate at 30°C is 11.7-fold higher than in the original model substrate. Replacing the reference growth rate with the meat-based value improved model performance, giving Bf = 0.90. Using the growth data generated in this study, 62 of the 87 challenge tests provided observed and predicted growth responses and were used for calibration. The calibrated reference growth-rate parameter for meat products was 15-fold higher than the value used for dairy and starch products. The calibrated model showed an accuracy factor of 1.20, indicating relatively low variation between observed and predicted growth rates.

For 14 challenge tests, both observations and predictions indicated no growth. In two cases growth was observed although no growth was predicted, while nine cases were fail-safe, with predicted growth but observed no growth. Observed lag phases ranged from 0 to 63 days. Relative lag time (RLT) analysis showed a highly variable relationship between μ_{max} and lag phase (days, median = 6.5, min = 0, max = 78), indicating that lag-phase prediction remains uncertain. Further model development and evaluation using an independent dataset will be completed before the conference.

A validated model for deli meats would meet an important industry need and support evidence-based product development, shelf-life assessment, and risk management for ready-to-eat meat products.

S8-P8: Risk-Based Decision Tool for Managing *Bacillus cereus* in Milk Powder Manufacturing Processes

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Bacillus cereus is an ever present pathogen in the hazard identification part of industrial risk assessments for dairy powders. Being ubiquitous in the environment, it is commonly present in raw milk and needs to be managed during the manufacture of milk powder both in terms of growth and cereulide production, since the latter once formed cannot be inactivated by heating or other types of processing.

The aim of this study is to build a quantitative decision-making tool for assessing the risk of growth and cereulide production of *B. cereus* during the manufacture of milk powder. To this end, literature was reviewed for the anticipated range of temperatures, holding times, pH and water activity during all steps of the milk powder manufacturing process along with suitable models/datasets for the growth/inactivation of this pathogen in milk. Furthermore, European guidelines and legislation on limits and control measures for *B. cereus* were reviewed to translate predictions into suitable questions for building a food safety decision tree. The purpose of the tree was to assess the risk of growth and cereulide production at each step of the milk powder production process.

Growth of *B. cereus* was predicted using a validated model in milk and a conservative threshold of 1,000 CFU/ml was used with regards to the risk of cereulide production based on the latest EFSA opinions on this pathogen and available scientific literature. The constructed process model consisted of 11 steps: 1) collection at the farm, 2) transport, 3) milk reception at dairy, 4) skimming, 5) standardisation, 6) pasteurisation, 7) evaporation, 8) spray drying, 9) secondary drying, 10) powder sifting and 11) packaging.

Storage temperature and time were identified as the most important factors affecting growth and therefore the best targets for interventions. In particular, the first 3 steps of the milk powder production process were found to be the most critical for ensuring that growth of *B. cereus* does not reach the minimum concentration associated with toxin production. The process model can also be used to determine the risk of cereulide production, as part of the requirements of EU Regulations 852/2004 and 853/2004 for companies to conduct internal risk assessments and manage relevant food safety hazards in the event of deviations from recommended storage temperatures and holding times.

S8-P9: Inactivation of *Escherichia coli* O157, O26, and O103 and Heat-Resistant *Enterobacteriaceae* During the Process of Grana Padano and Parmigiano Reggiano PDO Cheeses, and Predictive Model Validation

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Raw milk cheeses are susceptible to Shiga toxin-producing *Escherichia coli* (STEC), which can cause severe health complications such as haemolytic uremic syndrome. While long ripening generally ensures safety, scientific data is required to rigorously validate these margins. This study aimed to evaluate the inactivation of specific pathogens – *E. coli* strains (O157:H7, O26:H11, O103) and heat-resistant (HR) *Enterobacteriaceae* – during the production and ripening of Grana Padano and Parmigiano Reggiano cheeses, and to provide robust data to validate predictive models for food safety in long-ripened hard cheeses.

The experimental design involved three independent batches (replications) using approximately 5000 L of raw milk per batch. A challenge test was conducted by inoculating the milk with the target pathogens. Cheese wheels were sampled and analysed at multiple stages: within the first 48 h, after brine salting, and throughout a 9-month ripening period. Thermal profiles were continuously monitored during the entire process to characterise environmental conditions. A three-part predictive model was employed, integrating equations for growth, thermal inactivation, and non-thermal inactivation. While z-values were fixed based on literature (4.49°C for *E. coli* and 7.3°C for HR *Enterobacteriaceae*), the thermal inactivation parameters (D55°C) were estimated by fitting the experimental data from the first seven hours of the process. A growth/no-growth boundary was used to determine which model phase to apply during the dynamic temperature profiles.

The findings demonstrate that the cheese-making process and subsequent ripening guarantee a reduction of more than 6 log for all tested pathogens. Analysis of the microbial data showed a significant decrease in pathogen levels over time, with complete inactivation observed during the ripening phase. Estimated D55°C values were 9.05, 1.34, 2.58, and 111.63 minutes for O157, O26, O103, and HR *Enterobacteriaceae*, respectively. The model successfully predicted the total elimination of STEC within one month and the extended survival of HR *Enterobacteriaceae* (still detectable at 450 h), proving to be a reliable tool for fail-safe predictions.

The standard production and 9-month ripening process for these hard cheeses is highly effective in ensuring product safety by achieving substantial pathogen inactivation. These results support the use of predictive models to estimate the effects of changing environmental factors on pathogen reduction during cheese production.

S8-P10: Reservoir Potential and Virulence Profiles of Uropathogenic *Escherichia coli* in Raw Retail Meats Commonly Consumed in Taiwan

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Uropathogenic *Escherichia coli* (UPEC), a major subgroup of extraintestinal pathogenic *E. coli* (ExPEC), is recognized as the primary etiological agent of urinary tract infections (UTIs). Increasing attention has been directed toward the potential role of foodborne sources, particularly retail meats, as reservoirs of ExPEC strains; however, systematic evidence from Taiwan remains limited. This study aimed to characterize the occurrence and molecular features of UPEC in commonly consumed raw meats and to identify retail-associated factors that may influence its presence. A total of ninety raw meat samples, including chicken, pork, and beef, were collected from wet markets and supermarkets in Taiwan. Microbial analyses were conducted to quantify indicator organisms, followed by isolation of *E. coli*. Putative isolates were screened for UPEC-associated genetic markers (*c3509*, *c3686*, and *chuA*) using PCR. Confirmed UPEC isolates were further characterized by virulence gene profiling and phylogenetic classification. In addition, statistical modeling was performed to evaluate factors associated with UPEC detection. UPEC was detected across all meat types, with a markedly higher occurrence in chicken compared to pork and beef. Among recovered *E. coli* isolates, a substantial proportion exhibited UPEC-associated genetic signatures. The adhesin gene *fimH* was widely distributed, and isolates from poultry tended to harbor a broader repertoire of virulence determinants. Phylogenetic analysis indicated that group D was the predominant lineage. Multivariable analysis suggested that meat type, retail environment, packaging conditions, and overall microbial load were significantly associated with the likelihood of UPEC presence. These findings support the hypothesis that retail meats, particularly poultry, may act as a relevant reservoir of UPEC with virulence characteristics linked to extraintestinal infections. The study provides critical evidence for potential foodborne exposure pathways of UPEC in Taiwan, contributing to the development of quantitative microbial exposure assessment frameworks and facilitating the integration of retail-level variables into further risk modeling and risk-based control strategies.

S8-P11: Impact of *Listeria monocytogenes* Strain Variability on Growth Rates in Real Food Products

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Listeria monocytogenes is a psychrotrophic bacterium that poses a significant food safety risk, particularly in refrigerated ready-to-eat (RTE) products like smoked salmon and soft cheese. The EURL Technical Guidance Document states that challenge tests used to determine product safety should employ multiple strains selected based on defined maximum growth rates at low temperature (8°C), pH (5), and low water activity (0.9) but the recommended strains in the EURL document are primarily selected based on their performance in broth-based media. We have generated and compared detailed growth rate data in commercial smoked salmon and soft cheese products for multiple distinct *L. monocytogenes* strains. The test strains were selected to cover different genotypic and phenotypic characteristics such as CC-type, lineage, source and salt or organic acid tolerance in laboratory broth experiments. We have created unique sets of comparable strain-specific growth data on sensitive commercial products for multiple strains under the same conditions. Significant strain to strain differences in growth rates were observed at refrigeration (8°C), abuse (10°C) and room temperature (20°C). However, collectively growth rates at refrigeration and abuse temperatures for all strains in both products were generally found to be slower or in alignment with predictions from the predictive microbiology tools ComBase and Pathogen Modeling Program. These strain specific data which are limited in existing publicly available databases provide critical insights into growth rate variability in real food products and are important for designing effective challenge studies.

S8-P12: Risk Prioritization of Household Food Handling Practices in China Using Fuzzy Failure Mode and Effects Analysis

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Foodborne diseases continue to pose a major challenge to global public health and contribute substantially to the disease burden. Households represent a primary setting for foodborne diseases, where food handling practices playing a critical role, yet the relative risk associated with poor hygienic practices remains unclear. Failure Mode and Effects Analysis (FMEA) provide a structured framework for evaluating and prioritizing potential failures within a process or system, making it particularly suitable for assessing household food safety risks, where poor hygiene practices can promote the growth or transfer of foodborne pathogens and lead to illness.

This study proposes a Fuzzy-FMEA approach that integrates fuzzy logic into the traditional FMEA framework to prioritize the risks associated with poor hygienic practices in Chinese households. The Fuzzy-FMEA considers parameters across three dimensions: occurrence (the proportion of consumers engaging in poor hygienic practices), severity (the extent to which the practice may lead to foodborne diseases), and detection (consumers' risk perception of poor hygienic practices). The occurrence score was estimated using a national food handling practices survey, whereas the severity and detection scores were assigned through structured expert elicitation based on Cooke's classical model. Using the Mamdani inference mechanism, the Fuzzy Risk Priority Number (FRPN) was calculated to rank risky practices, with higher values indicating greater risk.

The results indicate that the highest-risk practices involve failing to use separate knives (FRPN = 7.00) and cutting boards (FRPN = 6.70) for raw and cooked foods, highlighting cross-contamination as a primary risk factor for foodborne diseases in Chinese households. In addition, the risks associated with insufficient reheating remain significant (FRPN = 6.02) and should not be overlooked. Household storage practices were identified as another risk factor, with keeping refrigerators above 4°C (FRPN = 5.57) and leaving fresh food at room temperature (FRPN = 5.56) were ranked fifth and sixth, respectively.

This study introduces a novel hybrid risk assessment approach that integrates fuzzy logic, national survey data, and structured expert elicitation to systematically evaluate household food handling practices. By prioritizing potential poor hygienic practices, this approach demonstrates both methodological innovation and practical value in enhancing household food safety management, offering a framework that can be adapted and applied in other countries with similar contexts.

S8-P13: Research and Design of a Predictive Model of Microbiological Risk for Food Industry.

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Microbiological contamination remains one of the major challenges in the food industry, where complex microbial communities can compromise safety and quality through food spoilage or pathogenic processes. In this study, we developed a strategy focused on a predictive algorithm for early detection of microbiological risk based on 16S rRNA gene metagenomic data, with the aim of reducing economic losses and mitigating public health hazards as an alternative for these well-stablished methods. This algorithm will allow the optimization of new cleaning and disinfection protocols on time to prevent new events.

For this purpose, initially an extensive sampling campaign was conducted all along a meat industry facility to characterize its own microbiome and identify critical contamination hotspots. Samples were analysed through 16S rRNA sequencing using Oxford Nanopore Technologies (ONT) complemented by classical culture-based plate counts to quantify contamination and ensure the robustness of the study. This dual approach enabled the identification of key microbial genera associated with contamination events and provided a comprehensive overview of the facility's microbial ecology.

Following this initial assessment, the identified hotspots were selected for a time study. An intensified sampling strategy was implemented, collecting 16S metagenomic data and production parameters, including processing conditions, operational practices and environmental variables. This dataset allowed us to explore temporal dynamics in microbial communities and their relationship with production factors and contamination events.

Using these integrated datasets, we developed an artificial intelligence-based predictive model capable of identifying early signals of microbiological risk. The algorithm was trained to detect patterns in microbial composition at the genus level and correlate them with conditions leading to contamination. The model demonstrated strong predictive performance, accurately anticipating shifts in microbial populations associated with increased risk scenarios.

Importantly, the algorithm provides actionable insights that can be integrated into routine monitoring systems, enabling proactive interventions before contamination reaches critical levels. This approach represents a shift from reactive to preventive microbiological control strategies in the food industry.

Overall, our results highlight the potential of combining high-throughput 16S metagenomics with AI-driven analytics to enhance food safety management. The proposed framework offers a scalable and adaptable solution for real-time risk assessment, contributing to improved product quality, reduced waste, and enhanced consumer protection.

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S8-P14: Temperature-Dependent Growth Kinetics of *Listeria monocytogenes* in Traditional Artisanal Cheeses: A Comparative Predictive Modeling Approach

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Traditional artisanal cheeses produced from raw or minimally processed milk present significant microbiological safety challenges, particularly regarding the growth potential of *Listeria monocytogenes*. This study aimed to comparatively evaluate the growth kinetics of *L. monocytogenes* in two traditional artisanal cheeses, Anari (a whey-based cheese derived from Halloumi production) and Xinotyri (a soft cheese produced from raw goat milk without starter cultures), under different temperature conditions using predictive modeling. Challenge tests were conducted using a three-strain cocktail in Anari and Xinotyri cheese. Samples were stored under isothermal conditions (5, 10, 15, 20, 25°C), and additional validation experiments were performed under dynamic temperature profiles. Initial pH values differed between the matrices (5.98 ± 0.01 for Anari and 4.23 ± 0.04 for Xinotyri), reflecting distinct physicochemical environments. Microbial growth was described using the primary Baranyi and Roberts model, while the effect of temperature on the maximum specific growth rate (μ_{max}) was modeled using the Ratkowsky square-root model. Clear matrix- and temperature-dependent differences in growth kinetics were observed. In Anari cheese, μ_{max} increased from 0.0229 h^{-1} at 5°C to 0.0612 h^{-1} at 25°C, with lag phases decreasing from approximately 36.6 h to 0 h. In contrast, Xinotyri cheese exhibited substantially lower growth rates at low and moderate temperatures, with μ_{max} values of 0.0045 h^{-1} and 0.0104 h^{-1} at 5°C and 10°C, respectively, accompanied by markedly extended lag phases exceeding 600 h. At higher temperatures, the differences between matrices were reduced, with μ_{max} values becoming similar (0.0663 h^{-1} in Xinotyri versus 0.0612 h^{-1} in Anari at 25°C), although lag phases remained longer in Xinotyri. The Ratkowsky model adequately described the temperature dependence of growth, with good model performance (R^2 = 0.93 for Anari and 0.87 for Xinotyri). The observed behaviour is likely associated with differences in intrinsic product characteristics, including pH and endogenous microflora, which influence pathogen adaptation and growth dynamics. Overall, *L. monocytogenes* growth was strongly matrix- and temperature-dependent, with Xinotyri exhibiting a pronounced inhibitory effect at low-to-moderate temperatures. These findings highlight the importance of product-specific predictive models for improving quantitative microbial risk assessment (QMRA) and supporting evidence-based safety management of traditional cheeses.

S8-P15: Prevalence and Distribution of Foodborne Pathogens in Raw Milk Cheeses: A Systematic Review and Meta-Analysis

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Raw milk cheeses are widely consumed globally and are valued for their sensory characteristics; however, their consumption is associated with increased microbiological risk due to the potential presence of foodborne pathogens. This study aimed to systematically evaluate the prevalence and distribution of pathogenic microorganisms in raw milk cheeses through a systematic review and meta-analysis. A systematic literature search was conducted following PRISMA 2020 guidelines using Scopus, PubMed, and Web of Science. After applying inclusion and exclusion criteria, 12 primary studies published between 2019 and 2024 were selected. Data on pathogen occurrence were extracted and analyzed using a random-effects meta-analysis model. Log odds were used as the effect size, while heterogeneity was assessed using I^2 statistics. The results revealed that *Escherichia coli* and *Staphylococcus aureus* were among the most frequently detected pathogens. Prevalence varied substantially across studies, ranging from as low as 1.0% for *Salmonella* spp. to over 80% for *S. aureus* and *E. coli* in specific regions. Notably, *Brucella* spp. exhibited particularly high prevalence in some studies (up to ~70%), highlighting region-specific risks. In contrast, *Listeria monocytogenes* and *Salmonella* spp. were generally detected at lower prevalence levels (< 10%). Higher prevalence rates were consistently reported in studies from regions in Africa and Asia, suggesting a potential influence of production practices and infrastructure. Subgroup analysis indicated that both geographical origin and pathogen type significantly contributed to the observed heterogeneity, with high between-study variability (I^2 > 75%). The overall pooled estimate from the random-effects model (log odds = 1.22; 95% CI: 0.98–1.46) indicates an increased likelihood of pathogen presence across studies, although variability remains substantial. No significant publication bias was detected based on funnel plot symmetry, supporting the robustness of the findings. Compared to earlier meta-analyses, this study provides an updated and product-specific synthesis based on recent data (2019–2024), enabling a more refined assessment of pathogen prevalence in raw milk cheeses. Overall, the study confirms that raw milk cheeses can act as important vehicles for foodborne pathogens, emphasizing the need for improved hygiene practices, targeted regulatory measures, and enhanced surveillance systems. The findings provide valuable input for microbiological risk assessment and support the development of evidence-based food safety strategies.

S8-P17: Development and Validation of a Predictive Growth Model of *Listeria monocytogenes* for RTE Products

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The presence of *Listeria monocytogenes* in RTE foods is increasingly important due to stricter food safety legislation, especially in Europe. Predictive models help to support safety documentation, guide validation studies, and to identify critical growth controlling parameters. In order to control *Listeria* in food product more complex food fermentation systems are used. These are often containing a blend of organic acids like lactate, acetate and propionate. However, the current available generic models often fail to accurately predict *Listeria* behaviour in presence of these complex systems. The work presented here elaborates on the build and validation of a *Listeria* predictive growth model in food.

Growth rates of ten *L. monocytogenes* strains were evaluated across seven environmental and preservative parameters: temperature, pH, water activity (aw), acetic acid, lactic acid, propionic acid, and ingoing sodium nitrite. Over 10.000 growth curves were obtained in BHI broth with Bioscreen C. For each absorbance growth curve, the maximum specific growth rate was determined by the Baranyi model. The Kerry model was constructed using the Gamma equations. Model parameters were estimated using the Levenberg–Marquardt algorithm to solve the nonlinear least squares optimization and minimize the total sum of squared errors (SSE) across all observations. To make the best prediction, the broth model was validated with unbiased food data. The validation dataset used came from literature and unpublished data from Dr. Dalgaard and Dr. Ross. Dataset contains growth rates of meat, sea food, dairy, broth and other foods (n = 400). A clear need to include a gamma food factor to force the food data (n = 320) to a bias factor of 1.

A *Listeria* control model was developed using broth data. After validation with food data and including a gamma food factor, the model can be applied in the meat, sea food and dairy industry to support safety documentation, guide design for challenge tests and knowledge building on critical growth parameters.

S8-P18: Matrix-Dependent Survival Kinetics of *Listeria monocytogenes* and *Salmonella* Enterica in Dairy and Plant-Based Kefir

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The rapid expansion of plant-based fermented foods requires a rigorous microbiological safety evaluation. Comparison with traditional dairy products enables a more informative assessment of matrix effects. The objective of this study was to investigate the survival kinetics of *Listeria monocytogenes* and various *Salmonella enterica* serotypes in dairy and plant-based kefir under different storage conditions. Challenge tests were conducted using separate three-strain cocktails (initial inoculum: ~6 log CFU/mL) for each pathogen, and the samples were stored at 6, 10, 12, and 15°C to simulate temperature storage conditions either recommended or abuse. Microbiological population changes and physicochemical parameters (e.g., pH and water activity), were monitored during storage. Survival data were fitted to the Weibull model to characterize inactivation kinetics and describe deviations from log-linear behavior. No growth of *L. monocytogenes* or *Salmonella* spp. was observed under all tested conditions; instead, progressive inactivation occurred, strongly influenced by the temperature and the food matrix. In dairy kefir, significantly higher inactivation rates were observed, with approximately 3-log reductions achieved within 13–18 days for *Salmonella* spp. and 22–31 days for *L. monocytogenes*, depending on the temperature. In contrast, plant-based kefir was characterized by significantly lower inactivation rates, demonstrating prolonged pathogen viability despite its lower pH. Weibull model parameters confirmed non-linear survival kinetics, with clear matrix- and pathogen-dependent differences. For *L. monocytogenes*, p values ranged from 0.91 to 2.24, indicating both shoulder and tailing behavior depending on the temperature levels. In contrast, *Salmonella* spp. exhibited consistent tailing behavior, with p values ranging from 0.19 to 0.37 in dairy kefir and from 0.10 to 0.23 in plant-based kefir, indicating enhanced persistence in the latter. These differences may be attributed to variations in the microbial ecology of the product and its matrix composition, with dairy kefir likely promoting stronger antimicrobial activity due to lactic acid bacteria and associated metabolites, whereas plant-based kefir providing conditions that enhance pathogen persistence. Overall, while neither kefir nor dairy matrix supported pathogen growth, the observed differences in survival kinetics underscore the necessity for matrix-specific validation and highlight the importance of considering matrix effects in quantitative microbial risk assessment (QMRA).

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S8-P19: Identification and Control of Microbiological Hazards in Reconstituted Novel Infant Milk Formula Enriched with Probiotics

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Although industrial processes for powdered infant formula are designed to ensure high levels of microbiological safety, outbreaks continue to be reported, highlighting that residual risks still exist. These incidents suggest that contamination does not only originate from raw materials, but may also enter during production and post-production stages, particularly during home reconstitution and storage. Furthermore, probiotics are increasingly incorporated into infant formulas, not only due to health benefits but also for their potential protective and competitive effects against pathogenic microorganisms. This study aims to identify the biological hazards associated with probiotic-enriched infant formula and to propose appropriate control strategies, with a focus on home preparation practices and the potential effects of probiotics on the behavior of pathogens.

A multidisciplinary approach was adopted, combining an in-depth review of the scientific literature and epidemiological databases with field observations, including visits to pilot plants and industrial facilities. Discussions with experts in the field of probiotics were also conducted to better understand their functional properties. In addition, several scenarios for preparation and storage at home were developed to reflect realistic consumer practices, including conditions likely to promote microbial growth.

The results highlight several sources of hazards throughout the production and reconstitution chain. At the industrial process level, pathogens such as *Salmonella enterica*, *Cronobacter sakazakii*, and *Listeria monocytogenes* were identified. Additional hazards are associated with formulation and post-process recontamination, including notably *Escherichia coli*, *Staphylococcus aureus*, and spore-forming bacteria such as *Bacillus cereus*, with a potential risk of cereulide production.

Finally, the reconstitution and home preparation phase appears to be a critical step, potentially promoting the contamination or proliferation of microorganisms such as *E. coli*, *Salmonella* spp., and *S. aureus*, particularly in cases of inappropriate reconstitution temperature, prolonged storage, or inadequate hygiene conditions. Control measures have been identified, including adherence to reconstitution and storage recommendations, as well as improved consumer information. Furthermore, certain probiotic strains of the genus *Lactobacillus* have been shown to have a protective effect, reducing the number of viable *C. sakazakii* cells by approximately 50% in co-cultures for five minutes of contact. A decision tree based on household practices is proposed to improve communication and hazard management.

This study highlights the importance of incorporating household practices into the hazard analysis of infant formula, as well as considering probiotic–pathogen interactions. Although focused on reconstituted infant formula enriched with probiotics, this approach can be extended to other food products that require preparation before consumption.

S8-P20: Microbial Responses to the Environment: Rethinking the Growth/No-Growth Interface

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One of the main objectives of predictive microbiology is to estimate the specific growth rate of a bacterial species as a function of its environment. Such kinetic models are less useful when nearing the boundary of growth, where the rate-predictions are increasingly uncertain due to experimental variability and lack of data.

An alternative approach is to describe bacterial behaviour by a model where the probability of growth plays the role of a response to environmental factors forming an E vector-space. A main characteristic of such a model is the growth/no-growth interface, E, where growth occurs at 50% probability. This interface can be estimated even from just binary growth/no-growth observations, via logistic regression.

We hypothesize that the 50% probability of growth corresponds to a small μ_crit specific growth rate, if the bacteria grow at all. Our main objective is to estimate the value of μ_crit and to leverage the μ(E)=μ_crit hypersurface E, for an improved definition of the growth/no-growth interface.

We use publicly available growth / no-growth data to construct an empirical probability model, whose 50% iso-probability contour curve provides an initial approximation of the interface. For the same set of environmental variables, a kinetic model is imported from the ComBase Predictor, from which the μ_crit critical value is determined via minimizing the distance between the 50% iso-probability and the μ=μ_crit contour curves. Finally, a weighted average of the two curves is combined for an improved definition of the growth / no-growth interface.

This study also intends to make a case that the methodology behind ComBase Predictor should be refined to utilize binary growth / no-growth data, too, to develop a more informative predictive tool.

S8-P21: Molecular Tools for Milk Safety: From Microbiome Profiling and Pathogen Detection to Species Authentication

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Milk and dairy products are staple foods consumed worldwide, yet they remain vulnerable to microbial contamination, viral transmission, and species adulteration, posing persistent challenges for food safety and public health. Ensuring the safety and authenticity of milk throughout the production chain demands versatile, efficient, and scalable analytical tools.

We present a series of automated molecular workflows developed on the Maxwell® paramagnetic bead-based platform, addressing key areas of milk safety. For microbiome surveillance, we demonstrate automated purification of bacterial DNA from raw milk, yielding high-quality extracts suitable for 16S sequencing and microbiome profiling. For pathogen detection, we show efficient recovery of both viral RNA and viral DNA from milk, including purification of Influenza A viral RNA from cow milk collected in transport medium, with sensitive downstream detection by qPCR. For food authenticity, we present automated DNA extraction from adulterated powdered camel milk enabling species identification by real-time PCR, supporting food fraud investigation.

Across these applications, we show that the automated approach on the Maxwell® instrument significantly reduces hands-on time while maintaining the nucleic acid quality required for diverse downstream analyses, from amplification-based detection to sequencing.

Looking ahead, additional molecular capabilities of the Promega portfolio hold promises for dairy safety monitoring. Viability PCR approaches could enable discrimination between live and dead microorganisms, providing a more accurate picture of microbiological risk than conventional PCR. This perspective highlights the potential for an even broader toolkit to serve the evolving needs of food safety laboratories and the dairy industry.

S8-P22: Mapping Consumer Concerns, Knowledge, Trust and Information Use Regarding Food Safety in Flanders

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Consumer perspectives on food safety play a crucial role in the effectiveness of risk management and communication. While these dimensions are often studied separately, integrated insights are needed to better inform targeted food safety interventions. This study provides a comprehensive assessment of these perspectives in Flanders (Belgium).

A cross-sectional online survey was conducted among 420 Dutch-speaking adults in Flanders using quota sampling for age, gender, and education. The questionnaire assessed multiple consumer perspectives on food safety, including factors influencing food choice, cognitive and affective concerns about food safety issues, trust in food chain actors to ensure the safety of foods on the market, food handling behaviour and knowledge, and the use of, trust in, and perceived misinformation across food safety information sources.

Absence of pathogenic microorganisms and taste were rated as the most important food characteristics when purchasing, preparing, and/or eating food. Microbial, chemical, and physical hazards were perceived as the most important food safety concerns, both cognitively (perceived risk) and affectively (worry). Trust was highest in neighbourhood shops, farmers, retail, and food safety authorities, and lowest in politicians passing food legislations, food trucks, and ready-to-eat delivery services.

Several critical gaps in food handling knowledge and behaviour were identified. While most respondents were aware of appropriate refrigeration temperatures, only 31% correctly identified that food is still safe to eat after the “best before” date. In addition, 42% believed that freezing eliminates all bacteria, and nearly half of respondents did not consistently wash their hands before food preparation. Moreover, 31% indicated that only poultry meat needs to be cooked thoroughly to ensure safety, and 43% assumed that food safety can always be judged based on sensory attributes.

Health professionals and government authorities were the most trusted and frequently used food safety information sources, whereas influencers, alternative media, and AI-based tools were widely perceived as sources of misinformation. Sociodemographic differences were generally limited across all studies food safety perspectives.

The findings highlight persistent knowledge gaps and unsafe practices that remain insufficiently addressed by current food safety communication. Interventions should prioritise correcting widespread misconceptions and promoting simple, actionable hygiene practices. Given the limited role of sociodemographics, communication strategies should be tailored to consumers’ value orientations, concerns and trust patterns, while leveraging highly used and trusted sources such as health professionals and national food safety authorities to maximise impact.

S8-P23: Predictive Models Enriched by Multimodal LLMs: Pipeline Development and Application to Shiga Toxin-Producing *Escherichia coli* Growth

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Pathogenic and spoilage microorganisms in food remain major challenges for public health, the environment, and global economy. To address these, the agri-food sector relies on quantitative tools like microbiological risk assessment and predictive microbiology. These tools enable industry, risk assessors, and researchers to anticipate microbial behavior, thereby supporting food safety and quality, as well as regulatory compliance. However, these tools would benefit from being continuously enriched, for instance using experimental data on microbial behavior or estimated growth parameters from literature. The challenge, therefore, lies in automatically analyzing and harmonizing data from scientific literature – a resource-intensive and error-prone task for humans that is now greatly facilitated by generative artificial intelligence.

This study aimed, first, to develop a data analysis pipeline enabling multimodal large language models (LLMs) to extract information from scientific documents and to create standardized, structured databases and repositories for experimental and model data; and second, to make microbiological predictions using these data. Finally, the pipeline was validated on growth models of Shiga Toxin-producing *Escherichia coli* (STEC).

We implemented an LLM-based information extraction (IE) pipeline that compiles a relevant corpus of scientific documents in food microbiology, then automatically extracts and structures key data from texts, figures, tables, and equations, and finally evaluates the extraction performance by computing metrics and grounding sources for traceability. The pipeline's performance was evaluated against manual expert extraction using a corpus of ten STEC scientific documents, both theses and articles. The extracted data, namely kinetics and primary and secondary fitted parameters, were stored in a kinetics repository and a database of model parameters derived from both the data and model fitting. These repositories were subsequently used to predict STEC growth using cardinal-type models. The method was also generalized across various food matrices, microorganisms, and predictive models. The code was written in Python and the LLMs used came from different platforms (e.g., Google, OpenAI, Anthropic).

The LLM-based IE pipeline achieved performance comparable to, or even superior to, those of a scientific researcher performing manual extraction, particularly in terms of accuracy. A Human-In-The-Loop approach proved optimal, utilizing expert knowledge to define expected data structures and validate extractions supported by grounded sources. The integrated data predicted the bacterial growth of STEC.

By combining AI and human expertise, this approach has the potential to facilitate the large-scale integration of literature data into predictive microbiology modeling research.

S8-P24: Microbial Diagnostics: Shifting from Taxonomy to Risk Exemplified by *Bacillus cereus* Group

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Bacillus cereus is a gram-positive, spore-forming bacterium, known to cause foodborne diseases and a variety of non-gastrointestinal diseases, including eye infections, endocarditis, and septicaemia. *B. cereus*, *Bacillus thuringiensis*, *Bacillus anthracis*, and several other closely related species compose the so-called *B. cereus* group, also known as *B. cereus* sensu lato.

The cytotoxicity of *B. cereus* has been attributed to multiple pore-forming exotoxins, the ionophore cereulide, and other virulence factors. Within this group, strains range from beneficial strains employed as plant growth promoters and biopesticides to those responsible for severe foodborne illness and, in rare cases, fatalities. Current taxonomy-driven criteria mandate food disposal when *B. cereus* group organisms exceed established thresholds, regardless of the strain's actual pathogenic potential, thereby contributing to significant food waste.

The MicRisk2030 initiative proposes to move current taxonomy-based diagnostics to risk-oriented diagnostics. Thus, the project aims to identify genetic markers that distinguish harmless *B. cereus* group strains from highly pathogenic ones. For this purpose, we assembled a representative strain panel with defined enterotoxicity profiles, subjected these isolates to whole-genome sequencing (WGS), and performed a genome-wide association study (GWAS) to elucidate the molecular basis of *B. cereus* pathogenicity and to pinpoint genomic markers associated with highly cytotoxic phenotypes. These biomarkers would enable the development of future risk-based diagnostic protocols capable of distinguishing pathogenic strains from harmless or beneficial ones. We will present findings from this ongoing study that provide the basis for future risk-based diagnostic protocols to differentiate pathogenic from harmless or beneficial *B. cereus* group strains, thereby supporting more proportionate risk assessment and reducing unnecessary food waste.

S8-P25: Whole-Genome Sequencing Supports *Salmonella* Surveillance in Native Fish Farming Across Pantanal and Cerrado Biomes, Brazil

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Genomic surveillance can strengthen food safety monitoring at the fish-farming stage; however, WGS datasets integrating fish and farm-environment matrices in Brazilian biomes remain limited. We applied whole-genome sequencing (WGS) and integrated genomic typing with spatial and matrix metadata to characterize *Salmonella* recovered from semi-intensive Tambaqui hybrids (*Colossoma macropomum*) farms in the Brazilian Midwest, spanning the Pantanal (wetland) and Cerrado (savanna) biomes, where Amazonian fish production is expanding and surveillance efforts benefit from integrating animal and environmental compartments. We sequenced 20 *Salmonella* isolates from fish farm locations across eight cities (3 in Pantanal and 5 in Cerrado biomes). Isolates originated from fish tissues and on-farm environmental sites (soil, gills, feces, organs, muscle), and were obtained through culture-based recovery followed by molecular confirmation targeting the *hilA* gene. Genomes were obtained using a short-read sequencing approach (Illumina MiSeq) with in silico serotyping determination via the SeqSero2 tool. This identified eleven serovars, dominated by *S.* Saintpaul (30.0%) and *S.* Matadi (20.0%), alongside *S.* Cerro, *S.* Abaetetuba, *S.* 4:b:-, *S.* Bredeney, *S.* Carrau, *S.* Javiana, *S.* Paratyphi B, *S.* Morehead, and *S.* Newport. Isolates originated from gills (35.0%), organs (40.0%), muscle (15.0%), soil/sediment (5.0%), and feces (5.0%), with an even distribution across Cerrado and Pantanal biomes (50.0% each). Core-genome (cgMLST) identified 13 distinct profiles, capturing within-serovar diversity and the occurrence of shared profiles across different cities distributed in both biomes. Across the dataset, 9/13 (69.2%) cgMLST profiles were observed in a single genome, whereas 4/13 (30.8%) were shared by two or more genomes from different cities, suggesting a possible common source of contamination. Pairwise Single Nucleotide Polymorphism (SNP) comparisons within selected serovars showed high genomic relatedness among *S.* Matadi genomes (12 SNPs) and a 14-SNP distance between the two *S.* Javiana genomes, while *S.* Saintpaul displayed heterogeneous SNP distances among the isolates compared. Virulence-associated screening indicated broad carriage of *Salmonella* pathogenicity islands (SPIs), with SPI-12 detected only in *S.* Saintpaul (three genomes) and SPI-14 absent in two genomes (*S.* Cerro and *S.* Morehead). Antimicrobial resistance screening detected *aac(6')-Iaa* in all genomes, while *fosA7* was identified in one *S.* Morehead genome. Plasmid replicons were detected in two genomes. In summary, integrating serotyping, cgMLST/SNP typing, and virulence/resistance screening with biome and matrix metadata establishes a genomic baseline for *Salmonella* surveillance in Brazilian aquaculture, supporting structured, data-driven monitoring of the fish-farming stage and its associated environments.

S8-P26: Prevalence of *Listeria monocytogenes* in Meat and Meat Products in Northern Italy: Data Supporting Quantitative Microbial Risk Assessment

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Listeria monocytogenes is a public health concern due to its high mortality rate and its ability to persist in food processing environments. Meat and meat products are frequently implicated as primary vehicles for human listeriosis, necessitating constant surveillance to ensure consumer safety. Despite its clinical relevance, few updated and comprehensive prevalence data are published in the scientific literature regarding specific meat categories and production chains. This study aimed to estimate the prevalence of *L. monocytogenes* across various meat categories, species, and sampling types to identify high-risk products and temporal trends and support risk-based food safety management.

From 2020 to 2025, a total of 25,755 samples were collected in the framework of the official control and HACCP activities. The samples were analysed according to AFNOR BRD 07/10-04/05 (real-time PCR), and, when positive, they were re-tested according to ISO 11290-1 (2017). Prevalence and 95% coefficient intervals (CI) were estimated using a Bayesian approach with a Beta (1,1) prior. Statistical significance was assessed using Pearson's chi-squared and Fisher's exact tests with Holm correction ($\alpha = 0.05$).

The overall prevalence of *L. monocytogenes* in meat and meat products was 8.5% (95% CI: 8.2%–8.9%). Non-official sampling showed a higher prevalence (10.0%) compared to official controls (8.0%). Temporally, a significant upward trend was observed, with prevalence rising from 5.4% in 2020 to a peak of 12.9% in 2024, before decreasing to 8.8% in 2025. Among FoodEx2 categories, meat preparations exhibited the highest contamination rate at 22.2%, followed by Fresh Meat (11.7%) and Meat Products (6.9%). Specifically, in fresh meat, pork showed the highest overall prevalence (15.5%) compared to beef (9.8%) and poultry (12.0%); in meat preparations a higher prevalence was found in pork (22.1%) and beef (21.1%), with specific high-risk subgroups such as fermented-dried pork preparations (40.7%) and beef hamburgers (29%); in meat products, while lower overall, salami (fermented; dried pork) maintained a significant prevalence of 14.2%.

The findings indicate a concerning increase in *L. monocytogenes* prevalence, particularly in processed meat preparations. These results emphasise the need for targeted food safety interventions, particularly for Ready-to-Eat products. Furthermore, the data can be used as inputs for Quantitative Microbial Risk Assessment studies, enabling more accurate modelling of listeriosis risks and the evaluation of control measures across the meat supply chain.

S8-P27: TemeRe: A Modular Stochastic Web Application for Predictive Microbiology and Risk-Based Decision Support in the Food Industry

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Most predictive microbiology tools remain deterministic and, when stochastic approaches are available, they are typically limited to expert users due to complexity and usability barriers, restricting their application for risk-based decision-making under real-world variability. This study presents TemeRe, an interactive, modular, web-based tool designed to operationalise stochastic modelling in predictive microbiology through a user-friendly interface. The objective was to enhance usability, transparency, and reproducibility while lowering barriers to the adoption of probabilistic approaches, enabling risk-based shelf-life estimation and sensitivity analysis as a complement to traditional challenge testing.

TemeRe was developed in R using the Shiny framework with a custom graphical user interface. The application follows a modular workflow architecture, allowing users to construct modelling pipelines by defining organisms, environmental conditions, and model linkages. It incorporates the revised ComBase broth models for 14 food-relevant microorganisms alongside additional growth and inactivation models within an expandable database.

The system integrates primary models (e.g. Baranyi–Roberts model, Bigelow D-value model) with secondary models describing environmental effects (temperature, pH, water activity, nitrites, CO_2) on kinetic parameters. Stochasticity is introduced by assigning probability distributions to key inputs (initial concentration, physiological state, temperature, pH, aw, antimicrobial factors, and time) and by accounting for uncertainty in growth and inactivation rates. Variability and uncertainty are propagated through Monte Carlo simulation (up to 10^6 iterations), enabling stochastic evaluation of microbial dynamics.

User-defined workflows are stored as structured model states, supporting reproducible scenario construction and simulation of food product trajectories across the production chain (farm-to-fork). Outputs include distributions of predicted microbial concentrations, probabilities of exceeding safety limits, shelf-life estimates under specified confidence levels, and sensitivity analyses identifying key drivers of variability.

By combining stochastic microbial modelling, simulation, and a modular user-driven architecture, TemeRe supports the transition from deterministic estimation to probabilistic decision support in the food industry. The app is designed to minimise technical barriers, enabling non-expert users to apply stochastic modelling without requiring advanced knowledge in predictive microbiology or programming. This facilitates broader adoption of probabilistic approaches in industrial practice and provides a practical complement to challenge testing for improved food safety management and reduced food waste.

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S8-P28: Fresh Produce as a Vehicle for *Campylobacter* spp. and *Cryptosporidium* spp.: A Meta-Analysis in the Context of Climate Change

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Fresh fruits and vegetables are increasingly recognized as important vehicles for foodborne pathogens due to their consumption without thermal processing. Among these pathogens, *Campylobacter* spp. and *Cryptosporidium* spp. pose significant public health concerns, given their low infectious dose and environmental persistence. In the context of global climate change, shifts in environmental conditions may further influence the transmission dynamics of these microorganisms. This study aimed to investigate the presence of *Campylobacter* spp. and *Cryptosporidium* spp. in fresh plant-based products through a systematic review and meta-analysis, while also exploring their association with environmental and climate-related factors. Following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, a systematic review and meta-analysis were conducted using three databases (Web of Science, Scopus, and PubMed) for studies published between 2004 and 2025. Eligible studies reported the detection of *Campylobacter* spp. and *Cryptosporidium* spp. in fresh produce, including vegetables, fruits, leafy greens, and herbs. Nations were divided into four biogeographical regions: Atlantic, Continental, Mediterranean, and Boreal, following the definition from the European Environment Agency. For *Campylobacter* spp., 22 studies were initially identified, of which 13 met the inclusion criteria and were evaluated. For *Cryptosporidium* spp., 26 studies were identified, with 20 included in the final analysis. Data on sample size, detection outcomes, and geographic origin were extracted. The analysis revealed heterogeneity in the occurrence of both pathogens across studies, with detection rates ranging from 0% to over 70%. *Campylobacter* spp. showed variable detection rates across different types of fresh produce, suggesting inconsistent contamination patterns and sensitivity to environmental conditions. Variability was observed among studies, likely reflecting differences in study design and environmental context. The findings suggest that pathogen prevalence is influenced by factors including sample size, geographic origin, agricultural practices, detection methodologies, and environmental conditions such as temperature and precipitation. Notably, the resistance of *Cryptosporidium* oocysts to environmental conditions and common disinfection methods contributes to their persistence in fresh produce. Additionally, the growing consumer preference for raw or minimally processed foods further amplifies the risk of foodborne infections. This study highlights variability and knowledge gaps in the occurrence of *Campylobacter* spp. and *Cryptosporidium* spp. in fresh produce, particularly regarding climate-related factors. Integrating climatic variables and standardized approaches is essential to improve risk assessment, strengthen food safety, and protect public health under climate change.

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S8-P29: Unravelling Salmonellosis Risk and Hygiene Indicators Level in Leafy Vegetables

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The reported prevalence of *Salmonella* in ready-to-eat produce is relatively low; however, its presence remains a significant public health concern, as fresh leafy vegetables are commonly consumed raw. The microbial quality of fresh-cut produce and pathogen survival are influenced by multiple interdependent factors, including product type, processing steps, packaging, storage conditions, and microbial interactions.

This study investigates the prevalence and detection of *Salmonella* in leafy vegetables and ready-to-eat salads in the Greek market and its association with *Enterobacteriaceae* populations and indigenous microbiota. To this direction, fresh leafy vegetables (i.e., lettuce, spinach, baby rocket, chicory greens, etc.) and packed salads (washed or unwashed) collected from the market across different seasons and analyzed for the detection of *Salmonella* using ISO 6579-1:2017 and real-time PCR. Presumptive *Salmonella* isolates are further characterized. In addition, the presence of *Campylobacter* and *Cryptosporidium* was evaluated by real-time PCR, while *Enterobacteriaceae* and total psychrotrophs are enumerated to assess hygiene indicators and overall microbiological quality.

Preliminary findings indicate a low risk associated with *Salmonella* prevalence in the analyzed samples. Similar results were obtained for *Campylobacter* and *Cryptosporidium*. However, variations in *Enterobacteriaceae* and psychrotrophic populations appear to be associated with factors such as season, product type, processing steps and storage conditions, suggesting their potential role as indicators of microbiological quality and processing impact. Overall, this study contributes to the current understanding of *Salmonella* associated risks in fresh produce, supporting the development of improved monitoring strategies and risk assessment models.

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S8-P30: Clinical Vulnerability to Listeriosis: Moving Beyond Traditional Risk Groups

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Certain population groups are disproportionately affected by foodborne illness, but definitions of "clinically vulnerable groups" have traditionally relied on broad categories (young, old, pregnant, immunosuppressed). This study reviewed current evidence to refine these definitions, with relevance to *Listeria monocytogenes*.

The review combined a narrative review of physiological and pharmacological susceptibility, consolidated of Scottish surveillance and unpublished prevalence data, and a systematic review of foodborne illness prevalence in developed countries. Additionally, expert panel consultations were conducted and a review of definitions used by food safety organizations. In-person discussion groups (n = 6) were undertaken throughout Scotland with individuals (n = 50) over the age of 65 years.

Listeriosis emerged as the pathogen with the strongest and most consistent evidence across clinically vulnerable groups. Scottish data included 166 confirmed listeriosis cases between 2012 and 2022, alongside data for other major foodborne pathogens. In Scotland, adults aged ≥ 65 years accounted for 68% of listeriosis cases despite comprising 20% of the population. Additional Scottish listeriosis cases were associated with cancer (19%), diabetes (10%), inflammatory bowel disease/IBS/Crohn's disease (3%), rheumatoid arthritis (1.2%), pregnancy (4%), and alcohol use disorder (4%), although data were incomplete for several groups. The systematic review included 138 studies, of which 58 reported on *L. monocytogenes*. Across the reviewed literature, 65–76% of listeriosis cases occurred in older adults, while pregnancy-associated cases were reported in 3–23% of studies. Listeriosis was also reported among people using proton pump inhibitors (16–50%), those with diabetes (7–29%), cancer (8–31%), cancer treatment (5–14%), HIV/AIDS (1–6%), transplant recipients (1–4%), alcohol use disorder (4–11%), rheumatoid arthritis (2–19%), and corticosteroid use (11–43%). While most current definitions target pregnancy and old age in general, experts advised that the relative that consumer messaging should be expanded to specific conditions and practical risk reduction. Discussion groups categorized risk communication containing expanded description of conditions and medication that lead to elevated risks to be more relatable.

The study identified PPI users, individuals with diabetes, rheumatoid arthritis, cancer, and IBD as clinically vulnerable, with listeriosis being particularly prevalent in these groups. Future definitions and food safety messaging should move beyond age alone and explicitly recognize underlying conditions and treatments associated with increased susceptibility to *L. monocytogenes*.

S8-P31: Converting Official Control Data into Probabilistic Risk Indicators: QMRA for *Listeria monocytogenes* in Retail Pre-Packaged Deli Products

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Listeria monocytogenes remains a major food safety concern in the EU, with incidence trends calling for more risk-based management. Ready-to-eat (RTE) products sold at small, independent retail establishments (e.g., delicatessens, butchers) represent critical exposure nodes, where hygiene constraints and equipment sharing increase the risk of re/cross-contamination during handling. Official control (OC) programs routinely generate large volumes of microbiological data from products and surfaces, yet these data are typically reduced to binary compliance outcomes, offering limited support for quantitative decision-making by competent authorities (CA).

This study developed a modular stochastic Quantitative Microbial Risk Assessment (QMRA) framework to convert OC data (surface/product testing, temperature records, shelf-life labeling) into probabilistic risk estimates, enabling risk-based inspection and intervention planning. The framework was applied to inspection data from pre-packaged RTE foods from the Barcelona metropolitan area. Module I estimated retail contamination probabilities through integration of OC data covering 76 establishments (170 environmental swabs across six surface types; 168 product samples, including cooked meat products and soft/semi-soft cheese) with stochastic transfer models. Surface-attributed contamination explained 87.8% of observed sliced meat contamination, 65.3% for pâté, and 43.0% for cheese, exposing transfer efficiency as the main determinant. Module II translated retail contamination levels into public health risk at the end of declared shelf-life by simulating pathogen growth under realistic cold-chain and product characteristics. Risk characterization was then performed for three population subgroups (healthy adults, elderly, and pregnant women). The compliance with the EU food safety microbiological criterion of ≤ 100 CFU/g at the end of shelf-life was also assessed.

At consumption, median per-serving illness probabilities ranged from 4.8×10^{-14} (pâté) to 4.0×10^{-15} (cheese) for healthy adults, 6.4×10^{-13} (pâté) to 7.7×10^{-14} (cheese) for elderly and 8.8×10^{-12} (pâté) to 9.5×10^{-13} (cheese) for pregnant women. The microbiological criterion limit was exceeded in 30–63% of the products at the end of shelf-life. Scenario analyses indicated that restricting shelf-life to ≤ 5 days would result in about 3 to 8 fold decrease in median listeriosis risk, and achieved > 95% compliance with the criterion. In contrast, reducing surface contamination would yield a more limited risk reduction.

This framework demonstrates how routine OC data can be converted into probabilistic risk indicators directly comparable with EU food safety criteria. By quantifying the contribution of specific retail sources to overall risk and propagating uncertainty, the model provides CAs with an auditable, transparent scientific basis for prioritizing inspections, implementing risk-based interventions, and enabling risk negotiation balancing public health with operational realities.

S8-P32: AI-Driven Sustainability Optimization for Secure Food Systems: The Athens Case Study

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The SOSFood project focuses on the development of an AI-driven system for assessing food sustainability through the use of interoperable data and advanced analytics. Its objective is to support improved decision-making across the food supply chain by leveraging predictive tools. The proposed solutions will be validated through three distinct case studies representing regional (Galicia), metropolitan (Athens), and national (Lithuania) contexts.

Within each use case, two predictive approaches for time-series analysis will be investigated: (1) single-response prediction, which aims to model and forecast future trends for a specific output variable; and (2) multi-response prediction, which simultaneously predicts multiple interdependent variables by capturing their underlying correlations.

For the Athens case study, the focus is placed on key parameters in baby food production, particularly the optimization of raw material selection that significantly impacts final product costs. The main objective is to mitigate price increases affecting consumers, while maintaining microbiological safety and compliance with regulatory requirements through predictive analytics. The methodology integrates: (*i*) data collection from price trends and proprietary databases on biological and chemical hazards in cereals and milk powder (e.g., mycotoxins, pesticides, chlorates); (*ii*) incorporation of external influencing factors such as seasonal variability, market demand, environmental events (e.g., droughts, floods), and socio-economic disruptions (e.g., pandemics, conflicts); (*iii*) predictive modeling for forecasting both cost and quality indicators; and (iv) the development of a decision-support system that balances economic, safety, and quality considerations in procurement strategies.

Expected outcomes include: (*i*) enhanced, data-driven procurement of raw materials; (*ii*) cost optimization without compromising product quality; and (*iii*) improved safety and consistency of products through predictive and real-time quality control mechanisms.

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S8-P33: Application of Flow Cytometry for Monitoring Yeast Populations During Alcoholic Fermentation and in Finished Wines

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Monitoring the viability and vitality of yeast populations during alcoholic fermentation, along with the chemical parameters of the must, is essential for optimising the winemaking process, ensuring wine quality, and enabling timely corrective actions to prevent sluggish or stuck fermentations. Monitoring *Brettanomyces* (*Dekkera*) yeasts in finished wines is important to prevent the formation of volatile phenols (e.g. 4-ethylphenol and 4-ethylguaiacol), which cause undesirable aromas such as horse sweat, leather, or barnyard notes that negatively affect wine sensory quality. Flow cytometry (FC) is a highly automated, rapid and sensitive analytical technique that enables qualitative and quantitative characterisation of yeast cells directly in grape must and wine by analysing fluorescence and light-scattering signals at the single-cell level. When combined with fluorescent dyes such as fluorescein diacetate (FDA) and propidium iodide (PI), FC enables discrimination between viable and dead yeast cells and provides information on the physiological state of yeast populations during alcoholic fermentation. The method also allows detection of yeast cells in the viable-but-non-culturable (VBNC) state, which cannot be detected using conventional plate count methods. Our experiments, involving single and mixed alcoholic fermentations, were performed using a CyFlow Cube 6 cytometer (Sysmex Partec GmbH, Germany) equipped with a blue laser (488 nm excitation wavelength) and five detectors. Yeast cells were analysed based on four optical parameters: forward scatter (FSC), side scatter (SSC), green fluorescent signal (FL1) and red fluorescent signal (FL3). Flow cytometry was compared with the plate count method using WL Nutrient Agar as described in the OIV compendium (OIV-MA-AS4-01, Section 4). The results confirmed that FC, in combination with FDA and PI staining, provides a precise and accurate method for monitoring the concentration and viability of *Saccharomyces* and non-*Saccharomyces* yeasts during spontaneous and inoculated alcoholic fermentations in grape must and Yeast–Malt medium over a concentration range of 10^6–10^8 cells/mL.

In addition, our experiments demonstrated that the same FC system, when combined with fluorescently labelled rRNA-targeted gene probes, as in the BrettCount® kit, enables rapid detection of *Brettanomyces* during alcoholic fermentation and in finished wines, with results comparable to the conventional plate count method using culture medium. However, reliable interpretation of flow cytometry data requires trained personnel with a good understanding of yeast physiology and cytometric responses associated with different yeast physiological states.

S8-P34: A Systematic Comparison of Outputs from Three Widely-Available Microbial Growth Models

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Mathematical models that predict microbial growth under defined conditions are a useful tool for academic researchers and commercial food scientists. Models to predict the behaviour of pathogens under a range of conditions can greatly reduce the number of experiments required when formulating safe food products and play an important role in microbiological risk assessment. However, the growth parameters predicted by different models for the same organism and conditions may differ, which can affect product formulation decisions and pathogen exposure assessments. In this poster a systematic comparison of the outputs from three free models is described.

The pathogens used to test the models were *Salmonella enterica*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Bacillus cereus*. The models compared were Pathogen Modeling Program (PMP, USDA), ComBase (developed by a consortium) and Food Spoilage and Safety Predictor (FSSP, Technical University of Denmark, *L. monocytogenes* only).

For each bacterium, the range of values for temperature, pH and salt concentration that were common between the models was determined. Maximum growth rate predictions were then performed and compared by varying one of the three parameters in turn while the other two remained fixed. All predictions were performed for aerobic conditions without lag phase. Additional experiments were performed by using the models to simulate the growth of pathogens in foods using typical values for the physicochemical parameters.

Predictions of maximum growth rate for *Salmonella* and *E. coli* showed good agreement between PMP and Combase. However, predictions for *B. cereus* and *S. aureus* differed substantially between the two models, with ComBase predicting growth rates more than double those predicted by PMP under certain conditions. In the case of *L. monocytogenes*, growth rate predictions from ComBase and FSSP were mostly in close agreement, while predictions by PMP were up to 25% higher. It was noted that the largest discrepancies in predicted growth rate were observed for those pathogens for which growth, rather than presence or absence, is most important. Strain differences and choice of growth medium are likely to be significant factors in discrepancies between models. These results show that the choice of model can affect safety assessments during product development and exposure assessments in microbiological risk assessment.

S8-P35: Development of a Risk Ranking Tool for Food-Hazard Pairs: Application to the Beef Sector in France

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Efficient food safety management requires a systematic approach to rank risks and optimize the allocation of public health resources, particularly for official controls. To support French risk managers in this process, the French Agency for Food, Environmental and Occupational Health & Safety (Anses) developed a methodology to identify and rank biological and chemical hazards, as well as food–hazard pairs, now implemented in the PrioR tool.

This methodology was applied to rank food–biological hazard pairs within the beef sector in France. A total of 28 biological hazards (16 bacteria, 4 viruses, 1 prion, and 7 parasites) were evaluated across 28 food categories. A structured filtering process—based on epidemiological data (2006–2022), regulatory criteria, and expert judgment—was applied to 784 potential food–hazard pairs, resulting in the identification of 98 relevant pairs for risk ranking.

The risk ranking relies on two macro-criteria: severity and occurrence. Severity was assessed using the two components of the Disability-Adjusted Life Years (DALY), namely Years of Life Lost (YLL) and Years Lived with Disability (YLD), derived primarily from European Centre for Disease Prevention and Control and World Health Organization estimates. Occurrence was estimated using a bottom-up approach to calculate the annual incidence associated with each food–hazard pair, integrating seven parameters: initial contamination, prevalence of contamination, pathogen evolution during storage, effect of final preparation, probability of high-risk scenarios, dose–response relationships, and annual portions consumed. Input data were compiled from French and European food monitoring programs, predictive microbiology models, scientific literature, and national food consumption surveys.

Food–hazard pairs were ranked using a multicriteria decision analysis based on the ELECTRE III method, accounting for data uncertainty and risk manager preferences. Several parameter sets and weighting schemes were evaluated to assess their influence on the final ranking and support risk manager decision-making. Results consistently identified Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* spp. in minced beef and meat preparations as top-ranked food–hazard pairs across all scenarios, including products intended for cooking. In contrast, *Listeria monocytogenes* and *Campylobacter* spp. rankings were more sensitive to weighting assumptions regarding severity versus incidence.

This work provides a robust, evidence-based tool to support risk-based decision-making and enable more targeted surveillance. Future developments include extending the methodology to other food sectors (e.g., poultry and pork) and implementing an operational interface for risk managers.

S8-P36: Differences in Growth Capacity Between *Listeria monocytogenes* Lineages I and II: A Multilevel Approach Under Different pH Conditions

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Listeria monocytogenes is one of the main concerns for the food industry, as it causes listeriosis, one of the most serious foodborne illnesses, with high rates of hospitalization and mortality, particularly among vulnerable groups. Despite improvements in regulations and controls, the incidence of listeriosis has risen in Europe since 2008, reaching a total of 2,041 cases in the European Union in 2024, the highest figure since 2007. This increase highlights the need to improve tools for assessing microbiological risks.

Four lineages are distinguished within *L. monocytogenes*, with lineages I and II being the most relevant to food safety. Currently, risk assessment and management approaches tend to treat the species as homogeneous, without taking into account phenotypic differences between lineages. However, recent evidence indicates that lineage II predominates in food and industrial environments, whilst lineage I is more common in clinical isolates, suggesting a possible specialization that has not yet been quantified in risk assessment.

This study analyses differences in growth performance between the two lineages under different environmental conditions. A multilevel model was employed that accounts for three sources of variability (between lineages, within lineage and within strain) alongside experimental uncertainty. The parameters were estimated from growth rates obtained using the double dilution method in 43 strains balanced across lineages, at pH 5–7 and 37°C. Gamma-type submodels were developed for the growth rate and the different levels of variability. Growth dynamics were monitored using a plate reader, whilst adjusting the pH of the culture medium.

The results obtained show a significant effect of environmental conditions on the three sources of variability, which is generally greater under conditions close to the limits of growth. Furthermore, a clear distinction is observed between the two lineages, allowing their differences in terms of growth aptitude to be quantified. These results highlight the importance of considering intraspecific heterogeneity in predictive modelling of *L. monocytogenes*. This information is highly relevant for improving the accuracy of microbiological risk models in food and represents a step towards a more realistic assessment of listeriosis risk, based on the biological variability of the pathogen.

S10 Different Perspectives on Challenging Topics of Food Microbiology in Changing World

S10-P1: Antifungal Potential of ß-Cyclodextrin-Winter Savory Essential Oil Microparticles in Tomato Juice

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Aspergillus species are common molds that contaminate tomatoes during cultivation, transport, and storage. They can survive and grow in processed tomato products, such as tomato juice, causing spoilage and potential mycotoxin production. Therefore, natural additives, particularly essential oils and their encapsulated forms, are gaining increasing attention due to their ability to provide controlled release of active compounds and improve food preservation. In this study, the antifungal activity of β-cyclodextrin (β-CD) microparticles loaded with winter savory (Ws) essential oil (EO) was evaluated in artificially inoculated tomato juice. Microparticles were obtained by the precipitation method using an EO:β-CD ratio of 20:80, selected based on previous *in vitro* studies. The selected formulation showed an encapsulation efficiency of approximately 70%, while SEM analysis revealed crystalline microparticles with dimensions mainly between 2 and 5 µm. Tomato juice samples were supplemented with β-CD/WsEO microparticles at a concentration of 20 mg/mL, while samples without microparticles served as controls. Samples were inoculated with *Aspergillus flavus*, *Aspergillus parasiticus*, or *Aspergillus ochraceus* and stored at room temperature for 10 days. Mold counts were determined on days 0, 2, 4, 6, and 10 and expressed as $\log_{10}$ CFU/mL. All experiments were performed in triplicate, and data were analysed by ANOVA followed by Tukey's post hoc test ($p < 0.05$). In addition, aflatoxin production (AFB1, AFB2, AFG1, and AFG2) by *A. flavus* and *A. parasiticus* was assessed by SPE-HPLC-MS/MS. The total number of molds gradually increased in control samples throughout storage. β-CD/WsEO microparticles reduced fungal growth after 6 days by 1.7 $\log_{10}$ CFU/mL for *A. flavus* and 1.2 $\log_{10}$ CFU/mL for *A. parasiticus*, while a stronger inhibition of *A. ochraceus* was observed after 4 days (3.4 $\log_{10}$ CFU/mL). The highest sensitivity was recorded for *A. ochraceus*, with a total reduction of up to 6.0 $\log_{10}$ CFU/mL after 8 days of storage. Significant reductions ($p < 0.05$) were observed in all treated samples compared with the controls. Furthermore, all analysed aflatoxins remained below the limit of quantification in tomato juice samples treated with β-CD/WsEO microparticles. These results demonstrate the potential of β-CD/WsEO microparticles as natural antifungal additives for improving the microbiological stability and safety of tomato juice, although their impact on sensory properties warrants further investigation.

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S10-P2: Microencapsulation of *Satureja montana* L. Essential Oil for Control of Pathogenic and Toxigenic Microorganisms: From *in vitro* Tests to a Food Model

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This study aimed to evaluate the antimicrobial potential of microencapsulated *Satureja montana* L. essential oil (SEO) against selected foodborne pathogenic and toxigenic microorganisms under *in vitro* conditions and within a food model system. The oil-in-water (O/W) emulsion was prepared by adding SEO dropwise into a biopolymer solution (gum Arabic and maltodextrin blend, 1:1 w/w) under high-shear mixing at 10,000 rpm for 5 min, followed by ultrasonication at 20 kHz with a 99% amplitude in a pulse mode (10:10 s (ON/OFF) for another 5 min. Microcapsules were produced using a spray dryer. The process parameters were maintained at an O/W emulsion flow rate of 0.192 L/h, an inlet air temperature of 120°C, and a compressed air flow rate of 600 L/h. The resulting microcapsules were collected and stored in sterile Petri dishes sealed with Parafilm at 8°C until use.

The *in vitro* antimicrobial activity of the microcapsules was assessed against *Escherichia coli*, *Salmonella* Typhimurium, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Candida albicans*, and *Penicillium expansum*. Assays were performed in Mueller-Hinton broth (for bacteria) and Sabouraud maltose broth (for yeast and mold) using an initial microbial inoculum of 106 CFU/mL. The microencapsulated SEO at a concentration of 15 mg/mL exhibited a bactericidal effect against all tested bacteria and a fungicidal effect against *C. albicans* and *P. expansum*.

To further evaluate its applicability in food matrices, the microcapsules (15 mg/mL) were incorporated into a model fruit-vegetable puree (50% apple, 20% carrot, 15% mango, and 15% banana puree) artificially inoculated with *E. coli*, *L. monocytogenes*, *C. albicans*, and *P. expansum*. Storage trials were conducted over an 8-day period at 37°C for bacteria and 25°C for the fungi. The microencapsulated SEO maintained its bactericidal/fungicidal efficacy against all tested microorganisms, confirming its stability and effectiveness within a complex food matrix.

These findings highlight the significant potential of microencapsulated SEO as a natural strategy for controlling foodborne pathogens and spoilage organisms. Future research will focus on evaluating the effectiveness of this system in additional food matrices and assessing its influence on the sensory, nutritional, and safety characteristics of food products.

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S10-P3: Nitrite Replacement in Processed Meat by Using Starter Cultures

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Nitrite is traditionally applied in meat processing due to its multifunctional role. It contributes to the formation of the characteristic cured flavor, supports the development of a stable cured color, and plays a central role in product safety by inhibiting *Clostridium botulinum*. However, nitrite can participate in reactions leading to the formation of potentially harmful nitrosamines. Considering these risks, the World Health Organization classified processed meat as "carcinogenic to humans" in 2015. Consequently, the European Union continues to promote a reduction of nitrite levels in processed meat products (EU VO 2023/2108). A replacement for nitrite must reproduce its essential chemical functions. In conventional curing, nitrite is reduced to nitric oxide (NO), which binds to myoglobin to form nitrosomyoglobin.

The patented BITEC Cotto® system is based on a comparable biochemical principle. The applied food culture, a *Staphylococcus equorum* strain, exhibits pronounced nitric oxide synthase (NOS) activity. This enzyme catalyzes the conversion of L-arginine to nitric oxide (NO) and L-citrulline. The necessary L-arginine is supplied through yeast extract, which serves as a nitrogen source and also provides freely available amino acids and peptides. This ensures sufficient substrate availability for the NOS-mediated reaction. The enzymatically generated NO subsequently reacts with myoglobin in the muscle tissue, forming nitrosomyoglobin derivatives analogous to those produced during conventional nitrite curing. This reaction is responsible for the development of the typical cured color and flavor, even in the absence of added nitrite.

In this study the food culture containing *S. equorum* was validated across multiple meat matrices, including cooked ham, cooked sausage and salami. Sensory tests as taste profiles and preference tests were done with products manufactured with the *S. equorum*. These tests show good acceptance of the nitrite free products and a comparable color and taste profile to the product with nitrite.

For food safety assessment, challenge tests were done with *Clostridium botulinum* and *Listeria monocytogenes* using either the food culture or nitrite. The results demonstrate the safety of the nitrite-free solution, which effectively inhibited the growth of both pathogens.

In addition, the cell counts of *Enterobacteria*, *Pseudomonades* and yeasts/molds were tested in salami produced with nitrite, with the *S. equorum* strain and without any food culture. Here it was possible to show that the cell counts in salami produced with the *S. equorum* were significantly lower than in the control without food culture and comparable to the cell counts of the nitrite control.

S10-P4: Chromatic Characteristics (OD420+520+620) Influence Pulsed Light Inactivation of Yeasts in Winemaking Conditions

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The use of sulphites in wine is questioned by both consumer and the sanitary authorities due to widespread exposure across nearly all consumer groups (EFSA). Furthermore, these issues are exacerbated by societal and environmental changes, such as the trend toward low-input products, sustainable practices, and climate change. The need for alternative methods makes it imperative to increase their inactivation efficacy in wine-making conditions. This study focuses on studying Pulsed Light (PL) technology as a non-thermal decontamination method that helps control microorganisms, improve hygiene, and reduce the need for sulphites. The matrix colour effect on cell viability was tested for pulsed light technology. The aim was to evaluate the UV-C dose applied and its efficacy on static conditions depending on the wine characteristics.

First, the UV dose applied was measured using the iodide/iodate actinometer method. A iodide/iodate mix was prepared, then underwent PL treatment, and the dose (J/L) was calculated from spectrophotometry data. The total UV-C dose applied to each sample was 411,18 J/L. Five different colored matrixes were prepared and inoculated with yeasts, then treated with PL at same fluence (50 pulses), total energy (4 KV) and frequency (3 Hz). The effect was studied in 3 strains of the spoilage microorganism *Brettanomyces bruxellensis* (CBS2499, AWRI1499, AWRI1608) and one strain of *Saccharomyces cerevisiae* (FX10). Cell viability after treatment was measured by total plate count in YPG supplemented with biphenyl and chloramphenicol. For the matrix, pH and colour (colour index) were measured pre and post treatment.

A decreasing tendency of PL efficacy with increasing colour intensity was observed. However, for some strains, the effect was more pronounced and different inactivation kinetics were observed. The strain AWRI1499 was the most sensitive, reaching almost a 2-log inactivation in the broth with the lowest colour index. Regarding the liquid's characteristics, pH wasn't affected by the treatment. However, colour intensity decreased significantly in all the samples tested.

S10-P5: The Role of Single-Cell Birth Kinetics in Shaping Population Dynamics and Food Safety

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The Latin prefix uni- has a profound dual meaning. It simultaneously signifies the unique one and the unified whole, perfectly captured in the relationship between a singular "unit" and a collective "unity". This symbolic link appears also in predictive microbiology. At the single-cell-level, each individual bacterial cell's behavior is unique and stochastic in nature. As its lineage proliferates, however, the single-cell-generated subpopulation exhibits unified and predictable behavior. Understanding this shift across scales remains a central challenge in microbial modeling and food safety, especially within the One Health concept.

Conventional predictive microbiology models usually rely on deterministic growth parameters that are applied to low cell concentrations, too, often ignoring the variability that is increasingly significant as the number of cells in a culture becomes less than about 30–100 cells. To address this limitation, a mathematical and computational framework is developed to link stochastic single-cell birth kinetics with population-level dynamics. Using branching process theory, it is possible to estimate population parameters (e.g., specific growth rate) from single-cell-level parameters (e.g., interdivision times). Unfortunately, for other parameters, like the lag phase, no closed-form equation is known and can be estimated via simulation only.

Laboratories have often reported a short lag phase after inoculation, even if the new environment was identical to the old one. This cannot be due to adaptation, but it can be explained by considering the age-distribution of single-cells at the time of inoculation, where age was defined as the proportion of the cell's lifespan elapsed from birth. Branching process theory reveals that an exponentially growing homogeneous population converges to a stable age distribution, characterized by an approximately 2:1 ratio of young to old cells. When a sample is inoculated in a new environment (e.g., via pipetting), this distribution is inevitably biassed toward younger cells, thereby causing a bias toward longer times until the first divisions. This bias induces a Pseudo-lag of a purely statistical, or "dry" nature, without any relation to "wet" causes, like the physiological adaptation to the new environment. As no closed formula is known for the distribution of Pseudo-lag, simulations were used to estimate its magnitude and impact on bacterial growth.

The transition from a stochastic unit to predictable unity hints towards the existence of a broader narrative in food safety: the unpredictability of the one becomes the law of the many. Probability distributions at the single-cel-level define the deterministic behavior of the cells at the population-level.

S10-P6: Fifty Shades of Red: *Staphylococcus xylosus* Stepping up to Reduce Nitrite Dependance in Meat Coloring

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The growing demand for clean-label meat products, together with concerns about the potential health risks associated with synthetic nitrite and nitrate, traditionally used as curing agents to ensure microbial safety, oxidative stability, and the characteristic red colour of processed meat, has driven the search for alternative strategies. Among these, coagulase-negative staphylococci (CNS) are promising candidates because of their ability to produce nitric oxide (NO) through the nitric oxide synthase (NOS) pathway, thereby contributing to the formation of stable red pigments in meat.

This study aimed to evaluate the presence of the *nos* gene and the related NOS enzymatic activity in 71 strains of *Staphylococcus xylosus* isolated from traditional fermented sausages produced without the use of commercial starter cultures. Genotypic screening by PCR analysis showed that 61 strains (86%) carried the *nos* gene. However, phenotypic analysis revealed that only six strains exhibited detectable NOS activity. Among the tested strains, *S. xylosus* 28U2C5 showed the highest enzymatic activity (1.40 ± 0.97 U/mL) and was therefore selected for additional functional characterization.

The chromogenic potential of *S. xylosus* 28U2C5 was assessed in a myoglobin model system supplemented with L-arginine and incubated at 17, 21, and 30°C. Spectrophotometric analyses (450–650 nm) revealed the evolution of absorption peaks in the 540–580 nm region, consistent with the formation of red myoglobin derivatives. During the first 72 h of incubation, both inoculated samples and controls showed similar spectral profiles suggesting the presence of oxymyoglobin. Subsequently, oxymyoglobin was progressively converted into metmyoglobin, as confirmed by its characteristic absorption spectrum. From day 7, control samples showed no relevant spectral changes, with metmyoglobin remaining the predominant form. In contrast, inoculated samples exhibited the reappearance of absorption peaks in the 540–580 nm region, likely associated with the formation of nitrosomyoglobin. The kinetics of pigment formation was affected by the temperature: the transition between myoglobin forms occurred earlier at 30°C, at intermediate times at 21°C with well-defined peaks after 7–10 days, and later at 17°C, where the peaks became evident only from day 17.

Overall, these results demonstrate that *S. xylosus* 28U2C5 has potential for application as a starter culture to reduce dependence on synthetic nitrite and nitrate in fermented meat products. In addition, these findings may help define the optimal time window for strain screening, as nitrosomyoglobin becomes distinguishable only after the disappearance of oxymyoglobin and the subsequent formation of metmyoglobin.

S10-P7: A Metagenome Catalogue Unearths the Contribution of Microbiome in Cheese Terroir

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Cheese microbiomes are shaped by production technologies and geographical factors, contributing to product identity and terroir. The terroir can be defined as the dynamic ensemble of physical, chemical, environmental, biological, and human factors that collectively shape the identity of the final product binding it to its geographical place of origin. Here, we integrated 1,593 cheese metagenomes, including publicly available datasets (*n* = 1,277) and newly sequenced Protected Designation of Origin and Protected Geographical Indication cheeses (*n* = 316), into a metadata-curated catalogue harmonised across 18 cheesemaking variables. Standardized metadata were collected using eAmbrosia (https://ec.europa.eu/agriculture/eambrosia/geographical-indications-register/) and original publications. The final data and metadata were integrated within the curated FoodMetagenomeDatabase (cFMD v1.3.1; https://github.com/SegataLab/cFMD/releases/tag/v1.3.1), and were made publicly available at a new repository called Metadata-curated Cheese Database (MetaCheeseDB; https://magliulo.github.io/metacheesedb). Our results showed that microbial communities varied consistently across cheese textures, animal feeding systems, and countries of origin, with microbial signatures associated with both technology and geography. Microbial biomarkers were indeed identified through explainable artificial intelligence analysis, which accurately classified cheese origin based on microbiome composition and identified key microbial drivers. Genome-resolved analyses uncovered extensive previously uncharacterised diversity. 4,170 high- and medium-quality metagenome-assembled genomes were reconstructed and > 28% of them were represented by unknown species. This catalogue advances understanding of cheese microbial ecology and provides a public resource for studying terroir, traceability, and functional potential of cheeses.

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S10-P8: Apple Pomace Extract in Short-Ripened Salami: Effects on Microbiological, Physicochemical and Sensory Characteristics and Efficacy in Inhibiting Foodborne Pathogens

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Fruit processing by-products are increasingly investigated as a sustainable source of bioactive compounds with antioxidant and antimicrobial properties that may improve food safety and quality while reducing the use of synthetic additives. Therefore, this study aimed to evaluate the effect of a 5% apple pomace extract obtained under supercritical CO_2 conditions on the microbiological quality, sensory and technological characteristics, of pork fermented sausages (salami). In addition, its antimicrobial efficacy was assessed through challenge tests against *Salmonella enterica* and *Listeria monocytogenes*.

Control and treated salami, produced following a commercial recipe, were inoculated with a starter culture (*Latilactobacillus sakei* and *Staphylococcus carnosus*) and ripened for 15 days. Microbiological analyses were performed at three production stages (1, 8 and 15 days) and complemented by 16S metabarcoding to further characterize the microbial profile. Physicochemical parameters (pH, aw, colour, texture) were also evaluated, and sensory differences were assessed through a triangle test involving 37 untrained participants. Additionally, challenge tests were performed by inoculating salami with a cocktail of *S. enterica* and *L. monocytogenes*, to achieve a concentration of 5 log CFU/g. Pathogens and lactic acid bacteria counts were monitored during production (1, 4, 8, 11 and 15 days), together with pH and aw.

Lactic acid bacterial and total mesophilic counts followed the expected fermentation pattern, with an initial increase followed by stabilization in both control and treated samples, while *Staphylococcus* spp. remained stable throughout production. 16S metabarcoding revealed no differences between groups, with the microbial population dominated by the starter bacterium *Latilactobacillus*. In treated samples, acidification proceeded more slowly but eventually aligned with the control, while aw decreased progressively in both groups. Colorimetric analysis revealed no significant differences in colour. However, the triangle test indicated a perceptible difference, mainly attributable to flavour and aroma, with the control preferred by two-third of participants. In challenge tests, *S. enterica* counts decreased significantly in treated samples (by 0.8 log CFU/g) from the end of fermentation onwards, whereas *L. monocytogenes* remained stable over time in both groups.

Overall, the apple extract was compatible with salami fermentation, did not adversely affect physicochemical or sensory characteristics, and contributed to the reduction of *S. enterica*, supporting its potential application as a natural preservative in fermented meat products.

S10-P9: Contamination Levels of Various Legumes with *Bacillus cereus* and *Clostridium* spp. Used as Ingredients in the Food Industry

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Legumes are crops of growing interest for current and future food systems. Their high protein and fibre content, combined with low environmental impact, make them attractive alternatives to animal proteins. However, they are prone to food safety issues, particularly microbiological contamination by spore-forming bacteria present in soil. *Bacillus cereus* and *Clostridium* spp. spores can contaminate legumes from cultivation through processing. Due to their heat resistance, spores persist in food matrices and may pose a risk to the safety of finished products.

This study aimed to assess contamination levels of *B. cereus* and *Clostridium* spp. spores in various legume species used as ingredients in mixed milk-legume products. Thirty samples representing five legume species (peas, yellow, green, and black lentils, and field beans) were analysed. Microbiological analyses were carried out according to ISO 7932:2005 and ISO 15213-1:2023 standards for enumeration of presumptive *B. cereus* spores and sulphite-reducing anaerobic spores (*Clostridium* spp.), respectively. Three analytical approaches were employed: *i*) enumeration of *B. cereus* spores; *ii*) enumeration of sulphite-reducing anaerobic spores; *iii*) qualitative analysis based on a preliminary enrichment step to detect spores below the detection limits of enumeration methods.

Results revealed predominantly low contamination levels. Nineteen of thirty samples contained *B. cereus* spore contamination below 100 CFU/g, with a maximum of 1,400 CFU/g, consistent with results reported by Kyrylenko et al. For *Clostridium* spp., 26 samples showed levels below 10 CFU/g, with a maximum of 30 CFU/g. However, qualitative analysis detected *B. cereus* in all samples, indicating widespread low-level contamination. In contrast, only 9 samples tested positive for *Clostridium* spp. spores. Among the five legume species tested, peas exhibited the highest contamination levels. No statistically significant association was demonstrated between contamination levels and cultivation practices; however, legume crops frequently attacked by insects appeared to show elevated contamination levels.

These results demonstrate that legumes can harbour bacterial spores at low contamination levels. This study contributes to improved microbiological profiling of legume ingredients in refrigerated mixed milk-legume products. To ensure safety of these products, future investigations will demonstrate the existence of a hurdle effect linked to thermal treatment combined with refrigerated storage and pH, which inhibits spore germination and growth resumption of spore-forming bacteria such as *Bacillus*. Although this effect is well-characterized for *Clostridium botulinum*, its demonstration and quantification remain necessary for various *Bacillus* species before integration into food safety and spoilage risk assessments.

S10-P10: Occurrence, Diversity, and Long-Term Persistence of Spoilage Moulds in Norwegian Food Production Environments

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Moulds represents a persistent challenge to food quality and safety across diverse food production environments, through both direct product degradation and the potential formation of mycotoxins. These issues are major contributors to production losses, extended processing time, and food waste, collectively resulting in significant economic impacts.

This study investigates the occurrence of moulds on food products and within selected processing environments across several food industry sectors in Norway, including the dairy, dry cured meat, and bakery industries. The aim was to identify critical points for mould growth and map potential contamination routes. In addition, we examined the long-term persistence of dominant mould species over decades and evaluated the effectiveness of existing hygiene and production-related control measures.

The mould isolates were collected from product and environmental samples, and the species were identified both morphologically and genetically by ITS or *benA* sequencing. The isolated moulds predominantly belonged to the genera *Penicillium*, *Aspergillus* and *Cladosporium*. However, the results revealed variation in species diversity between facilities, depending on the type of products produced, and certain species demonstrated remarkable persistence over decades in the production environment. The findings also showed that some production environments pose particularly high risks for mould growth, underscoring the need for preventive strategies tailored to both product category and specific operational conditions.

This study highlights the importance of improving our understanding of mould ecological niches in industrial settings and emphasizes the value of interdisciplinary approaches to food safety. The results may support the development of more effective control measures and improved guidelines for reducing mould related risks in the food industry.

S10-P11: *Listeria monocytogenes* Growth Potential on Nitrite-Free Steak Tartare

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Steak tartare is a raw meat product with a long tradition and a significant market value. Currently, to ensure product safety and stability, steak tartare formulations include nitrites. In the European Union, the regulatory limits for nitrites in meat products have been recently revised. Such changes reflect a clear trend towards a reduction of these additives due to their potential negative impact on human health. In this study a commercial natural ingredient, Tenerix, was evaluated as a prospective nitrite alternative in beef tartare formulation. The performance of the ingredient was tested considering its effects on product quality, microbial stability, and the potential inhibitory effects against *Listeria monocytogenes* during 20 days of storage in vacuum skin packaging at 4°C.

According to Hunter L*a*b* data, Tenerix was able to ensure colour parameters that were close to controls for Chroma (C*). This result makes it a viable choice for further recipe development. The monitoring of microbial populations showed reduced aerobic mesophiles and LAB counts (log CFU/g) compared to nitrite-added steak tartare. A higher pH was obtained throughout the experimental period (20 days), while aw values were comparable. In the challenge study with *L. monocytogenes*, carried out according to the EURL guidelines, the tartare formulated with Tenerix did not support *L. monocytogenes* growth ($\delta < 0.5$ log CFU/g), whereas the inoculum was always present in control samples.

These results indicate that Tenerix is a suitable alternative to nitrite salts, considering the promising results for C*, the reduction of aerobic mesophiles, and the results of the challenge test. Sensory data and process optimisation are needed to gain a comprehensive view of the possible technological transfer of this study.

S10-P12: Assessment of Cleaning Processes for Reusable Plastic Food Packaging Based on Microbial Growth on Food Residues

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At the beginning of 2025, the European Commission published the Packaging and Packaging Waste Regulation (PPWR) (EU 40/2025). This new regulation aims to reduce packaging waste and stimulate the transition toward reusable food packaging systems. However, this presents several challenges to the industry such as packaging design, standardization requirements, and the development of efficient return logistics systems. For food products, a key challenge is ensuring food safety in reusable food packaging, for which specific guidelines and regulatory frameworks are currently lacking.

This study investigates microbiological challenges associated with reusable plastic food packaging, with a particular focus on the assessment of industrial cleaning processes. Firstly, the growth of spoilage microorganisms commonly associated with food products was evaluated in the presence of different food type residues (sugar-rich [apple juice], fat-rich [cream 30% fat] and protein-rich [whole egg]). Each food residue was inoculated with a cocktail of spoilage microorganisms (2 log CFU/g), including yeasts, lactic acid bacteria, *Pseudomonas* spp., *Staphylococcus* spp., and *Bacillus* spp.

The inoculated food residues were applied onto polypropylene (PP) reusable food packaging surfaces and stored under controlled conditions (22°C with high [ca. 90%] and low relative humidity [ca. 60%]). Microbial growth was monitored over time to determine which specific spoilage organisms could proliferate the most. The objective of this experiment was to identify the most suitable incubation time, relative humidity condition, and microorganisms for assessing cleaning performance in subsequent experiments.

Selected microorganisms were subsequently used to assess cleaning performance on three packaging materials used in reusable systems: PP, polyethylene terephthalate (PET), and Tritan®. After controlled incubation (22°C, low relative humidity [ca. 60%]), the reusable plastic food packages were subjected to different industrial cleaning processes varying in temperature, time, and detergent. Cleaning performance was assessed using both traditional culture-based enumeration and ATP bioluminescence as a rapid indicator.

The results demonstrate that microbial survival and removal efficiency depend strongly on the type of food residue and its interaction with the packaging material and highlight the importance of residue–material combinations when defining adequate cleaning conditions for safe reusable food packaging systems. This work provides essential insights for developing evidence-based guidelines to support the transition from single-use to reusable food packaging.

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S10-P13: Essential Oils and Hydrolates: Designing a Winning Strategy for Successful Applications in the Food Industry

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The growing demand for clean label products has driven the search for natural alternatives to synthetic preservatives. Essential oils (EOs) and hydrolates, used as biopreservatives, have emerged as promising solutions for ensuring food safety and extending product shelf life. In fact, these biopreservatives exert activity against microbial cells by modifying the expression of genes that encode for different cellular functions, including destabilising cytoplasmic membranes, interfering with quorum sensing, and virulence factors. However, the lack of standardised research protocols and the inherent variability of these natural substances may compromise industrial applications, particularly in terms of consistency and effectiveness across different food products, which can lead to unpredictable results in food preservation and safety. Furthermore, the concentrations required to obtain antimicrobial activity are a key challenge for technological transfer because they can affect both product acceptability and cost when higher concentrations lead to undesirable flavours or tastes in food products.

This presentation aims to propose a procedure for the successful application of EOs and hydrolates in the food industry, based on several studies carried out by the authors in different food areas. Starting from the selection of aromatic plants, the steps of projects aimed at substituting food preservatives are described. The authors provide hints for solving problems that are crucial for the success of the project, such as the evaluation of the sensory threshold, the toxicological issues, the regulatory aspects, the mechanisms of action, and the dose reduction, also considering the risk associated with sub-MIC doses. Moreover, the authors propose applications where hydrolates, usually considered by-products of steam distillation, can provide the added value of water-soluble bioactive compounds with minimal sensory impact.

To investigate microbial reactions to treatments with biopreservatives, experiments carried out in gnocchi formulations are taken as a case study to describe the evaluation of the stress response in *Bacillus cereus* and *Bacillus subtilis* treated with *Thymus vulgaris* and *Origanum vulgare* subsp. *hirtum* EOs. In this study, many genes were downregulated at 6 h, indicating that the stressful condition extended the lag phase. However, the gene *spo0A* was upregulated from 6 h, suggesting an attempt to restore cellular communication and repair membrane damage. All in all, the two EOs mostly affected quorum sensing and cell membrane integrity, with variations in the genes involved.

S10-P14: Inhibition of Quorum Sensing by Plant Extracts from the Brazilian Cerrado Biome

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Foodborne diseases and microbial persistence in food-processing environments remain major food safety concerns, particularly due to antimicrobial resistance, spoilage, biofilm formation, and bacterial adaptive responses. In this context, plant-derived compounds have emerged as promising alternatives for sustainable food preservation, especially through anti-virulence approaches that reduce selective pressure associated with conventional antimicrobials. The Brazilian Cerrado biome represents an underexplored source of bioactive compounds with potential applications in food systems. This study evaluated aqueous and ethanolic extracts from *Tachigali subvelutina*, *Terminalia fagifolia*, *Anacardium occidentale*, and *Eugenia dysenterica* for antimicrobial and anti-quorum sensing (anti-QS) activity against *Pseudomonas aeruginosa* PAO1, a bacterium associated with biofilm formation, spoilage, and persistence in food-related environments. Plant extracts, supplied in powdered form by a Brazilian research institution, were evaluated for antimicrobial activity by broth microdilution. Anti-QS activity was investigated under sub-inhibitory concentrations (sub-MIC) by assessing the production of QS-regulated virulence factors, including elastase, protease, and rhamnolipids, relative to untreated controls. All extracts exhibited antimicrobial activity against *P. aeruginosa* PAO1, although anti-QS effects varied considerably among species. *T. subvelutina* demonstrated the most pronounced anti-virulence activity, markedly reducing elastase production and decreasing protease and rhamnolipid levels at sub-MIC concentrations. *A. occidentale* showed moderate inhibitory effects on virulence factor production, whereas *E. dysenterica* exhibited limited anti-QS activity despite antimicrobial effects. *T. fagifolia*, although showing relevant antimicrobial activity, displayed minimal interference with QS-regulated virulence traits. These findings suggest that antimicrobial activity does not necessarily correlate with QS inhibition, indicating that some extracts may primarily affect bacterial growth rather than signaling pathways associated with virulence regulation. Considering the role of quorum sensing in spoilage, persistence, and biofilm formation in food-processing environments, Cerrado-derived extracts, particularly *T. subvelutina* and *A. occidentale*, show potential as natural anti-virulence strategies for food preservation and food safety applications. Future studies should investigate their efficacy against foodborne and spoilage-associated bacteria in food-related systems.

S10-P15: Food Safety Culture in Artisanal Cheese Facilities in the State of São Paulo

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Foodborne diseases (FBDs) remain a global public health problem. Food Safety Culture (FSC) is an emerging concept aimed at improving food safety. Assessing FSC is essential to identify and prevent FBDs outbreaks more effectively than traditional methods. FSC encompasses aspects beyond programs such as Good Manufacturing Practices (GMP) and the Hazard Analysis and Critical Control Points (HACCP) system, including management of personal, human interactions, and behaviors that affect food safety. In the state of São Paulo, cheesemakers produce unique and diverse recipes (signature cheeses) that can challenge microbiological safety, requiring robust risk assessment and hygiene methods. Evaluating FSC in artisanal cheese facilities is crucial to address these issues and advance the safety of Brazilian artisanal cheeses. To our knowledge, no existing tool evaluates FSC in artisanal cheese facilities with the level of detail provided by a mixed-methods approach. This project adapts a mixed-methods instrument originally developed for food services. The methodology integrates quantitative tools, including questionnaires for leaders and employees and checklists assessing structural adequacy and sanitary risk, which are triangulated with qualitative participant observation. To accurately reflect the artisanal context, food service standards from the original instrument were replaced with targeted Brazilian artisanal dairy legislation in the structural checklist. Additionally, an artisanal cheese sanitary risk checklist was incorporated, and questionnaire elements (particularly knowledge and risk perception) were reformulated using specific regulations. The adapted questionnaires underwent content validation by five experts in FSC and artisanal cheese using the Content Validity Coefficient (CVC). Judges evaluated each item on a 5-point Likert scale across language clarity, practical pertinence, and theoretical relevance. A raw CVC was calculated and adjusted to remove bias. Items failing a final CVC cut-off of 0.80 were removed, resulting in a robust global validity score exceeding 0.92. Preliminary application in one facility revealed a 57.14% structural adequacy rate (indicating an environment needing improvements), while the sanitary risk checklist yielded a score of 4.33, classifying the process as high risk. Because FSC is a localized phenomenon built by each company's specific workforce, generating a state-wide panorama is not feasible at this stage. Therefore, application is currently limited to three facilities, prioritizing the rigorous adaptation and validation of the instrument over broad sectoral representation. Finally, while hygiene indicators reflect a specific point in time compared to the continuous construction of FSC, future steps will correlate these assessment outcomes with microbiological analyses from the visited facilities to strengthen practical relevance.

S10-P16: Mapping Climate-Related Food Practices: A Data-Driven Framework to Anticipate Microbiological Risks

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Climate change is expected to increase the burden of foodborne diseases while also reshaping food systems and consumer behaviors. Although research has focused on primary production and supply chains, its impact on consumer food practices, final link in the food chain, remains poorly understood.

In response to environmental concerns and constraints, consumers adopt diverse evolving food practices, including waste reduction, alternative sourcing, home production, and novel preservation strategies. These practices are also affected by climate-induced changes in food supply (availability, price, quality) and living conditions (weather, access to water and energy). They may inadvertently create new microbial contamination or exposure pathways through changes in food handling practices, including non-standard hygiene, storage conditions, and water use.

This study aims to identify and characterize emerging food practices linked to climate change, whether actively adopted or passively experienced. Within the SafeFood4ClimDiet project, we developed a multi-source, data-driven methodology combining scientific and grey literature review; media and social media exploration using climate- and food-related keywords; and semi-structured interviews with stakeholders and experts.

This approach produced a structured database of over 800 mentions on food practices, prior to deduplication. Practices were organized using an adapted consumption pathway framework with seven non-overlapping, chronologically ordered stages covering dietary choices, moving, acquisition, preparation, storage, consumption and waste management. We collected data on proactive adaptations (e.g., plant-based alternatives, local sourcing) and reactive behaviors in response to environmental disruptions (e.g., water reuse, modified storage). This approach also supports identification of risk management intervention points.

After consolidation, practices cluster around a limited number of dominant categories, mainly dietary choices and food acquisition (e.g. reduced animal-based or group purchase), with strong redundancy across sources. In contrast, practices related to storage, preparation or waste management appear less frequent but more diverse and original.

Climate-related changes in food practices are mainly driven by structural factors, social norms and public policies. The scientific literature documents established practices while the grey literature and press articles highlight context-specific and prospective trends in France. Social media (TikTok) identify individual level emerging practices with uncertain persistence (e.g. urban foraging, or raw foodism), but with potential public health implications if widespread rapidly or adopted by groups vulnerable to foodborne illnesses.

This research contributes to the development of microbiological risk assessment approaches for climate-related food practices. Ongoing work includes quantitative assessment of practice prevalence and persistence, supporting targeted food safety interventions and improved risk anticipation under climate change.

S10-P17: Integrating Climate Change Projections into a Slaughter-to-Fork Quantitative Microbial Risk Assessment of *Salmonella* in Poultry Meat

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Salmonella remains one of the leading causes of foodborne illness worldwide, with poultry products representing a major transmission pathway. Climate change is expected to influence environmental conditions and microbial dynamics, potentially affecting prevalence, growth and survival patterns of *Salmonella* along the food production chain.

We adopted a different perspective to decipher the higher risk of salmonellosis in poultry in summertime, we evaluated which climate-related variables impact on the risk using a slaughter-to-fork quantitative risk assessment model. The case study was focused on France and poultry as we could get data:

- Data on *Salmonella* contamination in poultry at the slaughterhouse based on surveillance and control plans
- Consumption of poultry

The model is innovative as its includes atmospheric temperature and humidity, consumer practices (use of barbecues), and the maintenance of the supply chain throughout the year. The model output is the risk of human exposure to *Salmonella* through poultry meat consumption. The model describes contamination dynamics along the poultry supply chain, including slaughterhouses *Salmonella* prevalence, distribution, retail and domestic storage and consumers' handling, and cooking practices. Particular attention was given to the representation of conditions that may promote bacterial growth or, conversely, lead to insufficient inactivation at different stages from slaughter to consumption. Variability and uncertainty in the input data were represented using appropriate probability distributions, and probabilistic risk estimates were obtained through Monte Carlo simulations.

The results confirmed a higher *Salmonella* risk during summer, identified principal contamination pathways and highlighted critical stages where risk mitigation strategies may be most effective. The results also emphasized specific steps along the chain where environmental conditions can facilitate *Salmonella* persistence or growth, or reduce the effectiveness of inactivation processes.

Moreover, different climate change scenarios (including temperature, humidity and precipitation) were evaluated to explore how future environmental conditions may influence exposure risk using the developed QMRA baseline model. By integrating these scenarios into the modelling framework, the study identifies stages that may become increasingly critical under changing climatic conditions.

By combining farm-to-fork risk assessment with climate change projections, this study provides a modelling approach to anticipate future food safety challenges and to support adaptive risk management strategies in the poultry sector. Overall, this different perspective approach enables the identification of vulnerable points along the slaughter-to-consumer continuum where climate-driven changes could increase the likelihood of *Salmonella* exposure.

S10-P18: Validation of the Washing and Drying Processes of Reusable Plastic Food Packages for Ready-to-Eat Food Products

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The enactment of the Packaging and Packaging Waste Regulation (PPWR, Regulation EU 2025/40) marks a shift in the European circular economy. The PPWR mandates a significant increase in the use of reusable packaging systems by 2030. As the food industry pivots from single-use to multi-use formats, a critical knowledge gap persists regarding the microbiological safety of food-contact surfaces. Especially for ready-to-eat food products, where no consumer preparation is required to aid in pathogen inactivation.

The NBN EN 17735:2022 standard provides a framework for the commercial washing, while empirical research on its application to diverse reusable food packaging materials remains scarce. Furthermore, while the washing phase is standardized, no guidelines are available for the drying process. This is still a critical step in preventing microbial regrowth, but it remains under-researched.

This study evaluates the cleaning and drying efficacy on a laboratory scale, in accordance with the NBN regulation, for three reusable plastic food packages: Polyethylene Terephthalate (PET), Polypropylene (PP), and Tritan®. Dirty reusable food packages inoculated with a surrogate or pathogenic strain(s) and a bovine albumin mucin starch mixture (BAMS), in accordance with NBN EN 17735:2022, were cleaned in a mini-lab-scale dishwasher and subsequently dried. Afterwards, the contact surface of the reusable food package was swabbed, and the microbial counts were enumerated. The washing and drying processes for the reusable food packages were validated using the surrogate *Enterococcus faecium* ATCC 6057, as specified in the NBN standard. The thermal and chemical (detergent) resistance of the surrogate is compared against the behavior of key foodborne pathogenic strains during both the washing and drying processes. By quantifying log reductions across a lab-scale washing and drying process, this study provides insights into validation guidelines for reusable plastic food packages.

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S10-P19: Soil Surface Sampling with Sterile Socks: A Novel Tool with Potential to Detect Soil Microbiome Shifts After Extreme Weather Events

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Extreme weather events such as heavy rain and flooding increasingly threaten both food safety and food security. While lost harvests can sometimes be replaced by supplies from other regions, the rising frequency of extreme events reduces the availability of safe alternatives. This shift challenges the balance between ensuring food safety and securing sufficient food supply, and it increases the need for updated risk assessments that reconsider acceptable safety margins. Such assessments require improved knowledge of microbial exposure of crops during primary production, including:

- Which additional microbes crops may be exposed to during and after extreme weather events;
- How long these microbes persist;
- Whether soil microbiomes change at the surface and deeper layers;
- Under which conditions increased microbial exposure could pose a food safety risk, especially when water or soil originates from polluted or densely populated areas.

The objective of this study was to develop a monitoring method capable of detecting changes in microbial exposure in fields and, ideally, to differentiate whether introduced microbes originate from natural environments, agricultural landscapes, densely populated areas, or polluted zones. The work consisted of two steps.

1. Identification of potential indicator strains: Soil samples from high population areas, agricultural fields, and natural environments were collected across four Norwegian counties. Samples were cultivated aerobically and anaerobically at multiple temperatures and dilutions to quantify colony-forming units. Colonies were identified using MALDI TOF.
2. Comparison of sampling methods: Surface sampling using sterile socks worn for ~200 steps was compared with single point soil sampling. Paired samples were collected from parks, fields, and natural areas in six counties. DNA was extracted and analysed by 16S rRNA sequencing.

Soil samples showed high microbial concentrations (> 10,000 CFU/g) and broad species diversity, including soil bacteria, food spoilage organisms, foodborne pathogens, and other human, plant, and animal pathogens. Sock samples contained fewer species but showed distinct dominance patterns compared to soil samples from the same sites. The 16S analysis occasionally indicated highly pathogenic species such as *Bacillus anthracis*, likely reflecting genus-level misclassification; thus, 16S data should be interpreted cautiously at the species level.

Sock sampling combined with 16S analysis is a promising method for monitoring soil-surface microbiomes. To detect changes following extreme weather events, sampling should be repeated over time and focus on regional patterns rather than individual farms.

S10-P20: One Heath – Emerge (OHE): An International MSc Program for Veterinarians, Food Engineers, Medical Doctors, Pharmacists, and Scientists

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The complexity of global health challenges necessitates a "One Health" approach, acknowledging the interconnectedness of human, animal, and environmental health. Effective responses to emerging risks require breaking down traditional silos. This requires professionals with advanced skills in cross-disciplinary collaboration and communication to address future health crises proactively. The lack of such cross-trained professionals necessitates the creation of comprehensive training programs.

The One Health – Emerge (OHE) program, evolving from the successful Man-imal Master's program, offers a cross-disciplinary MSc program for veterinarians, food engineers, medical doctors, pharmacists, and scientists. It includes a Master 1 and a Master 2 (with a work-study option), and PhD opportunities. The curriculum emphasizes the integration of human, animal, and environmental health pillars and develops operational capacity for cross-cultural collaboration.

Over ten years, the predecessor program, called Man-imal, graduated 110 students from diverse backgrounds. The OHE program, launched in 2023, builds on this success. It integrates courses from veterinary medicine, food engineering, and epidemiology. The international Master's 2 program (launched September 2024) trains managers to address complex public health challenges using a One Health approach. The program empowers a new generation of professionals to navigate the complexities of emerging health risks.

The OHE program directly addresses the critical need for professionals equipped to tackle global health challenges using a holistic "One Health" approach. By fostering collaboration and cross-disciplinary understanding, it strengthens our collective capacity to prevent and manage future health crises impacting human, animal, and environmental well-being. Graduates will be leaders in the field, better equipped to address the complexities of modern health issues.

This program represents a significant advancement in One Health education, offering a unique and comprehensive experience, integrating diverse disciplines, and training a new generation of leaders capable of addressing increasingly complex global health challenges. The program's international scope and impact on public health makes it highly newsworthy.

S10-P21: Exploiting Endogenous CRISPR-Cas System for Targeted Elimination of Tetracycline Resistance in *Bifidobacterium animalis* subsp. *lactis*: A Safe-by-Design Strategy

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Bifidobacteria are widely used probiotics in fermented foods and dietary supplements, contributing to both product functionality and consumer health. However, the occurrence of antibiotic resistance genes in probiotic strains represents a safety and regulatory concern. In particular, the tetracycline resistance gene *tet(W)* is commonly detected in *Bifidobacterium animalis* subsp. *lactis*. Its presence raises concerns regarding the potential horizontal transfer of antimicrobial resistance within the food system and the human gut microbiome. Consequently, the development of strategies to improve the safety profile of probiotic strains without compromising their functional properties is a key challenge for food microbiology and biotechnology.

In this study, we implemented a Safe-by-Design approach to selectively inactivate tetracycline resistance in *B. animalis* subsp. *lactis* by repurposing its endogenous CRISPR-Cas system. Genome editing was achieved through the targeted introduction of premature stop codons into the *tet(W)* gene. Whole-genome sequencing confirmed the accuracy of the editing process and revealed no relevant off-target mutations, demonstrating the reliability of this CRISPR-based strategy.

Phenotypic characterization showed a substantial reduction in tetracycline resistance, restoring susceptibility below EFSA microbiological cut-off values. Importantly, the edited strain retained key probiotic and technological characteristics, including tolerance to environmental stress conditions, exopolysaccharide production, auto-aggregation capacity, survival during simulated gastrointestinal digestion, and adhesion to intestinal epithelial cells. The preservation of these traits demonstrates that targeted removal of antibiotic resistance can be achieved without compromising probiotic functionality.

The stability of the antibiotic-sensitive phenotype was further confirmed under prolonged exposure to sub-inhibitory tetracycline concentrations, demonstrating the robustness of the modification for potential application in functional foods.

This study demonstrates a precise and safe strategy to mitigate antibiotic resistance in probiotics while preserving their key functional traits, supporting the controlled development of next-generation strains for fermented foods and dietary supplements.

S10-P22: Climate Change and the Potential Replacement of *Hanseniaspora uvarum* by *Hanseniaspora opuntiae* on Grape Berries: Implications for Wine Composition

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Climate change is increasingly affecting viticulture and wine production worldwide, influencing both grape composition and wine quality. In Slovenia, a study covering the period 2001–2025, based on nearly 70,000 wine samples from the official state database (Register of Grape and Wine Producers – RPGV), demonstrated a gradual increase in alcohol content, pH values, and sensory quality of wines, and a decrease in total acidity, depending on the variety and wine-growing region. These trends were accompanied by a reduction in total dry matter, particularly in white wines. Environmental factors such as climate conditions and vintage have also been shown to influence the diversity of microbial communities associated with grape berries. Recent observations in Slovenian vineyards suggest that the traditionally dominant non-*Saccharomyces* species *Hanseniaspora uvarum* is being partially replaced by the thermotolerant and invasive species *Hanseniaspora opuntiae*. To investigate the potential impact of this shift on wine composition, experimental fermentations were performed with both species individually and in co-inoculation with *Saccharomyces cerevisiae* in triplicate at two ratios (1:10 and 10:1), simulating spontaneous and inoculated alcoholic fermentations of grape must. Fermentation progress was monitored daily by measuring weight loss. Yeast populations were monitored using the plate count method and flow cytometry. Chemical parameters of the resulting wines (pH, total acidity, glucose and fructose, total dry matter, volatile acidity, glycerol, and alcohol) and volatile profiles (esters, C6 alcohols, higher alcohols, and ethyl acetate) were analysed by GC-MS. The results showed comparable fermentation kinetics but significant differences in the production of fermentation-derived metabolites and volatile aroma compounds. In particular, *H. opuntiae* demonstrated a strong ability to produce aromatic esters such as 2-phenylethyl acetate, whereas *H. uvarum* was associated with higher glycerol production. These results suggest that shifts in yeast community composition on grape berries may influence wine aroma profiles, especially in spontaneous fermentations and fermentations with delayed yeast inoculation used to emphasise the terroir of specific wine-growing regions. These findings highlight the importance of monitoring grape-associated yeasts, as shifts in their composition may ultimately affect wine composition and sensory quality.

S10-P23: Comparison of Next Generation Sequencing, RT-dPCR and RT-qPCR for the Detection and Quantification of Nov GI, Nov GII and HAV in Mediterranean Mussels

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Foodborne viruses are a major cause of foodborne illness worldwide, with shellfish representing an important source of infection due to their ability to bioaccumulate contaminants from surrounding waters and concentrate them within edible tissues. The increasing occurrence of viral pathogens in food highlights the need for sensitive and reliable detection methods. Although reverse transcription real-time PCR (RT-qPCR) is widely used, largely due to its inclusion in ISO standard methods, its limitations in absolute quantification, particularly its reliance on comparison with dsDNA plasmid standards, have driven the development of alternative approaches.

In this study, RT-qPCR, reverse transcription digital PCR (RT-dPCR), and next-generation sequencing (NGS) were compared for the detection of noroviruses of the genogroups I (NoV GI) and II (NoV GII), and hepatitis A virus (HAV) in Mediterranean mussels (*Mytilus galloprovincialis*). A pooled hepatopancreas sample from mussels, which had been confirmed negative for the target viruses by RT-qPCR prior to analysis, was spiked in two biological replicates with known concentrations of each virus to enable a controlled evaluation of method performance.

Results showed that RT-dPCR exhibited higher sensitivity and provided more accurate quantification than RT-qPCR, as it allows absolute quantification without the use of a standard curve. For qualitative detection, RT-dPCR and RT-qPCR showed comparable performance. NGS demonstrated limited sensitivity for virus detection in shellfish samples, likely due to low viral loads and the presence of abundant background nucleic acids; however, it provided broader insights into viral diversity and remains valuable for the detection of unknown or unexpected targets.

In conclusion, both RT-qPCR and RT-dPCR are suitable for routine screening of NoV GI, NoV GII and HAV in shellfish, with RT-qPCR preferred for qualitative detection and RT-dPCR for accurate quantification. NGS is better suited for genotyping and outbreak investigations but is less effective for detecting low-abundance viruses in complex food matrices. These findings highlight the importance of selecting appropriate detection methods for accurate identification and quantification of viral contaminants in shellfish, thereby supporting effective monitoring strategies and improving food safety.

S10-P24: Beyond QMRA: A Framework for Holistic, Food Systems-Oriented Risk Assessment

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Quantitative microbiological risk assessments evaluate the health impact of pathogens. However, the complexity of food systems demands for holistic, food system-based approaches that integrate microbiological risks with multiple dimensions like other health aspects, environment, and socio-economic factors, enabling the assessment of impacts across domains, and the support of evidence-informed risk management decisions.

The aim of this study was to design a comprehensive framework to guide the implementation of holistic risk assessments. The framework was built within the EU project HOLiFOOD by experts in various domains, based on lessons learnt from two complementary case-studies ran in parallel: lentil and poultry food systems. That brought solid experience and diverse perspectives but ensured the framework to be applicable to address different risk-management and research questions. The novelty of this work lies in offering step-by-step guidance on how to systematically combine existing methods across multiple domains into a single holistic risk assessment.

The guidelines are structured around four key steps: (*i*) definition of the assessment scope, (*ii*) the estimation and aggregation of food system impacts, (*iii*) uncertainty analysis and (*iv*) communication of results. The first step focuses on defining a structured assessment framework, capturing food system components (and their interconnectedness) that traditional QMRA might not consider. The guidelines provide specific criteria across multiple domains to identify and select relevant food system components, including the dimensions (microbiology, toxicology, nutrition, environment and economy) and their corresponding impact-indicators. The second step focuses on comparing impacts within the food system. The guidelines introduce approaches and metrics to estimate impact-indicators across multiple domains, as well as methodologies to visualize (e.g. radar plots) and aggregate results (e.g. multi-criteria decision analysis or monetization) in alignment with the scope and decision-context of the assessment. The third step introduces key considerations for the analysis of uncertainty in integrated frameworks that combine multiple dimensions and the fourth step describes good practices for communicating the results obtained to stakeholders.

Overall, the developed guidelines provide a general framework for conducting holistic risk assessments, supporting more comprehensive evaluations of the food system impacts and interventions.

S10-P25: Microbial Challenge Testing Against Botulinum Neurotoxin-Producing Clostridia: Improve Food Safety or Guarantee National Security?

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Microbial challenge testing is a widely accepted tool for enhancing food safety. This approach assesses the behaviour of a specific microorganism in a food product to validate the product or its production process. Microbial challenge testing can be conducted in accordance with the UNI EN ISO 20976 series; however, for microorganisms covered by Regulation (EC) 2073/2005, such as *Listeria monocytogenes*, the EU reference laboratory has developed specific guidelines and policy documents.

Botulinum neurotoxin-producing clostridia are not covered by the aforementioned regulation, and no specific EU-level rules have been published to date due to the absence of reference diagnostic methods and the rarity of botulism. However, given changes in consumer behaviour towards minimally processed foods and the increased availability of foods potentially at risk of botulism, the disease poses new concerns. The main objective of this work is to highlight this weakness in food legislation, propose guidelines for conducting microbial challenge tests against botulinum neurotoxin-producing clostridia and their toxins, and suggest an audit plan to control private laboratories and prevent the voluntary or accidental release of these agents.

Based on published documents and procedures for setting specific shelf-life studies in relation to *Clostridium botulinum*, and on peer-reviewed literature, the paramount issues included in the guideline are:

- Studies assessing the inactivation of neurotoxic spores can be conducted using a non-pathogenic surrogate; conversely, in other types of challenge testing, strains capable of producing the neurotoxins cannot be replaced, and toxin detection is mandatory, because the neurotoxins could be released into the food if the relative enumeration decreases compared to the inoculum.
- Studies require a cocktail of strains that cover all toxinotypes of interest, including both proteolytic and non-proteolytic strains.
- Strains have to be characterised and studied for their ability to grow in co-culture with the other component of the cocktail. The amount of toxins they can produce within the food matrix of interest should be evaluated prior to the study.
- The strains cannot be inoculated at the production facility; however, if the food product permits, they may be inoculated after packaging using the double-barrier technique to avoid altering the packaging atmosphere.

Because botulinum neurotoxins are the most dangerous poisons known to humans, laboratories must be accredited to UNI EN ISO IEC 17025, participate in interlaboratory exercises, maintain high biosecurity standards, and undergo biosecurity audits conducted by the national reference laboratory.

S10-P26: CATALYSE Action: Building Collaborative Networks to Accelerate Food Safety Innovation

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Introduction and objective: Food safety is a critical aspect of public health, economic stability, and consumer trust within the food sector. To address this challenge, extensive research and numerous innovative solutions have been developed in recent years. However, several barriers impede effective knowledge sharing among food system actors and the adoption of these innovative advances. The CATALYSE project aims to bridge the gap between industry, academia, and policymakers through the development of a Community of Practice (CoP) platform, to accelerate the dissemination of new knowledge and innovative solutions and increase networking among diverse actors in the food safety system.
Methodology: The CATALYSE CoP platform was developed as a collaborative environment to support interaction among food industry professionals, researchers, innovators, and regulators. A stakeholder engagement strategy, including surveys and semi-structured interviews, was implemented to identify key needs and priorities in food safety and to inform the development of a Community of Practice (CoP). Based on these insights, the platform was designed to integrate a structured knowledge database and educational resources in priority areas of food safety, aiming to facilitate knowledge exchange, collaboration, and dialogue within the CoP.
Results: A Stakeholder Engagement Strategy with 112 survey's responses, 85 one-to-one interviews and more than 260 interactions was performed; the key groups (industry, policymakers, researchers, and civil society) expressed their priorities and needs in food safety innovation. The collected data were systematically analysed to understand the main challenges and emerging concerns and used to design the platform.
Subsequently, 124 startups, spin-offs, and innovative solutions were mapped to strengthen connections, more than 10 matchmaking between innovators-industry were done fostering collaboration within the CATALYSE CoP platform network. In parallel, a structured knowledge database was developed, organizing scientific literature, innovations, and practical new solutions into thematic categories (Control measures, Rapid detection methods, Digitalization and AI tools, Traceability, Hygiene by design, Packaging innovation). This was complemented by the integration of educational resources, including more than 300 courses and training materials, including videos, guidelines, presentations, and webinars, to address identified knowledge gaps and support information exchange, considering different levels of complexity. Together, these components aim to enhance knowledge exchange, facilitate discussions, and promote active engagement within the CATALYSE CoP.
Conclusion: The CATALYSE CoP platform offers stakeholders interested in food safety innovation rapid, reliable access to emerging solutions—accelerating access to knowledge and applications across the entire food chain and improving collaboration and networking in the food ecosystem.

SO Methodological Approaches / Miscellaneous / Other

SO-P1: Quality of Philippine Lime (*Citrus microcarpa*) Juice Under Predictive Model-Based Pasteurization

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A predictive model based on response surface methodology (RSM) was developed to estimate the thermal reduction time (D value, D°C) of *Pichia anomala*, a heat-resistant spoilage yeast, in simulated fruit juice (SFJ) systems as a function of temperature (°C), pH, and natamycin concentration (ppm). The model was generated from thermal inactivation experiments and intended for application in beverage systems. Its utility was validated in Philippine lime (also known as Calamansi) juice (PLJ) by establishing a pasteurization process (P°C). Physicochemical, color, and microbial quality characteristics of the pasteurized PLD were evaluated and used as bases for assessing the utility of the developed model. Validation trials were conducted using a controlled isothermal thermal process (T = 75°C) in a laboratory water bath system, with PLJ samples adjusted to target specific pH and natamycin concentration (ppm). The predictive pasteurization (pP°C) was determined by combining the model-generated D°C with a target lethality of 5.00 $\log_{10}$ based on the Juice HACCP. The actual pasteurization (aP°C) was derived from experimentally obtained D°C values in PLJ. Results showed that key physicochemical properties of PLJ remained stable ($p > 0.05$) after pasteurization at 75°C. Color analysis indicated increased yellow chromaticity (b*) and chroma (C*) in thermally treated samples compared to the unpasteurized samples, while lightness (L*) was unaffected, and the overall color difference (ΔE*) remained below 1.0, suggesting minimal perceptible change. Microbiological analysis demonstrated that both aP75°C and pP75°C treatments effectively reduced total plate count (TPC) and yeast and mold counts (YMC) to levels compliant with national and international safety standards, with no detectable coliforms or *Escherichia coli*. These findings confirm that the RSM-based predictive model can design microbiologically safe pasteurization processes for acidic juices when combined with natamycin as a hurdle, while maintaining acceptable fruit juice quality. Further validation is recommended for juice systems with varying pH and compositional profiles.

SO-P2: Validation of AI-Coupled FTIR Spectroscopy for Typing of *Listeria monocytogenes* Serogroups

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Listeria monocytogenes is the cause of zoonosis listeriosis, most often transmitted by contaminated food, and represents a constant risk for food safety and human health in the world. Contamination of food with *Listeria* can occur at any stage, from production and processing to distribution of food, which emphasizes the importance of strict control. Epidemiologically, strain typing is a prerequisite for the determination of infection sources and the potential of the strain to cause wider epidemics. From a routine high-throughput point of view, it is essential to define the most informative, fastest, and most cost-effective currently available method for bacterial determination and typing. Our work aimed to validate AI-coupled Fourier Transform Infrared Spectroscopy (FT-IR) on IR Biotyper® (Bruker, Germany).

In total, 118 *L. monocytogenes* isolates were used in this study. All isolates were confirmed using a MALDI-TOF device. Molecular serogroup determination was performed using real-time PCR and GenoListeria to identify serogroups and clonal complexes. Whole genome sequencing (WGS) was performed to obtain detailed genomic characterization of the isolates. Conventional serotyping based on somatic and flagellar antigens was also performed to determine serotypes, providing essential information for accurate classification and interpretation of FT-IR spectral clustering. All these established methods were used as reference approaches for comparison and for the validation of the FT-IR-based typing approach for *L monocytogenes*.

The IR Biotyper® classified the 118 *L. monocytogenes* isolates into serogroups 1/2, 3, and 4x, with perfect reproducibility and agreement with standard serotyping methods. The method was also able to differentiate serotypes within the serogroups 3 and 4. This method offers a rapid and cost-effective solution for distinguishing *L. monocytogenes* serogroups. Its integration into routine laboratory workflow increases efficiency, improves pathogen surveillance, and contributes to food safety control systems. In addition, artificial neural network processing of FT-IR spectral data showed promising clustering capabilities that are comparable to whole genome sequencing depths to some extent, which will shorten the amount of time and decrease the number of strains and financial resources needed for epidemiological surveillance. More research in the future will investigate the possibility of differentiation within the 1/2 serogroup and the exact extent of its potential for epidemiological use.

Keywords: *Listeria monocytogenes*, epidemiology, FT-IR, bacterial typing, serogroups, food safety, AI, IR Biotyper®

SO-P3: Molecular Methods for the Quantitative Detection of Norovirus and Hepatitis A Virus on Food-Contact Surfaces

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In foodborne outbreaks, human norovirus and hepatitis A virus (HAV) pose significant public health risks. The contact of food with contaminated surfaces or utensils is considered to have a critical role in the spread of these viruses during foodborne outbreaks. While the ISO 15216 procedure provides molecular methods using swabbing to detect norovirus and HAV on food and food-contact surfaces, its validation was primarily performed on bell peppers, leaving a gap in standardized data for other surface materials.

Different sampling tools (swabs, wipes and sponges) and five elution methods were evaluated to select the optimal protocol based on direct inoculation with murine norovirus (MNV-1). The optimized protocol was then applied using two sampling tools across three surface types (glass, stainless steel, and plastic) to detect noroviruses and HAV, following ISO guidelines for analytical controls. Additionally, viral genome stability after sampling was assessed to determine the maximum storage time prior to analysis.

MNV-1 recovery rates ranged from 94.38% to 100.00% for swabs, 29.74% to 36.91% for wipes, and 23.87% to 39.69% for sponges, regardless of the elution method used. Ready-to-use swabs and sponges with direct lysis were selected for validation due to their varying adsorption capacities and their practical advantages for field use. Norovirus and HAV recovery rates ranged from 4.45% to 100.00% for swabs and from 8.29% to 100.00% for sponges depending on the surface tested. Across all sampling methods, Norovirus and HAV recovery rates were between 10.95% and 100.00% for glass, 4.45% and 100.00% for plastic, and 8.37% and 100.00% for stainless steel. The LOD_{95} ranged from 4.83 to 110.00 genome copies/cm² for swab sampling and from 5.26 to 120.00 genome copies/cm² for sponge sampling, depending on the virus inoculated. Analytical controls (process and RT-qPCR inhibition) were validated across all surfaces tested. Viral genome stability on sampling tools, inoculated at two concentrations near the LOD_{95}, was assessed after 3 days of storage at 4°C with detection rates of 86% for swabs and 69% for sponges.

These methods are suitable for routine hygiene monitoring and serve as a valuable complementary tool for foodborne outbreak investigations.

SO-P4: A Systematic Gap Analysis of FSSC 22000 Version 6 Using BRCGS and IFS Retail Standards as Comparative Benchmarks

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Consumers demand safe and quality food products, leading manufacturers to implement systems to achieve safer and higher quality food products. These systems are prescribed by Food Safety Management Systems (FSMS), quality and retail standards. These standards are audited by an accredited certification body, ensuring that food manufacturing companies comply with client requirements. Company X's current supplier quality assurance system, based on FSSC 22000 Version 6 requirements, was evaluated using a gap analysis to assess its effectiveness within the organization. The weak points in this system were identified, and retail standards, including Brand Reputation Compliance Global Standards (BRCGS) and International Featured Standard (IFS), were used for guidance to amend the current system. The new supplier quality assurance system underwent a gap analysis using the same checklist, and the overall score improved in terms of both the effectiveness of implementation and record-keeping. This approach can be used to improve other procedures in the FSMS and applied in other organizations to improve the current FSMS to enhance food safety and quality assurance.

SO-P5: Potential of Antifungal *Lactiplantibacillus plantarum* Strains as Bioprotective Cultures in Cheese Model

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Filamentous fungi, such as *Aspergillus* and *Penicillium*, are major contributors to dairy product spoilage, whereas antifungal lactic acid bacteria (LAB) provide a natural strategy to improve safety, quality, and shelf life of dairy products. This study investigated the antifungal efficacy of five *Lactiplantibacillus plantarum* strains (M3.1, M3.3, M3.6, R3.2, and R3.6) *in vitro* and in a cheese model, as well as the contribution of their metabolomic profiles to controlling dairy spoilage fungi. The antifungal potential was assessed using liquid co-inoculation assays against *Aspergillus niger* IOC207 and *Penicillium chrysogenum* IOC132, with fungal inhibition measured as biomass reduction over 12 days. Spore structural changes were evaluated by scanning electron microscopy (SEM). Metabolites were characterized through untargeted liquid chromatography–mass spectrometry (HPLC–MS). Antifungal effects were further confirmed in a cheese model inoculated with 10^5 CFU/mL of each *L. plantarum* strain and 10^2 spores/mL of each fungus, stored at 30°C for 7 days. All strains resulted in significantly reduced fungal biomass compared to controls ($p < 0.005$), with complete inhibition of *P. chrysogenum* and *A. niger* under co-inoculation conditions. Overall, SEM revealed extensive spore damage, including surface collapse and deformation, indicating impairment of fungal cell integrity. Metabolomic profiling identified 35 metabolites, mainly amino, organic and fatty acids, cyclic dipeptides, phenolic compounds, and esters. In the cheese model, all strains inhibited *P. chrysogenum* IOC132, while strains M3.3, M3.6, and R3.6 fully suppressed *A. niger* IOC207 growth during storage. These findings demonstrate that selected *L. plantarum* strains can effectively extend the shelf life and provide bioprotection to dairy products, offering a natural strategy aligned with current trends in food innovation.

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SO-P6: Assessment of the Effect of *Debaryomyces hansenii* on the Quality of Dry-Cured Sausages Using Low Field ^{1}H-NMR

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Microbial biopreservatives are within the most promising approaches to minimize the hazard related to ochratoxin A in dry-cured sausages. Nonetheless, they also can modify the sensory and nutritional attributes of the products. *Debaryomyces hansenii*, the most common yeast in dry-cured sausages, is being widely explored for its antiochratoxigenic activity, which does not adversely affect the sensory features of "salchichón". To date, the effect of this yeast on the metabolic profile of dry-cured sausages has not been evaluated. Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful technique for this purpose. The aim of this study was to evaluate the ability of low field ^{1}H−NMR to detect modifications of the metabolic profile, focused on the lipids, of dry-cured sausages because of using *D. hansenii* as antiochratoxigenic agent. Two different kinds of these foodstuffs, "salchichón" and "chorizo", were selected because of their different ingredients. They were ripened at the pilot plant of the Faculty of Veterinary Sciences (University of Extremadura, Cáceres, Spain). Two experimental groups were made: Dh (*D. hansenii* alone and combined with rosemary essential oil [REO], rosemary leaves [RL] or casings macerated with RL [CMR]) and noDh (non-treated batch and treatments with REO, RL or CMR). The eight batches were inoculated with *Penicillium nordicum* and *Aspergillus westerdijkiae*, the main ochratoxigenic moulds in dry-cured sausages. After the climate-controlled ripening of sausages for 25 days, lipids were extracted from surface mycelium together with the casing as previously reported for ochratoxin A and analyzed by 1H-NMR spectroscopy. The multivariate analysis of the 1H NMR data revealed a significant metabolic shift due to the yeast, especially in "salchichón". The fatty acid profile on the surface was evaluated since it influences nutritional value and oxidative stability throughout ripening. A marked and statistically significant reduction in polyunsaturated fatty acids (PUFA) content was displayed, accompanied with an increase in saturated fatty acids (SFA) in both matrices. Even though the positive impact of PUFA on human health have been reported, their presence in high levels can lead to increased lipid oxidation resulting in off-flavours and potential negative effects on the human health. On the contrary, higher SFA content contribute to a better oxidative stability. These outcomes have thus provided a more in-depth understanding of the effects of *D. hansenii* on the lipidomic profile of "chorizo" and "salchichón" and, consequently, on their sensorial, nutritional and safety attributes.

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SO-P7: *Fusarium* Mycotoxin Contamination in Wheat Grown in Croatia Over the Past Two Years

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Wheat represents one of the most significant cereal crops in human and animal nutrition. However, throughout cultivation, harvesting and storage, it is prone to mould contamination. Under favourable environmental conditions, such contamination can result in the production of mycotoxins, which are regarded as contaminants of food and feed. Global trends and long-term monitoring of cereals cultivated in the Republic of Croatia indicate an increasing prevalence of contamination with *Fusarium* mycotoxins and their metabolites. In addition to parent compounds, modified forms of mycotoxins are being increasingly reported. The aim of this study was to determine the occurrence of *Fusarium* mycotoxins and their metabolites in wheat samples intended for both human consumption and animal feed, harvested in the Republic of Croatia. A total of 62 wheat samples were analysed, including 29 samples from 2024 and 33 samples from 2025. The analysis encompassed 15 *Fusarium* mycotoxins and was performed using liquid chromatography–tandem mass spectrometry (LC-MS/MS). Among the analysed samples, deoxynivalenol (DON) and its metabolites (3-acetyl-DON, 15-acetyl-DON, and DON-3-glucoside), as well as zearalenone-14-sulphate, T-2 toxin, HT-2 toxin, and nivalenol (NIV), were detected with varying frequencies. Overall, 74.2% of the samples were contaminated with at least one mycotoxin. DON and its metabolites were the dominant mycotoxins detected. In 2024, DON was detected in 75.9% of samples, with a maximum concentration of 5652.1 µg/kg. Its metabolite 3-acetyl-DON was present in 30.3% of samples (maximum 354.4 µg/kg), while 15-acetyl-DON was detected in a single sample (603.5 µg/kg). DON-3-glucoside was found in 48.3% of samples, reaching a peak concentration of 704.1 µg/kg. In 2025, DON was detected in 48.5% of samples, with a maximum concentration of 2265.8 µg/kg, whereas DON-3-glucoside was present in 33.3% of samples, with a maximum concentration of 270.3 µg/kg. A comparison between the two sampling years revealed a decrease in both the occurrence and concentration of DON and its modified forms in 2025 relative to 2024. However, DON concentrations did not exceed the recommended guidance values for animal feed, while some samples exceeded the maximum permitted levels established for wheat intended for human consumption. Other mycotoxins were detected at low frequencies. In 2024, zearalenone-14-sulphate (10.3%) and T-2 toxin (6.9%) were detected; HT-2 toxin appeared in one sample in both years, while NIV was present in 6.1% of samples in 2025. Since the correlation between mycotoxin occurrence and climatic conditions was not investigated in this study, future studies should focus on this relationship.

SO-P8: Microbiological Quality Assessment of Croatian Post-Harvest Maize According to VDLUFA Method

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This study aimed to classify 67 post-harvest maize samples collected in 2024 prior to storage according to the VDLUFA method in order to provide insight into the microbiological hazards relevant to animal health. This method estimates the mycological quality of feedstuffs based on the presence of moulds and yeasts, which are grouped according to their potential impact on animal health. Mycological analyses were conducted on DG18 and DRBC agar, methods comparable to the HRN ISO 21527-2 standard. Identification at the genus level was based on the microscopic characteristics of the grown moulds.

The advantage of VDLUFA method is that samples are classified into four categories (I–IV) based on contamination levels of field (product-typical) moulds (e.g., *Fusarium* spp., *Cladosporium* spp. and *Alternaria* spp.), storage moulds (e.g., *Aspergillus* spp., *Penicillium* spp. and *Wallemia* spp.), moulds of the order *Mucorales* and total yeast counts. The VDLUFA method enables not only the assessment of mould and yeast contamination levels but also the prediction of potential mycotoxin contamination.

The results showed that 15% of samples were classified into category I, 25% into category II, 6% into category III, while 54% of samples belonged to category IV, indicating poor hygienic quality in the majority of the analysed maize. The primary reason for poor classification was excessive contamination with field moulds, particularly *Fusarium* species, as well as elevated yeast counts. Moulds of the order *Mucorales* were not detected in 66% of samples and were present only in relatively low counts in the remaining samples. The total count of moulds and yeasts ranged from 3.51×10^3 to 1.32×10^7 CFU/g, indicating a significant level of contamination already present at the post-harvest stage.

The results indicate substantial microbiological contamination of maize in Croatia prior to storage, highlighting the importance of proper drying, adequate storage conditions, and continuous microbiological monitoring. Feeding animals with poor-quality maize may lead to health disorders and reduced productivity. Furthermore, the presence of *Fusarium* species suggests a potential risk of contamination with *Fusarium* mycotoxins and their modified forms, which may further contribute to adverse health effects.

SO-P9: Extended Validation of ISO 10272 "Enumeration of *Campylobacter*"

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The validation status of microbiological reference methods is essential to ensure their reliable application across different food matrices. ISO 10272-2:2017 is currently validated for four food categories 'raw milk and dairy products', 'raw meat and ready-to-cook meat products except poultry', 'raw poultry and ready-to-cook poultry products', 'processed fruits and vegetables' and for the category 'primary production'. It requires validation of an additional food category to extend its scope to a broad range of foods according to ISO 17468:2023.

The objective of this study is to generate validation data for the food category "fresh produce and fruits" in accordance with the requirements of ISO 17468 and ISO 16140-2:2016. In 2026, the EURL-*Campylobacter* organised validation of ISO 10272-2 in combination with a proficiency test (PT) follow-ing the principles and criteria defined in the given standards.

This work is conducted within the framework of CEN/TC 463/WG 3 '*Campylobacter'* and aims to provide the data necessary to extend the validated scope of the method described in ISO 10272-2:2017. On 9 March 2026, test materials were distributed to 26 laboratories participating in the validation study. The test contains in total eight samples of shredded cabbage with three contamination levels of *Campylobacter* in duplicates, as well as two negative samples. Results from the validation are under evaluation and will be summarised in a report and in a new amendment to ISO 10272-2:2017.

Achieving a validation of the reference methods applicable to a broad range of foods is essential, as in-complete validation would require individual laboratories to perform additional validation according to ISO 16140-4 when applying the method outside its validated scope. The results will support extension of the method's validated scope, enhancing its applicability across diverse food matrices for microbiological testing laboratories.

SO-P10: Not Too Wet, Not Too Dry: Optimal Agar Plate Conditions for *Campylobacter* Cultivation

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Campylobacter spp. are Gram-negative, microaerophilic bacteria and represent one of the leading causes of foodborne bacterial gastroenteritis in many parts of the developed world. Compared to other foodborne pathogens, *Campylobacter* is highly sensitive to environmental stressors such as low pH, osmotic stress, high temperatures, and desiccation. Their poor survival on dry surfaces makes careful handling essential during laboratory cultivation.

While media composition and atmospheric conditions are well-recognized factors influencing growth, practical aspects of agar plate preparation and plating technique are equally important but often less discussed. Based on laboratory experience, agar surface moisture is a critical factor affecting colony formation and enumeration. Excessively moist plates promote swarming and colony spreading, resulting in merged colonies that are difficult to distinguish and count. Conversely, overly dry plates may reduce bacterial viability and recovery, leading to low colony numbers.

Plate moisture is highly related to spreading time and Gram-negative bacteria are known to be sensitive to extensive spreading, hence the duration should be limited to 5 sec according to the literature. However, laboratory experience indicates that spreading for up to 10 sec may serve as a practical indicator of adequate agar surface moisture.

Optimal plating conditions therefore require a careful balance between preventing swarming and maintaining bacterial survival. As a practical consideration, plates should be pre-dried before use in an incubator with lids slightly open. Drying time depends on plate size and initial moisture level: smaller plates (9 cm diameter) typically require 10–30 min of drying, whereas larger plates (14 cm diameter) may require 1–2 h to achieve suitable surface dryness. Plate moisture may also vary with plate age, as freshly prepared plates tend to be more humid than older plates, depending on storage conditions. During spreading, the bacterial suspension should be distributed evenly until the agar surface just appears/feels dry. Determining when the agar surface is sufficiently dry is subjective and relies on practical experience and operator judgment.

Water activity (aw) measurements were performed on agar plates after different drying conditions showing consistently high aw values (0.994–0.998), indicating that pre-drying primarily affected surface moisture rather than overall water availability. As a complementary measure, plates were weighed before and after drying, showing increased weight loss with higher temperature and longer drying time, consistent with enhanced surface evaporation.

These observations emphasize the importance of practical technique and operator experience for successful cultivation and enumeration of sensitive microorganisms such as *Campylobacter*.

SO-P11: Enhanced Inactivation of *Salmonella* Typhimurium and *Listeria monocytogenes* on Pork by Piezoelectric Cold Plasma Combined with Organic Acids and Nanoemulsions: Insights into Membrane and Oxidative Damage

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Salmonella Typhimurium and *Listeria monocytogenes* remain leading causes of foodborne illness, with pork representing a significant transmission vehicle. Cold atmospheric plasma (CAP) is a promising non-thermal intervention; however, its integration within established hurdle strategies and the mechanistic basis of combined treatments require clarification.

This study evaluated piezoelectric direct discharge CAP applied alone and in combination with organic acids (lactic and acetic acid) and citral- and cinnamon-based nanoemulsions against both pathogens on pork. Antimicrobial efficacy was quantified by culture-based enumeration, while cellular disruption was assessed using scanning electron microscopy and assays for membrane permeability, reactive oxygen species accumulation, lipid peroxidation, and malate dehydrogenase activity.

CAP treatment alone for 9 min led to approximately 0.6 log CFU/g pathogen reduction on pork. On pork, CAP for 9 min combined with organic acids or nanoemulsions at 10× minimum inhibitory concentration produced additive effects, enhancing pathogen inactivation by approximately 1.5 log CFU/g compared with CAP or antimicrobials alone under the same conditions. When CAP was applied before organic acids at inhibitory/bactericidal levels, combined treatments achieved significantly greater reductions (approximately 3.6 log CFU/cm²) than the reverse sequence (approximately 2.3 log CFU/cm²), demonstrating that application order influences antimicrobial efficacy.

Mechanistic assays showed that piezoelectric CAP induced multi-target cellular disruption in both pathogens. Membrane permeability increased by 50%, 65%, and 94% following 6, 9, and 15 min CAP treatments, respectively. CAP also promoted intracellular oxidative stress, evidenced by reactive oxygen species accumulation and significantly elevated lipid peroxidation markers in *S.* Typhimurium and *L. monocytogenes*. In addition, CAP significantly reduced malate dehydrogenase activity, indicating disruption of cellular metabolic function. Scanning electron microscopy further revealed marked morphological injury, including pore formation, cell shrinkage, and cytoplasmic leakage.

Collectively, these findings demonstrate that piezoelectric CAP can be integrated with organic acids and nanoemulsions to achieve enhanced pathogen inactivation on pork through complementary mechanisms. The sequence-dependent effects highlight the importance of treatment order in the design of plasma-based hurdle strategies for meat processing systems.

SO-P12: Cereulide Detection in Starchy Food Using MALDI Biotyper

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Cereulide is a heat stable toxin produced by emetic *Bacillus cereus*, which is commonly associated with carbohydrate-rich foods such as rice and pasta. Consumption of food products contaminated with cereulide can cause food intoxication with symptoms of nausea, vomiting and malaise, or even fatal liver failure in some cases. Current detection of cereulide relies mainly on LC MS/MS (ISO 18465: 2017), which is sensitive but resource demanding. No rapid routine method for detecting cereulide currently exists, highlighting an urgent need for developing fast and accessible screening tools. The aim of this study was to develop and validate a cereulide detection method for starchy foods using MALDI Biotyper® System (Bruker).

Cereulide detection is based on sodium and potassium adduct peaks (m/z at 1174 and 1190, respectively) using an optimised HCCA (α-Cyano-4-hydroxycinnamic acid) matrix with MALDI Biotyper®. The limit of detection (LOD) without food matrices was determined by serial dilutions of pure cereulide in acetonitrile. LOD in cooked rice and pasta was determined by spiking with known concentrations of cereulide. Acetonitrile was used to extract cereulide from homogenized food. Challenge test was conducted by inoculating cooked rice (ca. 2 log CFU/g) with heat-activated emetic *B. cereus* spores (BP-1 and F4810/72) and incubated at 23°C for up to 43 h, to mimic real contamination scenarios. Samples were analysed by MALDI Biotyper® and confirmed in parallel by LC MS/MS analyses performed by an external accreditation lab.

The LOD for pure cereulide without food matrices was 0.075 µg/mL, demonstrating the machine's inherent sensitivity. LOD in spiked food was 10 µg/kg in rice and 30 µg /kg in pasta, involving the impact of the cereulide extraction from food. Cereulide was successfully detected in a certified rice reference material (11.3 µg/kg). In rice challenge tests, cereulide was detected in both emetic strains from 21 h incubation, with LC-MS/MS confirmed concentrations of 178 µg/kg (BP-1) and 35 µg/kg (F4810/72). At 21 h, the concentration of BP-1 and F4810/72 was 7.6 log CFU/g and 7.3 log CFU/g, respectively.

The results demonstrated that MALDI Biotyper® System can serve as a rapid and accessible detection method for cereulide in starchy foods. Even though the method is not as sensitive as LC MS based methods, its accessibility and rapid performance highlight its potential for routine testing.

SO-P13: Occurrence and Diversity of Spore-Forming Bacteria in French Pâté Production

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Spore-forming bacteria are ubiquitous in natural environments and commonly encountered throughout the food processing chain. They can survive a wide range of food processing treatments and potentially lead to food safety and spoilage issues. Reducing nitrite and salt (NaCl) in deli meat products, including ready-to-eat pâtés, may enhance the germination and growth of spore-formers such as *Clostridium* spp., *Bacillus* spp. and related genera. Limited data are available on the diversity and contamination levels of these bacteria in French pâtés. To address this issue, a sampling campaign was conducted in three pâté-producing facilities, each visited at two sampling times between 2020 and 2021. The collected samples (*n* total = 115) comprised surface samples from processing area and equipment, raw materials (especially raw meats and various plant-based ingredients), in-process materials and pâtés. For each sample, aerobic and anaerobic mesophilic as well as psychrotrophic spore-forming bacteria were enumerated by using appropriate culture-dependent methods. A total of 608 isolates were recovered, and a selection of 95 isolates were identified by 16S rRNA gene sequencing. Results showed that more than half of the plant-based ingredients used in the pâté recipes exhibited relatively high spore counts (between 3.0 and 6.1 $\log_{10}$ CFU/g), mostly exceeding those found on raw meats. Spore-forming bacteria were detected in all sampled production areas, including critical zones for product contamination. Some pâtés collected at the end of processing were contaminated with spores, typically at low levels, reaching up to 2.6 $\log_{10}$ CFU/g. The highest phenotypic diversity among spore-forming isolates was observed for plant-based ingredients and environmental surface samples. They were mainly composed of the genus *Bacillus* and related genera (e.g. *Paenibacillus*). However, the actual abundance of anaerobic spore-formers was difficult to assess due to cultivation challenges. The few anaerobic isolates belonged to the genera *Clostridium* and *Lacrimispora*. Some species identified in this study have been previously reported to cause meat and/or processed-meat product spoilage. Among the collected isolates, 14 from diverse sources were presumptively identified as *Bacillus cereus*, known to cause foodborne illness. This work provides valuable insights into the primary sources and diversity of spore-forming bacteria in French pâté production, which will help implement effective strategies to mitigate product contamination.

SO-P14: Reduction of Nitrite in Canned Meat Products: Consequences for Safety and Shelf Life

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Nitrite is a critical preservative in processed meat products, inhibiting spore germination, growth, and toxin production by *Clostridium botulinum*, while also suppressing spoilage-associated clostridia. Recently EU legislation has lowered the maximum permitted ingoing nitrite levels in foods of animal origin to reduce consumer exposure to potentially harmful nitrosamines. For shelf-stable canned meat products, this raise concerns as to whether microbiological safety and shelf life can be maintained at reduced nitrite concentrations, particularly because product stability depends not only on the microbial contamination of ingredients and thermal processing, but also on intrinsic preservation factors such as salt and ingoing nitrite content.

Historically, Codex Alimentarius recommendations for equivalent safety following changes in recipes or heat treatment were based on the addition of 150 ppm sodium nitrite. Under the revised legislation, the permitted ingoing sodium nitrite level is reduced to 120 ppm for products receiving a thermal process of F0 ≤ 3 (heat treatment equivalent to 121.1°C for 3 min or less), and to 82 ppm for products with F0 > 3. However, the scientific basis for maintaining equivalent safety and shelf life at these lower nitrite levels remains limited.

To address this knowledge gap, we used a luncheon meat model inoculated with spores of *Clostridium sporogenes*, a well-known spoiler of canned meat, but also accepted as surrogate for mesophilic *C. botulinum*. Meat batters were filled into metal cans, thermally processed at F0 = 1 to >3, and incubated at 37°C to perform an accelerated shelf-life test. Sodium nitrite was tested at 0–150 ppm in combination with 2.0–3.6% water phase salt. Clostridial growth was quantified on Reinforced Clostridial Medium, and spoilage was assessed as the time required for 50% of cans in a batch to become blown.

Our results show that reducing sodium nitrite from 150 ppm to 118–120 ppm has only a limited effect on the lag phase prior to spore germination, clostridial growth, and spoilage. Ongoing studies are evaluating whether supplementation with organic acids can enable a further reduction in nitrite levels.

The study will generate new evidence to support the reformulation of canned meat products at reduced nitrite levels without compromising safety or shelf life. This knowledge will help the meat processing industry comply with updated legislation while ensuring microbiological safety and product stability.

SO-P15: A Novel Multiplex qPCR for Species Identification of *Campylobacter* Isolated from Wild Ducks

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The genus *Campylobacter* is the most common cause of bacterial gastroenteritis in humans and represents a serious public health problem. Wild waterfowl, particularly mallards (*Anas platyrhynchos*), are significant environmental reservoirs and potential vectors of *Campylobacter* species, which makes it important in the One Health concept. *Campylobacter* is considered one of the most significant health hazards for consumers of wild duck meat.

Although monitoring of presence and antimicrobial resistance is mandatory in domestic animals, data on *Campylobacter* in hunted birds are scarce. A novel pentaplex qPCR method has recently been developed at our department to identify the most important thermotolerant *Campylobacter* species occurring in humans, birds, and ruminants: *C. jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. fetus*.

The method, validated on both target and non-target reference strains, was used to identify isolates from the digestive tracts of wild ducks hunted in the Czech Republic and was compared with the results of a previously used multiplex PCR (Denis et al., 1999), which allows simultaneous detection of *C. jejuni* and *C. coli*.

Analysis of the 2015 baseline data revealed a clear dominance of *C. jejuni* (147 out of 154 isolates, 95.5%) over *C. coli* (2 out of 154 isolates, 1.3%) in wild ducks. Five samples (3.2%) harboured mixed cultures. The results of the novel qPCR method showed a high level of concordance with the multiplex PCR (99%).

The study will continue with a detailed analysis of prevalence across different hunting grounds and the antimicrobial resistance of the isolates. The 2026 sampling results from the same hunting grounds will provide insight into changes in prevalence, resistance patterns, and genomic lineages over the past decade.

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SO-P16: A Rapid, Highly Sensitive and Specific Detection of *Clostridium perfringens* in Meat Samples Using RPA-CRISPR/Cas12a System

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Clostridium perfringens is an important foodborne pathogen responsible for a range of enteric diseases in both humans and animals. The presence of antimicrobial resistance genes further highlights its public health significance. Current approaches for detecting *C. perfringens*, such as culture-, biochemical-, and nucleic acid-based techniques, are often time-consuming, complex, and lack high sensitivity. Hence, rapid and sensitive detection methods are needed. Here, we demonstrate development of a highly sensitive and specific detection platform integrating recombinase polymerase amplification (RPA) with the CRISPR/Cas12a system for rapid identification of *C. perfringens*.

The RPA method was developed and optimised to target a conserved region of a gene in *C. perfringens*. The RPA products were subsequently used to develop the CRISPR/Cas12a system. Various parameters including CRISPR RNA (crRNA), fluorescence probe concentrations were further optimised for the CRISPR/Cas12a reaction. The analytical sensitivity and specificity of the developed RPA-CRISPR/Cas12a assay were also estimated against different foodborne pathogens. Varying numbers of *C. perfringens* cells were spiked into different meat (beef, chicken, pork and lamb), which were then used to validate the optimised assay. To increase the sensitivity of the assay, *C. perfringens* spiked samples were enriched for 4 h and 8 h. The results of the developed RPA-CRISPR/Cas12a assay were compared to those obtained using a previously established PCR method.

The newly developed RPA-CRISPR/Cas12a method showed no cross-reactivity against 52 bacterial species commonly found in food, indicating high analytical specificity for *C. perfringens*. The RPA-CRISPR/Cas12a assay detected 1 fg (equivalent to 0.3 genome copies) of *C. perfringens* genomic DNA, demonstrating higher sensitivity than the RPA alone (10 fg) and the PCR (10 pg) assays. Similarly, the detection limit of the RPA-CRISPR/Cas12a assay for *C. perfringens* cells spiked into different meat samples ranged from 1 to 10 CFU/g using purified DNA, which was higher than that obtained by the PCR method (10^3 to 10^4 CFU/g). Furthermore, results from the spiked-in samples showed that the RPA-CRISPR/Cas12a assay could detect as low as 1 CFU/g of *C. perfringens* in beef, chicken, and lamb meat samples after 4 h of enrichment using purified DNA as template, whereas it was unable to be detected by the PCR method.

Overall, the results suggest that the newly developed RPA-CRISPR/Cas12a assay is highly sensitive and specific for detecting *C. perfringens* and could potentially be used in the food industry for rapid detection.

SO-P17: Relationships Between Heat Resistance, Colony Formation, and Germination in (*Para)Geobacillus* Spores

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Bacterial spores of the genus (*Para*)*Geobacillus* are highly heat resistant and can survive commercial sterilization processes. The correct optimization of thermal processing remains challenging due to inaccuracies in plate count–based quantification methods, which tend to underestimate spore levels. This underestimation may result from limitations in germination and/or subsequent outgrowth. This study aimed to assess the relationship between heat inactivation kinetics and colony recovery efficiency (i.e., the proportion of microscopically determined spores able to form a colony in a reference rich growth medium) in several strains of *Geobacillus stearothermophilus*, *Geobacillus thermodenitrificans*, *Parageobacillus thermoglucosidasius*, and *Parageobacillus toebii*, as both high heat resistance and low recovery efficiency are characteristic features of this genus. Additionally, the correlation between colony recovery efficiency and the extent of germination in the same liquid recovery medium was evaluated (i.e., the proportion of microscopically determined spores able to transform into germinated cells with loss of refringence).

Heat resistance, expressed as DT values at different temperatures, showed high variability across species and strains, with *G. stearothermophilus* consistently exhibiting the greatest resistance. Within species, strain-dependent differences in heat resistance were primarily associated with shoulder length. Colony-forming efficiency varied widely among strains, independently of species, ranging from 2.5% to 89.8%, and was generally low compared to values typically reported for mesophilic spores (~95%). No correlation was found between either DT or shoulder length values and colony recovery efficiency. Germination assays indicated that most strains germinated efficiently (> 90%), suggesting that limitations in colony formation are more likely linked to outgrowth rather than early germination events. These findings highlight the need to improve methods for enumerating thermophilic (*Para*)*Geobacillus* spores in foods without requiring outgrowth.

SO-P18: Prevalence, Genetic Diversity, and Antimicrobial Resistance of *Listeria monocytogenes* in Dairy Products in the Western Cape, South Africa

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Listeria species are ubiquitous in nature and food processing environments. *Listeria monocytogenes* is a foodborne pathogen and the causative agent of human listeriosis, with dairy products recognised as a major source of outbreaks worldwide. The use of antibiotics in dairy cattle farming, e.g., tetracycline (readily available over the counter in South Africa), may contribute to the carryover of antimicrobial resistance in *L. monocytogenes* found in dairy products. Despite these concerns, there is a scarcity of data on *L. monocytogenes* (and other *Listeria* spp.) in dairy products in South Africa. The aim of this study was to investigate the prevalence, genetic diversity, and antimicrobial resistance of *L. monocytogenes* in dairy products from Western Cape, South Africa, whilst observing the prevalence of other *Listeria* species that co-existed in the dairy samples.

Dairy products (*n* =79), including raw milk, pasteurised milk, cream, butter, soft cheeses, and desserts, were obtained from a ready-to-eat (RTE) food factory and an independent testing laboratory from April 2025 to February 2026. The samples were collected on RAPID'L.MonoTM chromogenic media and Brilliance Listeria agar plates. The presumptive positive *Listeria* species (*n* = 56) were isolated using culture-based methods such as plating on RAPID'L.MonoTM chromogenic media and Brain Heart Infusion agar for purification. For confirmation, the presumptive positive *L. monocytogenes* isolates underwent genomic DNA extraction and were screened for the haemolysin (*hly*) gene using polymerase chain reaction. All positive *L. monocytogenes* isolates were screened for phenotypic susceptibility to seven antibiotics with clinical and veterinary significance (meropenem, gentamicin, tetracycline, chloramphenicol, ampicillin, erythromycin, sulfamethoxazole/trimethoprim) using Mueller-Hinton agar + fastidious supplements (horse blood + NAD) (MH-F) for a Kirby-Bauer disc diffusion test. The minimum inhibitory concentration breakpoints were examined using the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Referenced breakpoint values for *Staphylococcus* spp. were used for gentamicin, chloramphenicol, and tetracycline due to the absence of values for *L. monocytogenes*.

Of the 56 isolates, 35 (62.5%) were confirmed positive for *L. monocytogenes* (soft cheeses, butter, cream and desserts). Nine isolates had intermediate resistance to tetracycline. All isolates were susceptible to the remaining six antibiotics. Based on phenotypic observations, five isolates (8.9%) were presumptive positive for *Listeria innocua*, while 16 (28.6%) were presumptive for *Listeria ivanovii* and *Listeria welshimeri*.

The high prevalence of *Listeria* species in these dairy products is concerning, as they are RTE and receive no further processing. Whole genome sequencing of selected isolates is ongoing, with analyses of resistance and virulence genes to follow.

SO-P19: Potential of *Bacillus velezensis* for the Biological Control of *Listeria monocytogenes* in Hydroponic Leafy Green Nutrient Solutions

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Recirculating nutrient solutions pose a critical food safety risk in hydroponic systems as they can serve as reservoirs and transmission pathways for foodborne pathogens such as *Listeria monocytogenes*. This pathogen may disseminate and persist within the system through biofilm formation, transfer to edible plant tissues under conducive environmental conditions, or internalise into plant tissue following root exposure. Biological control has been widely utilised in hydroponic cultivation to manage phytopathogens and offers a sustainable alternative to chemical disinfectants, which may negatively affect plant growth or disrupt beneficial microbiota. *Bacillus velezensis*, a rhizosphere-associated plant growth-promoting bacterium, has emerged as a promising candidate due to its ability to produce antimicrobial compounds, including bacteriocins, with inhibitory activity against foodborne pathogens. It is already used commercially in hydroponic systems for plant disease control, typically applied to achieve concentrations of approximately 10^4–10^6 CFU/mL in nutrient solutions, although these levels may fluctuate under recirculating conditions. Despite its established antimicrobial and plant-beneficial properties, there remains a significant research gap regarding its direct efficacy in controlling *L. monocytogenes* in hydroponic systems. This study aimed to evaluate the effectiveness of *B. velezensis* in directly controlling *L. monocytogenes* in a hydroponic lettuce nutrient solution by evaluating the inhibitory effect under controlled conditions. In this study, 10^4 CFU/mL *B. velezensis* and 10^3 CFU/mL *L. monocytogenes* were inoculated into 100 mL of lettuce nutrient solution (pH 6.02; EC 1.2) and nutrient broth in triplicate and incubated at 25°C with agitation (80 rpm) for 48 h to simulate hydroponic conditions. Samples were collected at 6, 18, 24, and 48 h, serially diluted in peptone buffered saline, plated on the selective media Rapid L'Mono (BioRad) and incubated at 37°C for 24–48 h for enumeration. Treatment with *B. velezensis* resulted in substantial reductions (4–5 log CFU/mL) in *L. monocytogenes* populations (18, 24, and 48h) ($p < 0.001$), with low variability observed among treatment replicates. Meanwhile, *L. monocytogenes* populations in untreated nutrient solution reached approximately 7 log CFU/mL. These findings demonstrate that *B. velezensis* can significantly suppress *L. monocytogenes* in hydroponic nutrient solutions. This study highlights the potential of biological control as part of a food safety strategy in hydroponic systems, while underscoring the need for further research to optimise application strategies and evaluate its effectiveness under commercial conditions. Ongoing work is investigating the efficacy of *B. velezensis* at higher concentrations to further elucidate dose-response relationships and highlight its application for food safety management in hydroponic systems.

SO-P20: Compensatory Evolution in the Epigenetic Network of Yeast

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Epigenetic networks are key regulatory elements in cellular physiology, yet their resilience to genetic perturbation and the spectrum of adaptive responses remain incompletely understood. Here, we systematically disrupted 29 epigenetic regulators in *Saccharomyces cerevisiae* and assessed phenotypic and molecular changes before and after adaptive laboratory evolution (ALE). Growth assays showed that approximately 35% of knockout (KO) strains retained wild-type (WT) growth, indicating substantial mutational robustness. In all selected growth-defect KOs, ALE restored fitness to WT levels, demonstrating strong phenotypic resilience. Multi-omics profiling revealed diverse molecular trajectories underlying this recovery. Some evolved lines displayed large-scale or moderate proteomic and metabolomic reprogramming, whereas others exhibited only subtle changes. Although the molecular changes varied, all evolved lines ultimately converged on the WT phenotype. A detailed case study of a fitness-impaired strain lacking a subunit of the COMPASS complex showed that most evolved lines adapted through metabolic and regulatory rewiring, reflecting non-WT states, while a single line followed a WT restoration pathway. These findings highlight the plasticity of epigenetic networks and reveal multiple, distinct molecular mechanisms that allow cells to overcome regulatory disruption.

SO-P21: Raising the Bar on Food Safety in Pork-Based Meal Kits

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Pork is an important agricultural commodity that is a common protein source prepared from a raw state by consumers. While food safety recommendations exist, consumers often overlook safe cooking practices. Meal-kits are boxes of fresh ingredients (fresh vegetables, raw protein, etc.) with step-by-step instructions for domestic preparation. While meal-kits have the potential to provide nutritious foods and improve cooking skills, the food safety and nutritional implications of pork-containing meal-kit recipes are unknown. The objective of this study was to evaluate the presence/quality of food safety instructions and the nutritional content in pork-containing commercial meal-kits in the US.

Unique recipe cards (*n* = 1,330) were collected from US meal-kit suppliers (*n* = 10, > 90% of market). A Qualtrics-based data extraction tool captured recipe food safety content and nutritional information. Recipes were stratified by raw protein source; pork-containing recipes were analyzed. Food safety practices (hand hygiene, cooking temperatures, cross-contamination prevention) were evaluated using the Partnership for Food Safety Education 'Safe Recipe Style Guide.' Descriptive statistics were calculated and compared to the Dietary Reference Intakes.

Of animal protein-based recipes (*n* = 993), nearly one-fourth contained pork (*n* = 240). Of those, over 75% omitted chilled storage instructions. Most recipes (89%) lacked hand hygiene advice, despite nearly half (*n* = 109) directing consumers to use bare hands during meal preparation. Additionally, 42% instructed consumers to pat the raw pork dry with paper towels prior to preparation without additional instruction to prevent cross-contamination. All cooking adequacy advice within recipe instructions relied on consumer subjective judgment (color, doneness). While nearly half of recipes omitted minimum internal cooking temperatures entirely (44%); of those listing internal temperatures, 72% were within footnotes. Only 18% of recipes listed a food thermometer in the required equipment, yet no recipes provided instruction on their use. More than half of pork cuts were processed (51%); bacon was included most frequently (*n* = 48). Pork-containing recipes provided 140–1,400 kcal/serving (median: 700–735 kcal/serving whole vs. processed pork). Nearly one-fifth of pork-containing recipes (*n* = 44) exceeded 1,500 mg sodium/serving.

While meal-kits are an avenue to increase consumption of high-quality protein, they currently lack adequate food safety instructions. This study found that, while pork comprises a large proportion of animal-based meal-kit recipes, common preparations are processed, and recipes may exceed recommendations for nutrients of concern (i.e. sodium). This study provides specific recommendations to help health educators inform consumers about safe pork-based recipe handling.

SO-P22: Discovery of Polyethylene Terephthalate-Degrading Enzymes in the Gut Microbiome of Black Soldier Fly Larvae

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The increasing accumulation of plastic waste poses serious ecological and health risks. Polyethylene terephthalate (PET) is among the most widely used plastics and contributes significantly to this problem. Addressing this ecological challenge requires innovative and sustainable strategies. A promising approach focuses on insects able to biodegrade plastics thanks to their gut microbiome. Indeed, the black soldier fly (BSF) microbiome has proved to biodegrade polystyrene and polyethylene and is an interesting candidate for the discovery of novel PET-degrading strains and enzymes.

To investigate the potential of the BSF larvae microbiome in PET biodegradation, samples were collected from both the posterior midgut and the hindgut of larvae reared on PET. As controls, larvae reared on standard diet and on agar were used. The effects of PET exposure on the gut microbial community and functional potential were then explored using shotgun metagenome sequencing. The metagenome-assembled genomes from the BSF larval gut samples were analyzed to predict protein-coding genes and screened using statistical model to identify key enzymes associated with plastic degradation, including PETases, MHETases, hydrolases, cutinases, and esterases.

The results showed a clear shift in the gut microbiome following PET exposure. Several potential PET-degrading enzymes were identified, and the plastic-based diet significantly influenced the relative abundance of microbial taxa harboring these genes, thereby shaping the overall functional potential of the bacterial community. *Proteobacteria* was the phylum most notably affected, showing a relative increase in the genera *Providencia* and other members of the *Enterobacteriaceae* family, including *Citrobacter* and *Klebsiella*.

Overall, this study suggests that BSF may serve as bioreactors for the growth of PET-degrading taxa. Our results might drive further isolation of microbial strains and the development of new biotechnological solutions for plastic recycling and bioremediation.

SO-P23: Different Molecular Approaches to Characterise Norovirus in Shellfish

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Noroviruses (NoV), member of the *Caliciviridae* family, are the causative agents of acute viral gastroenteritis and foodborne illness in humans. NoV infection can occur after the ingestion of a low infectious dose (10^1–10^3 viral particles). The most common symptoms are vomiting, diarrhoea and abdominal cramps, with a self-limiting course; however, vulnerable populations may experience severe dehydration or even death. The primary route of transmission is faecal-oral, occurring through the consumption of contaminated foodstuffs and water. The foods most implicated in outbreaks in European countries are shellfish, consumed raw or undercooked.

In this study, two distinct sequencing approaches were evaluated for the molecular characterisation of NoV in shellfish: metabarcoding based on next-generation sequencing (NGS) and the Sanger method. The target for sequencing was the ORF1/ORF2 junction region (RdRp-VP1). During 2023–2024, a total of 179 shellfish samples positive for NoV by real-time PCR (ISO/TS 15216-2:2019) were further analysed using Sanger sequencing. Also, the NGS metabarcoding method was employed on the Illumina MiSeq platform.

A total of 25 samples were successfully typed by Sanger sequencing, whilst 27 samples were typed using the NGS metabarcoding approach. The two methods showed a concordance rate of 61%. Among the sequencing results, GII.17 was the most prevalent genotype (33.3%).

The results suggest that the NGS-based metabarcoding approach is a more suitable method for the characterization of norovirus (NoV) in molluscs. This is due to its high-throughput capability (providing a large number of reads per sample) and its ability to detect the presence of different genotypes within the same sample. Moreover, a single sample analysed by the NGS metabarcoding approach revealed a co-infection that was not detected by the Sanger method.

This study, conducted under sub-optimal conditions (i.e. a complex sample matrix and low viral concentration), demonstrates the potential of the NGS approach in cases of foodborne outbreak investigations. In particular, high throughput molecular characterization can support the correlation between the NoV genotypes identified in food samples with those detected in human specimens.

The NGS approach shows to be highly useful for the molecular characterisation of NoV strains across a wide range of food matrices and water sources typically contaminated with low viral concentration.

SO-P24: Ecological Role of Bacteriophages in Fermented Vegetables: Implications for Microbial Community Dynamics

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Fermented vegetables are generally produced through spontaneous fermentation, which involves the successsive development of various microorganisms naturally present in the raw material. The initial community, mainly composed of *Enterobacteria* and yeasts, is progressively replaced by heterofermentative lactic acid bacteria (LAB), such as *Leuconostoc mesenteroides*, and then by homofermentative LABs, such as *Lactiplantibacillus plantarum*. Bacteriophages, viruses that specifically infect bacteria, may play a critical role in shaping this complex microbial dynamic. Their capacity to modulate and regulate bacterial populations has been extensively documented across a wide range of environments. However, despite this well-established ecological role, their specific involvement in vegetable fermentation processes remains insufficiently characterized and warrants further in-depth investigation. In this work, we investigated the hypothesis that bacteriophages play a significant role in shaping microbial communities during vegetable fermentation.

We first established a comprehensive catalog of viruses present in three different fermented vegetables, namely Sauerkraut, fermented carrots and fermented turnips, by using a combination of shotgun and viral metagenomics. This revealed a high viral diversity and structural differences in the communities depending on the vegetable used as raw material. Subsequently, experimental spontaneous fermentations carried out under controlled conditions enabled the characterization of viral population successions throughout the course of the fermentation process. These analyses revealed a viral dynamic that closely follows that of bacterial communities. Such temporal relationship supported the hypothesis that bacteriophages may exert a regulatory influence on bacterial populations, potentially contributing to the structuring of the microbial ecosystem during fermentation. Finally, a synthetic microbial ecology approach was implemented using a model of fermented carrots inoculated with a simplified microbial community composed of three bacterial strains to assess the effect of one single virulent phage on the community behaviour. Our results demonstrated the active replication of the phage during the fermentation process, together with a significant reduction of the level of the sensitive strain, confirming the regulatory role of viruses in shaping microbial communities of fermented vegetables.

Altogether, this work provided novel insights into the microbial ecology of fermented vegetables and confirmed the ecological importance of phages in microbial dynamics during fermentation. Beyond its fundamental scientific contribution, these findings also open new perspectives for applied research and industrial practices, particularly in the optimization and control of fermentation processes. A better understanding of phage–bacteria interactions could ultimately contribute to improving product consistency, safety, and quality, while supporting the development of more sustainable and efficient fermentation strategies in the agri-food sector.

SO-P25: *Clostridioides difficile* Isolated from Food Products, Food-Producing Animals, and Wildlife in the Western Cape, South Africa

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Clostridioides difficile, an emerging zoonotic pathogen, was previously only limited to hospital environments. However, a rise in community-acquired *C. difficile* infections (CA-CDIs) may suggest that the pathogen could be linked to food-producing animals, wildlife (game and game birds) and associated food products. The purpose of the study was to determine the prevalence and genetic profiling of *C. difficile* associated with food-producing animals, game, and associated food products in South Africa.

Faecal samples were collected from food-producing animals (*n* = 80), game (*n* = 20) and game birds (*n* = 10) across ten different animal production systems in the Western Cape of South Africa. Retail product samples (*n* = 45) included dairy products, pork, beef, chicken, venison, leafy greens, and coleslaw. *C. difficile* was isolated using maximum recovery diluent, a dilution series was prepared and plated onto *C. difficile* moxalactam norfloxacin agar with 5% defibrinated horse blood (CDMN-HB), and incubated at 37°C for 48 h under anaerobic conditions (Whitley A20 Anaerobic Workstation). To account for *C. difficile* spores, samples were placed in an enrichment broth and incubated anaerobically at 37°C for up to seven days, plated onto CDMN-HB and then incubated anaerobically at 37°C for 48 h. Presumptive positive colonies were identified based on morphology and confirmed by PCR targeting the *tpi*-gene. To determine the toxin profiles of selected isolates a multiplex PCR was performed based on a reference protocol targeting *tcdA*, *tcdB*, *cdtA*, and *cdtB* toxin genes.

C. difficile was detected in 37.3% (*n* = 41/110) of animal faecal samples, of which pig (80%), dairy cattle (52.4%) and poultry (36.8%) had the highest prevalence of the pathogen. Furthermore, it was detected in 35.4% (*n* = 17/48) retail food products; chicken (*n* = 4/5), pork (*n* = 2/5), venison (*n* = 3/5), game bird meat (*n* = 3/5), cottage cheese (*n* = 1/5), coleslaw (*n* = 2/5) and leafy greens (*n* = 2/5). The majority of the isolates (83%) (*n* = 39) associated with food-producing animals were toxigenic. Isolates carrying both toxin genes (*tcdA*+ and *tcdB*+) as well as binary toxin genes (*cdtAB*+) were mainly associated with pig (*n* = 14) and dairy cattle (*n* = 3). Further ribotyping and whole-genome sequencing of these isolates are ongoing. This is the first study to isolate *C. difficile* from the agri-food sector in South Africa. Therefore, this study further highlights the presence of *C. difficile* in the agri-food sector with toxin profiles that can cause severe infections and may be associated with hypervirulent strains. This may pose a threat to individuals in close contact with food-producing animals, and may contribute to CA-CDIs.

SO-P26: Determination of Culturable Bacteria in Haemolymph, Hepatopancreas and Gills of the Invasive Species Atlantic Blue Crab (*Callinectes sapidus*) from a Sardinian Coastal Lagoon (Western Mediterranean)

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The ongoing expansion of the invasive Atlantic Blue Crab (*Callinectes sapidus*) in the Western Mediterranean highlights the need to evaluate its potential influence on the Mediterranean fishery sector and its role as possible reservoir and vector of marine pathogens. The present study aimed to determinate the diversity of culturable bacteria in haemolimph, hepatopancreas and gills in blue crabs collected in the S'Ena Arrubia Lagoon (Sardinia, Italy), the first Sardinian brackish environment colonized by this invasive species since 2017. From June to September 2025, 10 blue crabs were sampled using baited traps. For each specimen, morphological measurements were taken and total weights were recorded. Primary isolation of culturable bacteria from haemolimph was performed on Thiosulfate-Citrate-Bile Salts-Sucrose Agar (TCBS) for *Vibrionaceae* and on Marine Agar 2216 for other marine heterotrophic bacteria. Plates were incubated at 22 ± 2°C for 72 h and examined daily for bacterial growth. Dominant pure colonies were selected and then plated onto Columbia Blood Agar (CBA) and identified by MALDI-TOF MS. Hepatopancreas and gills samples were homogenised, decontaminated with 1.5 % hexadecylpyridinium chloride (HPC) and inoculated onto Löwenstein-Jensen and Stonebrink media for the presence of Non-tuberculous mycobacteria (NTM). Cultures were incubated at 28 ± 1°C and 37 ± 1°C and monitored for up to two months. Colonies were screened for acid-fast bacilli using Kinyoun staining, and positive colonies were subcultured for species identification. The average size of the individuals analysed was 15.38 ± 1.59 cm in carapace width (between lateral spines) and 226.45 ± 84.40 g in total weight. Among ovigerous females, the mean egg weight was 7.36 ± 5.83 g. The bacteriological analysis revealed the predominancy of marine heterotrophic bacteria such as *Planococcus* spp., *Psychrobacter maritimus* and *Pseudomonas* spp. in 40% of the samples. The presence of species belonging to the genus *Vibrio* was not pointed out. Culturable colonies of NTM were observed from gill (20%) and hepatopancreas (30%) samples of blue crabs. Molecular analyses are currently ongoing to confirm their identity. This study provides the first evidence of culturable haemolymph, hepatopancreas and gills-associated bacteria of invasive Atlantic Blue Crab populations from the Sardinian coastal lagoons (Western Mediterranean), underscoring their potential influence on crab physiology, ecological influences, and biosecurity risks for the Mediterranean fishery sector.

SO-P27: Multidisciplinary Framework on Moldy Foods in Consumer Households: From Consumer Behaviour to Risk Assessment of Mycotoxins in Moldy Foods

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Food waste occurs worldwide and throughout the entire food chain. Edible food waste amounted to 3.8 million tons in France in 2023, a third being produced by households. While many factors cause food waste, mold contamination is common at the consumer level. Yet, limited knowledge on fungal diversity of moldy foods in households exists. Moreover, molds may produce mycotoxins that migrate into foods thus leading to safety concerns for consumers. We first used a citizen science strategy to collect moldy foods from consumers, identify mycotoxin-producing molds and determine consumer behaviour regarding moldy food handling strategies (discard or consume without removal or with trimming at varying distances from the visible fungal lesion). Over 500 moldy foods were collected among fruits and vegetables, cheeses or other dairy products, jams, bakery products and dry cured meats. Around ~50% of the identified molds are known mycotoxin-producing species. From this data, 6 food-fungal species associations were then selected for mycotoxin migration studies including *Alternaria alternata* – tomato, *Penicillium expansum* – apples and *Penicillium verrucosum* – jam. For each association, the food was contaminated by a mycotoxin(s) producing isolate using point inoculations onto the surface and stored at temperatures to mimic consumer storage conditions (8°C for fridge conditions and 20°C for room temperature conditions). Thallus diameter was monitored and mycotoxin concentrations were quantified depthwise and widthwise when the mold colony reached 1, 2, 3, 4 or 5 cm in diameter. This was based on a worst case scenario and 3D migration strategy we previously developed.

Toxin production was estimated according to mold size and migration into each food was characterized, enabling a spatial migration pattern at different points in the product depending on visible thallus size, distance from the thallus and storage temperature. Coupled with food consumption data from the latest French food consumption survey, we are currently determining the extent of mycotoxin exposure occurring among the French population, which will also be based on multiple behavioural scenarios that we determined using two successive consumer surveys. These surveys involved 350 and 1000 consumers to better understand food storage and handling strategies and we identified multiple at-risk consumer practices.

To our knowledge, this is the first time such a multidisciplinary strategy integrating consumer behavioural and consumption data with fungal growth, mycotoxin production and spatial migration modelling is applied.

SO-P28: Metagenomic Analysis for the Characterization of Yellow Spot Defect in Sliced Cooked Ham

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Yellow spots on sliced cooked ham can indicate chemical changes, like oxidation or bacterial spoilage, with the latter often appearing on vacuum-packed or modified atmosphere products. Yellow spots combined with other indicators of spoilage are generally a reason to discard the product. In this study nine samples of sliced cooked ham (produced in Italy) packaged in a protective atmosphere, both with and without defects, from two different companies were analyzed.

The samples were initially subjected to microbiological analysis to attempt to identify the origin of the defect. At the same time a metagenomic analysis was performed using the molecular technique of next-generation sequencing (NGS), which allows the identification of all species present in a sample by sequencing a specific 16S rRNA hypervariable regions (V2–4–8 and V3–6, 7–9).

A number of bacterial DNA fragments ranging from 6.672 to 121.917, depending on the sample analyzed, were sequenced in the cooked ham. The average length of the sequenced fragments was 250 bp. By evaluating the quality of the Alpha Diversity index, it can be stated that at least 90% of the bacterial populations were described in all samples.

Nineteen genera were identified, mostly Gram-positive. The most represented genus in terms of relative abundance was *Leuconostoc*, followed by the genus *Lactobacillus*. However both genera were present in all samples analyzed regardless of the presence of the defect.

The results were then analyzed at the species level; 26 species belonging to the previously described genera were identified. The most representative microorganism in terms of relative percentage abundance was *Leuconostoc carnosum*, present indiscriminately in all samples except one. Evaluating the results by focusing on the microorganisms present exclusively in all samples with a defect, come into view that the species *Leuconostoc gelidum* was present in all the spoiled samples but absent in those without the defect.

Several studies confirm that *L. gelidum* is able of growing at low temperatures and can produce a pigment that causes yellow coloration in meat products. Its ability to survive in production environments through which it can contaminate the finished product, is also reported. This issue has a significant economic impact, and its resolution can be of great help to the food industry.

SO-P29: ArcMark: A Marker-Based Database for Archaeal Detection in Shotgun Metagenomic Food Samples

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Archaea constitute a relatively underexplored domain of microorganisms, despite their widespread distribution across diverse environments, including marine ecosystems, soils, and host-associated niches. Although their presence has been reported in various food matrices, comprehensive insights into their occurrence and diversity within these contexts remain limited. Shotgun metagenomic sequencing offers the opportunity to characterize entire microbial communities, underscoring the need for robust databases and bioinformatic pipelines specifically designed for the detection and quantification of archaea.

In this study, we developed a targeted approach based on marker sequences derived from 50S ribosomal proteins, obtained from 635 archaeal genomes representing 444 taxa. The ArcMark database and pipeline showed high sensitivity and precision in detecting archaeal sequences across simulated mock communities.

ArcMark was subsequently applied to 639 shotgun metagenomic datasets from several sources, including animal and environmental samples available in GenBank, with a subset of 360 Illumina samples derived from plant- and animal-based foods. Our analysis revealed that 8% of the examined food samples contained archaeal reads. Alpha-diversity analysis showed higher Chao1 and Fisher indices in fermented fish compared with cheese, milk, and olive samples. Notably, halophilic taxa were predominantly detected in salt-rich fermented foods. Genera such as *Haloarcula*, *Halobacterium*, *Halococcus*, *Halorussus*, *Halolamina*, *Halapricum*, and *Halobellus* were identified in olives and fermented fish, highlighting the ecological relevance of halophilic archaea in these environments. Although both fermented fish and olives harboured halophilic taxa, their overall community composition differed significantly (PERMANOVA, p-value < 0.05).

Overall, this work establishes a curated archaeal marker database and a dedicated bioinformatic pipeline for archaeal detection in shotgun metagenomic datasets, providing a foundation for future studies on their distribution and functional roles in food systems.

SO-P30: Culture to Metagenomics: Genetic Diversity of *Listeria monocytogenes* and Its Coexistence with *Pseudomonas* spp. in a Ready-to-Eat Facility

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Listeria monocytogenes persists in food processing environments via biofilm formation. This poses a significant risk to consumers of RTE products, which are ingested without a prior heating step to mitigate microbial contamination. This study investigates the prevalence and diversity of *L. monocytogenes* within an RTE facility and its relationship with the resident microbiota, specifically focusing on coexistence with *Pseudomonas* spp. and broader microbial communities through metagenomic analysis.

Sampling was conducted across three events (September 2025 – February 2026) in a RTE facility located in the Western Cape, South Africa. Environmental swabs were collected from the high-risk produce section, including food-contact and non-contact surfaces (Zones 1–3). Environmental samples (*n* =105) were analysed via traditional culture methods for *L. monocytogenes* (ISO 11290-1:2019) and *Pseudomonas* spp. (ISO 13270:2017). This yielded 42 *L. monocytogenes* isolates on RAPID'L.Mono™ chromogenic media (Bio-Rad) and presumptive *Pseudomonas* spp. on Cephaloridine-Fucidin-Cetrimide (CFC) agar. Of these, *Pseudomonas* (*n* = 22) isolates were selected for identification using the VITEK® 2 Compact system (bioMérieux) for Gram-negative bacteria. This revealed a diverse population, including *P. aeruginosa*, *P. fluorescens*, *P. putida*, and *P. alcaligenes*. Other micro-organisms identified included *Serratia*, *Stenotrophomonas*, *Aeromonas*, *Acinetobacter*, and *Yersinia* species. Molecular confirmation via polymerase chain reaction targeting the *hly* housekeeping gene, successfully identified all *L. monocytogenes* isolates. Antimicrobial susceptibility testing (AST) was determined via the Kirby–Bauer disc diffusion method on Mueller-Hinton (MH) supplemented (MH-F) agar (ISO 20776-3). Minimum inhibitory concentration breakpoints were determined using the European Committee on Antimicrobial Susceptibility Testing guidelines for *L. monocytogenes* with referenced *Staphylococcus* spp. breakpoints applied for gentamicin, chloramphenicol, and tetracycline due to unavailable values for *L. monocytogenes*. Isolates (*n* = 42) revealed resistance to tetracycline (TET; 7%), erythromycin (E; 5%), and ampicillin (AMP; 2%), while intermediate resistance was observed for TET (28%). Ongoing studies will further identify and characterise the Gram-negative isolates via ID and AST (VITEK® 2 Compact system). Furthermore, whole-genome and metagenomic sequencing are in progress to fully elucidate pathogen resistance, virulence mechanisms and microbial community dynamics.

Ultimately, mapping the distribution of these isolates identifies critical contamination reservoirs within the food processing environment, as *L. monocytogenes* was recovered from both food-contact surfaces and equipment utilised in final processing stages. Observed resistance to E, AMP, and TET is significant, as these contaminated surfaces interact directly with products prior to distribution. These findings will inform targeted sanitation interventions to mitigate cross-contamination risks.

SO-P31: Microbiota-Based Prediction of *Campylobacter* Contamination on Broiler Carcasses Using Machine Learning

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Campylobacter remains the leading cause of zoonotic infections in Europe, with poultry identified as the primary source of human exposure. Despite ongoing control measures, contamination levels on broiler carcasses remain high. Emerging evidence suggests that carcass-associated microbiota may influence pathogen presence/survival, yet this relationship is still poorly understood for *Campylobacter*. This study aimed to assess whether *Campylobacter* contamination status on broiler carcasses can be predicted from microbiota profiles at slaughter and after refrigerated storage, using a tree-based machine learning approach.

A total of 314 broiler carcass samples was analysed. Microbiological data consisted of culture-based quantification of *Campylobacter* combined with bacterial community profiling using 16S rRNA gene metabarcoding. Two Random Forest classification models were developed using data collected immediately after slaughter (D0) and after 7 days of refrigerated storage (D7). Samples were classified as "Positive" when *Campylobacter* levels exceeded 1 $\log_{10}$ CFU/mL and "Negative" otherwise.

Both models demonstrated robust predictive performance, with an accuracy of 75.9% at D0 and D7, and AUC values of 0.83 and 0.79, respectively. At D0, key predictive taxa included *Barnesiella viscericola*, *Faecalibacterium* spp., *Bacteroides* spp., and *Ruminococcus* spp., which were positively associated with *Campylobacter* presence. In contrast, *Lactobacillus* spp., *Lactobacillus pontis*, *Psychrobacter* spp., and *Limosilactobacillus* spp. showed a negative association. After refrigerated storage (D7), predictive taxa shifted and included *Pseudomonas*, *Flavobacterium*, *Psychrobacter*, *Lactococcus raffinolactis*, *Shewanella*, *Aeromonas*, and *Acinetobacter*. Among these, *L. raffinolactis*, *Shewanella* spp., and *Pseudomonas* spp. were positively associated with contamination, whereas *Flavobacterium* spp. and *Acinetobacter* spp. were negatively associated.

This study demonstrates the potential of microbiota-informed machine learning models to predict *Campylobacter* contamination on broiler carcasses. Identified bacterial taxa could serve as indirect microbiome-based indicators, opening new perspectives for targeted monitoring strategies and improved risk management in poultry production.

SO-P32: Magnetic Particle-Assisted Bacterial Capture in Cheese Matrices Under Challenging Conditions

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Food processing and storage environments expose microorganisms to stress conditions such as acidic pH, high salt concentrations, and low temperatures, which can alter bacterial cell surface properties and influence capture-based detection strategies. These variations may compromise the efficiency of conventional sample preparation approaches. This study aimed to evaluate the bacterial capture efficiency of chitosan-coated magnetic nanoparticles (CS-MNPs) under short-term individual stress conditions and long-term combined stress, and to assess their applicability in a real food matrix.

Six representative foodborne bacteria with diverse cell envelope characteristics (*Salmonella* Typhimurium ATCC 14028, *Salmonella* Enteritidis ATCC 13076, *Escherichia coli* ATCC 25922, *Listeria innocua* ATCC 33090, *Staphylococcus aureus* ATCC 33591 and *Klebsiella pneumoniae* ATCC 13883) were investigated under pure culture conditions. Bacterial cells were exposed separately to acidic pH (5.5), high salt concentration (5.5% NaCl), and low temperature (10°C) for 3 h, as well as to a long-term combined stress condition (acidic pH, high salt, and low temperature for 5 days). Capture efficiency was determined by comparing bacterial counts before and after magnetic separation.

For real food validation, brined white cheese samples were artificially contaminated with *E. coli* ATCC 25922 and *L. innocua* ATCC 33090 at different inoculation levels. Following CS-MNP-based capture, microbiological enumeration was performed, and capture efficiency was calculated based on the reduction in recoverable bacterial counts.

CS-MNPs demonstrated consistently high and stable capture performance across all tested conditions, with capture efficiencies ranging between 40% and 95% depending on the bacterial strain and stress type. Under short-term individual stress conditions, only minor reductions in bacterial viability were observed (generally below 0.5 $\log_{10}$), indicating mild to moderate stress effects. In contrast, long-term combined stress did not produce consistent reductions in bacterial viability suggested possible adaptive responses. Despite these variations, capture efficiency remained largely unaffected across all conditions. In several cases, stress exposure resulted in increased capture efficiency (up to ~10–15%), likely due to stress-induced modifications in bacterial surface properties.

Validation experiments in brined white cheese confirmed that CS-MNPs effectively captured bacteria across different contamination levels, demonstrating reliable performance in complex food matrices.

These findings demonstrate that CS-MNP-based bacterial capture is robust against environmental stress conditions commonly encountered in food systems. The observed decoupling between bacterial viability and capture efficiency highlights the suitability of this approach as a reliable preconcentration and sample preparation strategy for food safety monitoring in real-world applications.

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SO-P33: Layer-by-Layer Alginate Coatings Enriched with *Lacticaseibacillus* Probiotics for Postharvest Strawberry Preservation

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Strawberries are highly perishable due to their fragile epidermis and soft texture, making them prone to mechanical damage during postharvest handling. They are also susceptible to microbial contamination during ripening, which reduces their shelf-life. The multi-layer coating technique, based on sequential immersion in oppositely charged polyelectrolytes, provides better adhesion than conventional mono-layer active packaging dipping methods. This study evaluates alginate-based probiotic coatings applied via the layer-by-layer technique on the microbial quality, physicochemical properties, and sensory attributes of strawberries during refrigerated storage.

Strawberries (var. Victory) were coated using a layer-by-layer method involving 5 sequential dipping steps, each lasting 2 min. For coatings containing probiotic cultures, the dipping sequence followed the order: 1% calcium chloride ($CaCl_2$)→ 1% sodium alginate (SA)→ 1% $CaCl_2$→ 1% SA supplemented with either *Lacticaseibacillus casei* Shirota (EC-LC) or *Lacticaseibacillus rhamnosus* GG (EC-LR) (ca. 8.0 log CFU/mL)→ 1% $CaCl_2$. Uncoated strawberries (C) and multi-layer coated strawberries with SA but without probiotic cultures (EC) were also studied. Strawberries were stored in macro-perforated PET with cap trays at 4°C for 14 days. Various parameters were monitored i.e., weight loss, visible decay, pH, titratable acidity (TA), total soluble solids (TSS), ripening index (TSS/TA), color (L*, a*, b*), texture (Fmax;N via penetration), mold counts, total viable counts, as well as organic acids and sugars by HPLC-UV/RI. Additionally, sensory evaluation was conducted, and the viability of both probiotic species was confirmed throughout storage.

Visible decay increased in the order C> EC> EC-LR> EC-LC during storage. Weight loss was significantly higher ($p < 0.05$) in controls than in coated samples. TSS accumulation was slower ($p < 0.05$) in probiotic-coated strawberries (EC-LC and EC-LR) than in controls, indicating delayed ripening. This was further supported by the significantly lower ($p < 0.05$) concentrations of sucrose and glucose, particularly in EC-LC samples, compared to controls. Color parameters (a* and b*) showed less variation in probiotic treatments than in controls. Firmness in EC-LC strawberries was 2–3 times higher than in controls from day 5 onward, demonstrating improved texture retention. The presence of probiotic cultures significantly ($p < 0.05$) inhibited fungi by 1.5–2.0 log CFU/g compared to controls, while they remained viable at approximately 6.0 log CFU/g throughout 14-day storage. Sensory evaluation (odor and color) indicated that EC-LC samples remained acceptable up to day 14, compared to day 9 for controls.

Layer-by-layer assembled alginate coatings enriched with *Lacticaseibacillus* probiotics may offer new perspectives for extending the shelf-life of strawberries, while also providing functional properties.

SO-P34: Spectral Insights: Assessing FTIR for High-Resolution Identification and Source Attribution of Foodborne *Campylobacter* spp.

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With over 168.000 confirmed cases in 2024, foodborne campylobacteriosis was the most reported zoonosis within the European Union. It is primarily caused by *Campylobacter jejuni* and *Campylobacter coli*. Yet, *Campylobacter*-associated gastroenteritis has increasingly been associated with also other emerging *Campylobacter* species such as *Campylobacter lari* and *Campylobacter fetus*. Source attribution of cases is known to be difficult, complicating outbreak analysis and leaving a high proportion of cases with an unidentified source. One of the contributing factors to his low success rate on finding the infection-associated *Campylobacter* is the standard method of *Campylobacter* detection that relies on the fully characterisation of, often, only a single sample isolate. In combination with the observation that *Campylobacter* diversity at retail level may consist of more than one subtype, the degree of success in resolving an outbreak is even more hindered.

Fourier Transform Infrared (FT-IR) spectroscopy is an emerging microbial phenotyping method that makes use of the biochemical characteristics of a micro-organism under study. Its beyond (sub)-species level characterisation allows high-resolution strain discrimination. Together with its speedy high-throughput possibilities at a low cost, FT-IR could be a good complementary tool for a first more exhaustive screening of multiple isolates, dereplicating an initial large set of isolates to as subset of unique isolates for subsequent more detailed characterisation via whole genome sequencing. When access to both food and *Campylobacter* case isolates, FT-IR could possibly also allow to provide a first indication whether or not a food and clinical isolate might represent the same strain.

From 2026 on, the National Reference Laboratory (NRL) for *Campylobacter* and for foodborne outbreaks at Sciensano, Belgium, performs – in addition to MALDI-TOF MS – FT-IR analysis on all *Campylobacter* isolates it retrieves in food samples or that it receives (e.g., from official monitoring). This way a reference database is being established composed of the FT-IR profiles of a highly diverse (e.g., longitudinal, geographical, source of isolation) set of *Campylobacter* strains. With this we aim at (*i*) evaluating the accuracy of (sub)species identification (by comparing with MALDI-TOF MS results); (*ii*) assessing the level of discriminatory power for subtyping (based on obtained WGS data); and coupled to this (*iii*) assessing the added value FT-IR can provide for source attribution and foodborne outbreak analysis.

We will present the first results on accuracy of (sub)species level identification and preliminary data on the level of discriminatory power.

SO-P35: The Optimisation of Sustainability for Secure Food Systems – SOSFood

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In the present environmental crisis, the food system must evolve to be more productive, inclusive, sustainable, and resilient. SOSFood will utilise data exploitation and AI-driven technologies to create a comprehensive representation of the EU food system and develop customised predictive tools to facilitate informed decision-making among all stakeholders in the food chain. This will be achieved through a multifaceted, multi-actor, and multi-scale approach, supported by its multidisciplinary consortium of experts from the private and public sectors, academic research, and representatives of the food system, including consumers, producers, and industries. Despite numerous initiatives undertaken in this regard, none have comprehensively addressed the overarching system or sent a dependable and accessible message to the intended audience. Hence, SOSFood will result in a consolidated food data space focused on sustainability and health and decision-making tools adapted to each level of the chain (a predictive dashboard displaying data and predictions for industries, and a mobile app for consumers comprising an eco-healthy fingerprint visualization plot, a greening indicator of industries, reformulated European recipes promoting safe and healthy, local and seasonal ingredients and general recommendations), following these steps: (1) creating a multi-actor network gathering social, political, legal, economic, technological, food, health, environmental and climatic data, promoting transparency and data-sharing, (2) mapping the food system scenario with a multidimensional strategy, exploiting the interoperability of data with advanced impact analysis and innovative AI-technologies, (3) co-designing solutions i.e., decision-making tools fitting the context and priorities of each user, validated through field case studies to ensure viability and representativeness.

Keywords: sustainable safe food system, One Health, data science, AI technologies, machine learning

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SO-P36: Fast Detection of Toxigenic *Bacillus cereus* in Plant-Based Foods Using LAMP Assays

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The increasing consumption of plant-based foods has raised concerns regarding their microbiological safety, particularly in relation to *Bacillus cereus*, a spore-forming bacterium capable of producing toxins linked to foodborne illness. Effective and rapid methods for detecting toxigenic strains are essential to support food safety monitoring and risk assessment.

This study describes the development and validation of Loop-Mediated Isothermal Amplification (LAMP) assays targeting four key virulence genes associated with emetic (*cesA*) and enterotoxigenic (*hblD*, *nheB*, *cytK-1*) activity. The assays were evaluated using both fluorescence-based (qLAMP) and colorimetric detection formats. Specificity and sensitivity were assessed using a diverse panel of bacterial strains and serial dilutions of genomic DNA. The applicability of the method was further investigated in artificially contaminated plant-based matrices and in a set of 72 commercial products.

All assays demonstrated high specificity, with no cross-reactivity observed in non-*Bacillus* strains. The *hblD*, *nheB*, and *cytK-1* assays showed complete agreement with reference methods, whereas the *cesA* assay produced a single false-positive result. High analytical sensitivity was achieved, with detection limits down to very low DNA concentrations and successful amplification in spiked food matrices at contamination levels as low as 10^1 spores/mL.

Screening of commercial plant-based products using the colorimetric approach yielded predominantly clear positive or negative outcomes, while a subset of samples showed intermediate signals. Subsequent qLAMP analysis confirmed that some of these ambiguous results corresponded to low-level gene presence, whereas others were negative. Among the detected targets, *nheB* was the most prevalent, followed by *hblD*, *cytK-1*, and *cesA*.

Overall, the developed LAMP assays provide a rapid, sensitive, and practical tool for detecting *B. cereus* toxin genes in plant-based foods. The colorimetric format is particularly suitable for initial screening, while fluorescence-based confirmation enhances reliability in borderline cases. This combined approach offers a valuable strategy for improving microbiological surveillance in the growing plant-based food sector.

SO-P37: *Enterococcus faecium* as a Surrogate for *Salmonella* in Cocoa Nib Heat Inactivation – Stable, Scalable Preparation for Process Validation in Cocoa and Other Low-Moisture Foods

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Salmonella is a key pathogen of concern in cocoa and chocolate production. Raw cocoa beans may be contaminated at low levels, with approximately 8% of samples testing positive. To ensure safety, processing includes inactivation steps (e.g. roasting, steaming), targeting a ≥ 5-log reduction of the pathogen. For process validation purposes, surrogate organisms such as *Enterococcus faecium* are used. However, spiking in production facilities requires large quantities of biomass. Using a dry powder is practical and mimics the dry state of *Salmonella*. In contrast, agar-based production is labor-intensive, difficult to scale, and wet biomass can alter product moisture, affecting inactivation efficiency. The aim of this study was to produce stable *E. faecium* preparations at scale for industrial use and to evaluate shelf life and heat resistance compared to *Salmonella*.

E. faecium NRRL B-2354, a biosafety class I organism, was cultivated in a fermenter. Biomass was harvested, freeze-dried, and stored in sealed sachets at 4°C. Viability was assessed periodically for up to one year. The heat resistance of this surrogate was tested on dry cocoa nibs with low background levels (total viable counts < 2 log CFU/g). Nibs were first equilibrated to defined moisture contents prior to inoculation, and freeze-dried powders were then applied at a 1% (w/w). Inoculated samples were subjected to heat treatments (2–10 min) at 78°C or 110°C, depending on the nib moisture content, targeting partial inactivation with survivor levels above the detection limit (10^2 CFU/g).

Lyophilized *E. faecium* powders yielded approximately 3×10^{10} CFU/g. Viable counts remained stable over a storage period of one year at 4°C, indicating good shelf-life stability of the preparation. In heat inactivation experiments on cocoa nibs, survival of *E. faecium* was influenced by moisture content, with higher survival observed at lower moisture levels. Overall, the inactivation kinetics of *E. faecium* on cocoa nibs were comparable to reported values for *Salmonella*, and experimental work performed earlier by the authors, supporting the suitability of *E. faecium* as a surrogate for process validation in cocoa processing.

Freeze-dried *E. faecium* exhibited high storage stability and heat resistance comparable to *Salmonella* on cocoa nibs. The dry formulation enables reproducible application for validation of the heat kill step used to sterilize cocoa nibs. The preparation is also applicable to other low-moisture foods where pathogen inactivation and moisture-dependent heat resistance are critical factors.

SO-P38: Genomics-Based Technologies for *Listeria monocytogenes* Detection

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The accurate and reliable detection of foodborne pathogens is a key priority in the food industry to ensure compliance with food safety standards and to safeguard public health. Conventional culture-based methods, such as ISO 11290 for the detection and enumeration of *Listeria monocytogenes*, remain the gold standard, however they are time-consuming and may delay risk management decisions. Molecular-based detection methods targeting genes such as *hlyA* , *lmo1030*, *prfA* and *inlA*, for *L. monocytogenes* offer faster alternatives but they often rely on enrichment steps, and these widely used targets demonstrate high sequence similarity to other *Listeria* species such as *L. ivanovii* and *L. innocua* which can compromise the specificity of the assay. The selection and evaluation of robust genomic targets specific to *L. monocytogenes* is therefore essential to support reliable detection strategies. This study aimed to evaluate candidate genomic regions for the specific detection of *L. monocytogenes*. An *in silico* pipeline was applied to evaluate candidate genes for *L. monocyotogenes* from the 2025 MetaPhlAn database for their suitability as diagnostic targets. These were analysed alongside conventional targets (*hlyA*, *prfA*, *lmo1030* and *inlA*). Species specificity was assessed using BLAST against publicly available NCBI genomes. Gene prevalence across approximately 77,000 *L. monocyotogenes* NCBI genomes was evaluated using ABRicate. Multi sequence alignment was performed to identify conserved regions. Primers were designed for the selected candidate genes using rprimer and they were assessed for predicted specificity using Primer-BLAST and for secondary structure formation using OligoAnalyser. Several candidate gene sequences derived from MetaPhlAn demonstrated high predicted specificity for *L. monocytogenes*, with no off-target hits observed in silico. Primer sets designed for these targets showed complete specificity, with no predicted amplification in non-target organisms. These represent promising targets for molecular assay development and may contribute to more reliable detection strategies for *L. monocytogenes*. Further experimental validation is required to confirm assay performance. In contrast, analysis of the conventional target genes demonstrated high sequence similarity in other *Listeria* species. Primer design for these targets produced variable outcomes for specificity: with predicted off target amplification observed for hlyA and *inlA*, but not for *prfA* and *lmo1030*. These findings suggest that achieving specificity for conventional targets may depend on the careful selection of appropriate regions within the gene.

SO-P40: Resilience Building in Food Safety Management: Influence of the External Food Business Environment and Response Strategies to Microbiological Food Safety Challenges

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Food businesses are operating in a rapidly changing environment. The external business environment is exerting drivers, being Social, Technological, Environmental, Economic and Political (STEEP) developments, thereby challenging food safety management systems (FSMS), their performance and related food safety output. This research investigates how the external business environment affects microbiological food safety performance, focusing on how these influences are perceived and handled by food businesses. In addition, it explores the response strategies that food businesses implement to manage and adapt to these external pressures.

The driver-pressure-state-impact-response (DPSIR) analytical framework was applied to establish the relation of drivers, which are perceived as most relevant to food businesses, the resulting food safety pressures, and how they may induce misalignments in implemented quality control (QC) and assurance (QA) activities. Response strategies were identified to anticipate on, cope with, and learn from external disruptions (food safety shocks). Data were collected through structured questionnaires and group discussions, organised through both moderated online sessions and in-person workshops with stakeholders of the Belgian food processing industry.

Legislation, policies, and governance was identified as most relevant driver for consideration within the FSMS. In addition, climate change was highly ranked on importance to consider and feasibility to respond, when considering food safety shocks. Furthermore, five food safety pressures were identified as cause of undesired change related to the increasing presence or emergence of food safety hazards. Disruptive changes and related food safety shocks were mainly perceived related to the supply of raw materials and stakeholder requirements. Efforts in managing uncertainty start with awareness on and increased understanding of the complex and dynamic nature of these shocks. Therefore, a holistic approach is needed to strengthen resilience in food safety management.

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SO-P41*: Bacillus thuringiensis* on Minimally Processed Fresh Produce at Retail Level

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Bacillus thuringiensis is one of the most widely used microbial biopesticides and is likely to gain further relevance in the agrifood systems transformation. However, as a member of the *Bacillus cereus* group, all *B. thuringiensis* exhibit the genetic basis for enterotoxin production and their occurrence on minimally processed fresh produce raises concerns for potential cases of toxicoinfections. This study aimed to assess the prevalence and genetic diversity of *B. thuringiensis* on retail fresh produce to better understand its potential role in food contamination and risk. A total of 280 fresh produce samples, including leafy greens, vegetables, and fruits, were collected from a retail distribution centre. Samples were analysed by selective cultivation and PCR-based characterization of presumptive *B. cereus* group isolates, including screening for enterotoxin genes, and *B. thuringiensis*-associated insecticidal toxin genes (*cry* genes). Isolates were further characterized using FTIR spectroscopy and whole-genome sequencing for phylogenetic analyses. *B. thuringiensis* was detected in 22% of produce samples. All samples harbored at least one enterotoxin gene. Isolates predominantly showed biopesticide-associated profiles, and 20/22 sequenced isolates showed close genomic relatedness to commercially used biopesticide strains. These findings underscore that residues of biopesticides lead to the widespread occurrence of *B. thuringiensis* on retail fresh produce.

SO-P42: Nutrient Conditions Shape *cytK1* Expression Dynamics and CytK-1 toxin Production in *Bacillus cytotoxicus*

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Foodborne toxicoinfections associated with *Bacillus cytotoxicus* have resulted in severe or even fatal diarrheal illness caused by the CytK 1 toxin. Its thermotolerance may allow it to escape routine screening, increasing the importance of understanding CytK 1 regulation. Contamination with the pathogen is almost exclusively associated with dried, starch rich products such as potato-based foods as well as with insect derived foods. Although *B. cytotoxicus* is frequently detected in these products, only comparatively few outbreaks have been reported. However, documented cases include severe and even fatal disease. This discrepancy suggests that environmental conditions and matrix composition may influence CytK 1 production and cytotoxicity. In this study, we investigated environmental factors shaping *cytK1* expression by comparing nutrient conditions in CGY and minimal media with different carbon sources. Eight strains of *B. cytotoxicus* were compared, including the reference strain NVH 391 98. Gene expression was measured using qPCR, and CytK 1 production and activity were assessed via ELISA and Vero and CaCo-2 cell cytotoxicity assays at 0, 1, and 5 h. QPCR results indicate strain specific and condition dependent differences in *cytK1* expression. Several strains peaked after 5 h in CGY supplemented with fructose, whereas others showed higher expression as early as 1 h in CGY without added sugars. These findings suggest that *cytK1* expression in *B. cytotoxicus* is highly variable across strains and growth conditions. We therefore hypothesize that specific food relevant matrix components, such as starch and its breakdown products, modulate CytK 1 production and cytotoxic activity.

SO-P43: Challenges in Determination of the Concentration of Non-Viable Bacteria in Postbiotic Food Supplements

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Postbiotics are preparations containing intentionally inactivated (non-viable) microbial cells and/or cell components, with or without metabolites, that confer health benefits to the host (Salminen et al., 2021). They may modulate the immune system, influence host gene expression, or contribute to the maintenance of intestinal barrier integrity. In contrast to probiotics, which consist of live microorganisms, prebiotics, which serve as substrates for beneficial microbes, and synbiotics, which combine both, postbiotics offer advantages in terms of stability and safety due to the absence of viable cells. Consequently, their use in food and pharmaceutical products is increasing in response to demand for stable, easy-to-handle formulations.

Despite their growing market presence, the quality and compliance of commercial postbiotic products remain insufficiently investigated, particularly with respect to the accuracy of declared microbial composition, raising concerns regarding product efficacy and consumer trust. The aim of this study was to assess the consistency between labeled and actual content of inactivated microorganisms in selected commercial postbiotic products using a combination of analytical approaches: plate counting, quantitative real-time PCR (qPCR), digital PCR (dPCR), and flow cytometry.

Fourteen products available on the market were analysed. Nine declared a single species of bifidobacteria or lactic acid bacteria, three declared *Lactobacillus acidophilus* and *Lacticaseibacillus casei*, and two declared four lactic acid bacteria species. Cultivable bacteria were detected on MRS agar in 6 out of 14 samples (67–567 CFU/g), which is unacceptable for postbiotic products. Based on both dPCR and qPCR targeting different genes, the detected quantities of target bacterial species were lower than the labeled values in most cases (13 out of 14 products). While the detection levels of qPCR analyses were generally higher from those of dPCR, the values remained substantially below the declared concentrations. In contrast, flow cytometry showed better agreement with labeled claims: six products met the declared bacterial content, three were slightly below, and three contained markedly lower amounts than claimed.

The discrepancies observed between flow cytometry and PCR-based methods may result from the effects of microbial inactivation on DNA isolation, DNA integrity and/or amplification efficiency, a hypothesis that warrants further investigation. Overall, this study highlights frequent inconsistencies between manufacturers' claims and the actual composition of commercial postbiotic products, underlining the need for improved analytical methods and clearer regulatory frameworks to ensure product quality and build consumer confidence.

SO-P44: Efficacy of UV and Biocide Treatments on the Inactivation of Spoilage Fungi in the Dairy Industry

Mélanie Cadoret[1], Van Son Ho[1], Philippe Dantigny[1], Emmanuel Coton[1]
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Fungal contamination is a major concern in various food production chains, particularly in the dairy industry, where contamination can occur at different stages. To limit this risk, cleaning and disinfection procedures are routinely implemented. However, some commonly used disinfectants can generate undesirable by-products and may not meet the increasing consumer demand for natural solutions. Although environmentally friendly biocides are available, their efficacy can be limited. Therefore, physical treatments are increasingly considered as complementary approaches. Moreover, current disinfection protocols are typically established on the base of the sensitivity of hydrated conidia, whereas real contamination scenarios more often involve airborne dry conidia.

This study investigated the efficacy of environmentally friendly biocides (ethanol, acetic acid and peracetic acid) and UV irradiation against four major dairy spoilage fungi: *Cladosporium cladosporioides*, *Penicillium biforme*, *Penicillium roqueforti* and *Mucor circinelloides*, as well as *Aspergillus brasiliensis* ATCC 16404 as a reference strain. Both hydrated and dry (i.e. produced at aw 0.95 and mechanically harvested without liquid contact) conidia were exposed to different biocides or UV for various exposure duration. Initial spore concentrations were determined using a Malassez counting chamber and post-treatment viability was assessed by thallus enumeration but also flow cytometry using propidium iodide as a membrane integrity marker, providing a faster alternative. Inactivation was quantified as $\log_{10}(N/N0)$ and modelled using the Weibull equation. For *A. brasiliensis*, dry conidia were more sensitive to alcohol-based biocides and to UV than hydrated conidia, suggesting that combined biocide/UV treatments could be of interest. For the dairy spoilers, both physiological states showed sensitivity to the tested biocides, with no viable conidia remaining after 2 min of treatment, regardless of the biocide used with the exception of *P. roqueforti*, for which dry conidia were more resistant than hydrated conidia remaining viable for up to 20 min with alcohol and up to 3 min with 14% acetic acid. Regarding UV treatment, hydrated conidia were generally more sensitive than dry conidia across all four spoilage fungi. Interestingly, when no viability was observed on Petri dishes, membrane integrity estimated in flow cytometry was not compromised in 100% for all conidia, thus raising question of the accuracy of both methods (either over- or under-estimation). This divergence should be further studied to establish the reality of the treatment efficacy.

SO-P45: Effect of *Salmonella* Secretome on *Campylobacter* Survival

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Campylobacter and *Salmonella* are major causes of zoonoses worldwide, primarily because of their high prevalence in broiler chickens. Previous studies have shown positive interactions between these pathogens, leading EFSA to emphasize the need for control programs targeting their co-colonization. *In vitro* research has demonstrated enhanced survival of *Campylobacter* in co-culture with *Salmonella* under unfavourable conditions, while increased colonization efficiency of chicken ceca is observed *in vivo*.

This work aims to elucidate the role of the *Salmonella* secretome in *Campylobacter jejuni* survival in PBS, biofilms, and on chicken skin, particularly via extracellular vesicles (EVs). Both bacteria release EVs during their life cycles, which play important roles in bacteria-bacteria interactions, biofilm formation and facilitate host persistence. We first observed that *Salmonella* volatile organic compounds (VOCs) give no significant effect on *C. jejuni* survival. However, *Salmonella* supernatant and purified EVs, enhanced survival and even enabled *C. jejuni* multiplication as observed by growth curve analysis during 17 h under aerobic conditions in PBS at 37°C. Moreover, confocal laser microscopy combined with quantitative image analysis indicated that *Salmonella* EVs modified structure of *C. jejuni* biofilm. Finally, enumeration on specific agar showed that *Salmonella* EVs promoted *C. jejuni* survival on chicken skin at 4°C for 72 h. Our results suggest that *Salmonella* EVs enhance *C. jejuni* survival, implying the existence of bacterial interactions that could promote host colonization.

SO-P46: The Synergy of Salt Reduction, Improved Packaging, and Omics Techniques in Enhancing the Biofunctional Dynamic of Kalamata Natural Black Olives (SPO-KAL-OLIVES)

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The scope of the SPO-KAL-OLIVES project includes the integrated development and evaluation of innovative strategies to improve the nutritional, functional, and safety profile of Kalamata natural black olives. The project adopts a multidisciplinary approach combining food microbiology, analytical chemistry, molecular biology, and food engineering to address critical challenges associated with high sodium content, product standardization, shelf-life stability, and sustainability in table olive processing.

Specifically, the project focuses on controlled fermentations using a *Lactiplantibacillus plantarum* starter culture under three brining conditions: (*i*) 7% NaCl (control), (*ii*) 25% NaCl substitution with KCl, and (*iii*) 25% NaCl reduction. Fermentation dynamics will be monitored through physicochemical analyses (pH, acidity, salt content), microbiological enumeration (lactic acid bacteria, yeasts, *Enterobacteriaceae*), sensory assessment, and advanced omics approaches. Metataxonomic analysis (16S rRNA and ITS sequencing) will be employed to elucidate microbial community succession, while high-resolution metabolomics will enable the comprehensive profiling of bioactive compounds, including phenolics, organic acids, and volatiles.

The most effective sodium-reduction strategy will be selected and further evaluated through two innovative packaging approaches. The first involves preservation in virgin olive oil enriched with essential oils and hydroxytyrosol-rich extracts recovered from fermentation brines, promoting both antimicrobial activity and circular economy practices. The second approach involves the application of chitosan-based edible coatings incorporating natural antimicrobials, combined with modified atmosphere packaging. During storage (up to 18 months), product quality, microbial stability, and metabolic evolution will be systematically assessed, together with sensory evaluation by a trained panel.

The safety of the final product will be addressed through challenge tests with *Listeria monocytogenes*, *Salmonella* spp., *Bacillus cereus*, and *Staphylococcus aureus*, followed by predictive modelling and quantitative microbiological risk assessment to estimate pathogen behavior and risk at consumption.

The proposed integrated approach is expected to deliver a reduced-sodium, functionally enhanced table olive product with extended shelf-life and assured safety. Furthermore, the valorization of phenolic-rich fermentation by-products aligns with circular economy principles, contributing to sustainability in table olive processing. Overall, SPO-KAL-OLIVES provides a multidisciplinary framework combining fermentation control, advanced analytics, and innovative preservation technologies to support the development of next-generation fermented foods.

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SO-P47: *Enterococcus faecalis* ST35VF: A Bacteriocin Producer at the Intersection of Benefit and Risk

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The genus *Enterococcus* belongs to the group of lactic acid bacteria and comprises microorganisms that are commensals of the gastrointestinal tract of humans and domestic animals. Despite concerns regarding the pathogenic potential of certain species, *Enterococcus* spp. may also exert beneficial effects on human, animal, and environmental health and are intentionally used in several food and biotechnological products. This study aimed to investigate the probiotic and pathogenic potential of *Enterococcus faecalis* ST35VF, isolated from Bulgarian Feta cheese; its technological capacity for bacteriocin production; the identification of the produced bacteriocin; and the evaluation of cytotoxicity, hemolytic activity, and antioxidant potential. It was identified that the strain produces a metabolite with antimicrobial activity against *Listeria monocytogenes* strains; this metabolite was confirmed as a bacteriocin after treatment with proteinase K. This bacteriocin proved stable to variations in temperature and pH, as well as to the presence of chemical compounds (Tween 20 and 80, NaCl, milk, and SDS). Its activity was specific to *L. monocytogenes* only in solid media. *E. faecalis* ST35VF showed good grow in conditions mimicking an *in vitro* GIT model and can produce diacetyl. Regarding strain safety, predominate production of L-lactic acid was recorded; the virulence genes *efa*, *is16*, *asa*, and *gel* were detected; strain demonstrated resistance to clindamycin, gentamicin, kanamycin, and streptomycin; does not have proteolytic activity, not produce biogenic amines, and not degrade mucin. Mass spectrometry verified that the strain produces two bacteriocins, which were semi-purified, and cytotoxicity against Vero cells was evaluated, yielding an IC_{50} value of 53.71 ± 0.15 µg/mL. Furthermore, no hemolytic activity was observed with purified bacteriocin, with a hemolysis percentage of 3.72% at 100 µg/mL; the DPPH antioxidant activity was 76.49 ± 0.87%.

Although *E. faecium* ST35VF demonstrated antagonistic activity against *L. monocytogenes* strains, with bacteriocins showing an adequate safety profile, the production of this metabolite is very low. This microorganism has demonstrated virulence factors, which limit its use as a probiotic, despite possessing desirable characteristics for this purpose, such as survival in a simulated gastrointestinal system. Therefore, this study highlights concerns regarding the genus *Enterococcus* and demonstrates that bacteriocin production in this context may represent a potential virulence-associated trait.

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SO-P48: NF Validation Study of the RAPID'Campylobacter Method for Enumeration of Campylobacter spp. in Meat, Poultry, and Production Environmental Samples Without Confirmation

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Campylobacter spp. are among the leading causes of foodborne gastroenteritis. C. jejuni, C. coli, and C. lari are most frequently associated with human infections. These bacteria are commonly found in food producing animals, making foods of animal origin, especially poultry, major sources of contamination. Due to the low infectious dose and public health impact, reliable enumeration of Campylobacter in foods and processing environments is essential. Chromogenic media allow direct detection and enumeration through enzyme-based colony differentiation.
Objective: The objective of this study was to evaluate the performance of RAPID'Campylobacter Agar, a media enabling direct enumeration of Campylobacter spp. (C. jejuni, C. coli, C. lari), in food and production environmental samples, according to ISO 16140-2:2016 and ISO 16140-2:2016/Amd 1:2024.
Methods: The candidate method was compared with the reference method ISO 10272-2:2017. Three categories were tested: meat and meat products, poultry and poultry products and production environmental samples
Both naturally and artificially contaminated samples were analyzed. Relative trueness and accuracy profiles were assessed. Agar plate stability after refrigerated storage (2–8 °C) for 3 days was also evaluated.
Results: Across all categories, the bias between the candidate and reference methods ranged from 0.04 to 0.03 log CFU/g, including after 3 days of refrigerated storage. Differences in standard deviation ranged from 0.29 to 0.31 log CFU/g. Accuracy profiles for all matrices remained within the acceptability limits (±0.5 log CFU/g).
Conclusion: RAPID'Campylobacter Agar provides accurate enumeration of Campylobacter spp. in food and production environmental samples. Results are obtained within 40 hr using a single step procedure without confirmation. The method was shown to be equivalent to ISO 10272-2:2017 and is suitable for routine monitoring applications.

PRECONFERENCE

International Life Science Institute Europe (ILSI)

Cereulide: From Bacteria to Toxicity

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From strain-level variability and inherent taxonomic complexity to changing food-production processes, novel ingredients and increasingly sophisticated toxicological studies, cereulide continues to surprise us. Both the toxin itself and its producer, Bacillus cereus sensu stricto—and, more broadly, the B. cereus sensu lato group—continue to challenge our understanding of the properties, contributing factors and conditions that ultimately lead to emetic foodborne intoxication.

In food microbiology, some facts do not age well. Yet some safe harbours remain and must not be forgotten. They need to be revisited, repeated and thought through again as microorganisms, technologies and food systems continue to change. Recent outbreaks and contamination events remind us that cereulide risk is not confined to foods traditionally associated with emetic B. cereus. Unexpected food matrices are emerging, including ingredients produced through modern biotechnological processes. Fermentation-derived oils, proteins and other microbial biomass-derived products that are subsequently incorporated into a wide range of foods can create new ecological and processing contexts in which B. cereus contamination, growth and cereulide formation need to be considered.

Cereulide therefore provides a striking example of the interplay between what we thought we knew, what remains fundamentally true, and what continues to surprise us. Understanding this interplay requires us not only to embrace new knowledge, but also to return repeatedly to the fundamentals of food microbiology—and to rethink them in the context of how we produce and formulate the foods of today and tomorrow.

MicRISK 2030

AI-Assisted Risk Negotiation: The Concept and Opportunities

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Future sustainable food systems must navigate a complex landscape that extends beyond food safety and food security. They must also account for health-related expenses, environmental impacts, biodiversity preservation, and the reduction of food waste. However, traditional taxonomy-based approaches to microbial food safety, as exemplified by the Bacillus cereus group, struggle to address such complexities and uncertainties.

Recent advancements and emerging technologies, such as AI-assisted multi-agent negotiations, offer promising prospects for enhancing risk analysis and management in this context. We propose Risk Negotiation – an AI-assisted interdisciplinary framework to balance trade-offs and arrive at an equilibrium of interests in a participatory setting. This integrated holistic approach could make a crucial contribution to developing sustainable and resilient food systems, devise One Health solutions, and achieve the UN SDGs. This symposium will highlight the current challenges in risk analysis arising from an academic, regulatory, and food industry perspective using the example of the B. cereus group, which comprises a wide variety of strains ranging from highly pathogenic foodborne outbreak strains to strains used as biopesticides, plant growth promoters, and probiotics. We will discuss how Risk Negotiation can be utilized as a strategic decision-making tool to balance agrifood system trade-offs.

Funding: Swiss National Science Foundation

Round Tables

RT 1: Artificial Intelligence and Big Data in Food & Gut Microbiology

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The rapid development of artificial intelligence (AI), machine learning, and large-scale biological datasets is transforming food microbiology and microbiome research. Advanced computational approaches increasingly support the integration and interpretation of complex datasets, pattern recognition, predictive modelling, and risk assessment, creating new opportunities to improve food safety, understand microbiome dynamics, and support evidence-based interventions.

This roundtable brings together experts working at the interface of predictive microbiology, food safety, microbiome sciences, bioinformatics, and AI to discuss the potential and limitations of these emerging approaches. Particular attention will be devoted to the application of AI and machine learning for the analysis of complex microbiome datasets, identification of biologically meaningful patterns, development of predictive models, and support of decision-making in food safety and gut microbiology research.

Beyond the technical aspects, the discussion will address a number of important scientific and societal questions. As AI systems become increasingly capable of generating predictions and recommendations, where should the boundary between automated support and human decision-making lie? Which aspects of food safety assessment and management can appropriately be delegated to algorithms, and which require human supervision, accountability, and ethical judgement?

The panel will explore key challenges of uncertainty assessment, model explainability, robustness and transferability across biological systems, data quality and bias. Examples from food safety and gut microbiome research will provide a basis for discussing how AI can enhance scientific discovery and decision quality without obscuring uncertainty or diminishing human responsibility.

RT2: Predictive Microbiology Snapshots: Turning Microbial Data into Scientific Stories

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The proposed roundtable builds upon the established activities of PreMiSS, a bi-weekly, community-driven forum that facilitates structured scientific exchange on a broad spectrum of topics of predictive microbiology, quantitative risk assessment, and computational food science. Designed primarily for early-stage researchers (ESRs), PreMiSS provides a low-barrier, discussion-oriented environment that prioritises critical dialogue and ideas exchange between peers over one-directional presentation. Beyond simple knowledge exchange, PreMiSS functions as a capacity-building platform, supporting the development of scientific communication skills, critical thinking, and peer interactions. By connecting ESRs from various research institutes and Universities, it serves as a dynamic self-organised networking space for interdisciplinary engagement.

In this context, the proposed roundtable aims to extend the PreMiSS ethos into a structured, interactive format at FoodMicro 2026. By bringing together diverse perspectives to critically examine current challenges and future directions in the field, the session seeks to:

Rethink how scientific knowledge is constructed and communicated: Move beyond results to examine the underlying reasoning, assumptions, uncertainties, and decision points that shape research outcomes, and how these can be best framed/discussed across different disciplines.

Build empathy between complimentary disciplines and perspectives: Facilitate dialogue between experimental microbiology, modelling, data science, and risk assessment to better understand how different approaches can be integrated when addressing complex food safety problems.

Spotlight ESRs: Provide a high-visibility platform for students and young scientists to act as discussion leaders and moderators.

Engage the Audience: Transition from a standard lecture format to a "Science Café" atmosphere that encourages "raising eyebrows", thought-provoking scientific storytelling and lively discussion.

Session outline (90 min)

The session is structured as an interactive roundtable built around the themes of networks and interdisciplinarity. It opens with a short framing by the chairs, who introduce the overarching theme and set the expectations for active audience participation.

Rather than a sequence of standalone presentations, the session is organised around three “Snapshot” contributions by PreMiSS members, each acting as a catalyst for discussion. Following each snapshot, the audience is actively engaged through moderated dialogue, guided questions, and interactive input.

The discussion is designed to be cumulative: insights from each segment are carried forward, enabling cross-comparison between perspectives. The session will conclude with a brief reflection on the main takeaways and an open invitation for early-stage researchers to engage with the PreMiSS community.

RT3: Innovative Fermentations: Balancing Tradition, Safety, and Innovation

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This roundtable will examine innovations and bioprotection in microbial food fermentations from both European and global perspectives. Novel food fermentations largely rely on complex microbial communities whose management, enabled by advances in microbiology, analytics, and biotechnology, is opening new opportunities for the development of sustainable, functional, and tailored fermented foods. The roundtable “Innovative Fermentations: Balancing Tradition, Valorisation, and Innovation” will focus on innovative microbial applications in wine technology and on developing fermentation processes for sustainable alternatives to vulnerable commodities such as cocoa.

An open discussion, moderated by experts in diverse fermentation technologies, will explore innovative fermentation systems employing selected and indigenous starter cultures, non-conventional microorganisms, and microbial consortia, focusing on their impact on product quality, valorisation, sustainability, and sensory properties. Particular attention will be given to balancing innovation with traditional practices, preserving product authenticity, and meeting changing consumer expectations.

The discussion will be followed by a guided tasting showcasing selected fermented products that illustrate the microbial strategies and fermentation approaches explored during the panel.

Satellite Symposium / Organised by QIAGEN GmbH

Applied Food Testing with Molecular Tools – Featuring digital PCR

Jan-Hendrik Rohde[1]
[1]*QIAGEN Wrocław Sp. z o.o., Poland*

Food testing laboratories are under increasing pressure to deliver faster, more sensitive, and more reliable results. This presentation examines how modern molecular technologies, including automated sample preparation, PCR, digital PCR (dPCR) and next-generation sequencing can help overcome research challenges such as complex sample matrices, low-abundance targets and the need for standardized testing.

Special focus will be placed on the QIAcuity Digital PCR system and its application in research on food safety and quality testing. We will explain how dPCR and the QIAcuity work and why it enables reproducible and accurate absolute quantification of up to 12 targets at once without the need for standard curves, even in difficult matrices. Finally, detection of foodborne microorganisms, allergens, GMOs and food authenticity markers will be discussed, and we will highlight how dPCR can improve results for these applications.

Satellite Symposium / Organised by MARITIM

Culture-Based Approaches to Overcome Waterrelated Bottlenecks: Accelerating *Pseudomonas aeruginosa* and *Clostridium perfringens* Analyses

Cruz Ramos Hugo[1]
[1]*Maritim*

Not received.

AUTHOR INDEX