Abstracts

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[O.1] UNLOCKING THE POTENTIAL OF SUNFLOWER MEAL: FERMENTATION WITH BACILLUS SUBTILIS FOR FOOD APPLICATIONS

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Introduction: There is a growing interest in alternative protein sources, as conventional animal-based proteins are not sustainable for feeding the global population. Sunflower meal (SM), a by-product of sunflower oil production, is a protein-rich material with potential applications in food formulations. However, its utilization is limited due to challenges such as its distinct green color, reduced protein digestibility, and the presence of anti-nutritional factors. Fermentation has emerged as a promising approach to enhance the functionality and sensory properties of plant-based proteins. Thus, this study aims to investigate the effect of fermentation by Bacillius subtilis on SM proteins in terms of techno-functional properties and protein profile changes.

Methods: Proteins were extracted from SM via alkaline extraction (pH 9) using an ultra-Turrax homogenizer and followed by fermentation with Bacillus subtilis at 28°C for 48 h. During fermentation, microbial growth, total soluble protein concentration, and pH changes were monitored. Changes in protein profile were visualized using SDS-PAGE. After fermentation, the final product was concentrated using a rotary evaporator and lyophilized. Functional properties, including emulsion stability and activity, as well as water-holding capacity (WHC) and oil-holding capacity (OHC) were assessed.

Results: During the first 24h of the fermentation, the change of color in the medium from green to yellow was very noticeable. The pH of medium tended to decrease (pH 6.71) during fermentation, then increased to 7.51 at the end of fermentation. The soluble protein concentration decreased from 12.7 to 9.6 g/L at 24h and remained stable at 9.71 g/L at 48h. Changes in protein profiles were demonstrated by SDS-PAGE results, where proteins between 29-42 kDa were completely hydrolyzed during fermentation and new protein bands ~51 kDa appeared after 24h. Regarding techno-functional properties, both unfermented and fermented samples showed no WHC, while OHC decreased from 3.1 to 0.73 g/g following fermentation. A slight increase was observed in emulsifying index from 170.3 to 175.3 m2/g while emulsion stability index increased from 110.01 to 138.8 min.

Conclusion: SM is a valuable by-product that supported the growth of B. subtilis without the need for additional carbon sources. Fermentation proved to be an effective approach to modify the distinct green color of SM proteins while inducing structural changes. Future studies should investigate the effect of fermentation on nutritional, sensory, and bioactive properties of SM proteins as well as other techno-functional attributes. SM has significant potential as a sustainable microbial food ingredient and in the valorization of agro-industrial by-products.

[O.2] THE NEUROACTIVE POTENTIAL OF GRANA PADANO AND PARMIGIANO REGGIANO CHEESE MICROBIOME

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Introduction: Grana Padano (GP), Trentingrana (TG), and Parmigiano Reggiano (PR) are among the most widely recognised Italian Protected Designation of Origin (PDO) cheeses. Their long ripening process (≥12 months) is characterised by strong proteolysis. During this time, the cheese microbiome plays a chief role in metabolising amino acids, leading to the formation of flavouring and neuroactive compounds. Thus, the present study was designed as a multiomic approach in order to profile the microbiome, the volatilome, and the proteome of GP, TG and PR cheeses.

Methods: We sampled PDO GP (n=42), TG (n=18), and PR (n=60), and then assessed the microbiome by high-throughput shotgun metagenomic sequencing, the volatile organic compounds (VOCs) by gas chromatography/mass spectrometry (GC/MS), and proteome by liquid chromatography–mass spectrometry/mass spectrometry (LC-MS/MS). Bioinformatic, statistical, and machine learning analysis were performed to investigate the metagenome, volatilome, and metaproteome.

Results: Taxonomic profiles revealed a clustering of PR and TG vs GP, suggesting the existence of two groups of cheeses, as also demonstrated by a supervised machine learning classification model. Functional metagenomic analysis further identified protein–coding genes involved in the biosynthesis of neuroactive molecules, including gamma-aminobutyric acid (GABA), serotonin, catecholamines, acetylcholine, indole-3-propionic acid, and conjugated linoleic acids. The presence of the predicted neurotransmitter peptides was also assessed and quantified. By merging metagenomics, volatilomics, and metaproteomics data, a second supervised machine learning analysis helped to explain those features that were most impactful in predicting the two groups of cheeses.

Conclusions: Our results demonstrated that, although GP and TG belong to the same PDO disciplinary, their microbiome strongly differ. In addition, long–ripened cheeses are potential sources of psychobiotics, underscoring their importance in promoting mental well-being.

Acknowledgments: This work was partially funded by the project HOLOGRAM, funded by the European Union - NextGenerationEU, NRRP Missione 4 “Istruzione e Ricerca” - Componente C2, Investimento 1.1, “Fondo per il Programma Nazionale di Ricerca e Progetti di Rilevante Interesse Nazionale (PRIN)” (P2022X8A9M) and by the European Union - NextGenerationEU, NRRP - Mission 4, Component 2, Investment 1.4 - National Biodiversity Future Center - CN_00000033 (D.M. Prot. 1034 of 17/06/2022). This work also exploited the infrastructure METROFOOD-IT, that received funding from the European Union - NextGenerationEU, PNRR - Mission 4 “Education and Research” Component 2: from research to business, Investment 3.1: Fund for the realisation of an integrated system of research and innovation infrastructures - IR0000033 (D.M. Prot. n.120 del 21/06/2022).

[O.3] HARNESSING FERMENTATION BY BACILLUS AND LACTIC ACID BACTERIA FOR ENHANCED TEXTURE, FLAVOR AND NUTRITIONAL VALUE IN PLANT-BASED MATRICES

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Our work explores the transformative potential of fermentation in elevating the quality of plant-based matrices to match the desirable attributes of traditional dairy and meat products. As the demand for sustainable products without animal welfare issues increases, fermentation emerges as a key process to enhance the organoleptic properties and nutritional content of plant-based analogs. A selection of Bacillus subtilis, lactic acid bacteria strains, and combinations thereof, showed potential for improving the organoleptic and nutritional characteristics of fermented plant bases. In four different legume-derived matrices, fermentation improved texture, degraded undesirable plant carbohydrates, and removed off-flavor compounds, while producing desirable dairy-associated compounds. The degradation of the undesirable beany off-flavor-causing compound hexanal appears to be a universal phenomenon, as every tested strain as well as their combinations exhibited the capability to decrease the hexanal content albeit with varying efficiency. Some LAB strains were found to be capable of producing carotenoids and might hence have the potential for tailoring fermented plant-based matrices for specific applications, such as yellow cheese or red meat analogs.

[O.4] MANNAN IN KOMAGATAELLA PHAFFII-BASED RECOMBINANT MILK PROTEIN PRODUCTION: CHALLENGES AND FUTURE DIRECTIONS IN SEPARATION STRATEGIES

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Introduction: The production of recombinant milk proteins from yeast Komagataella phaffii via precision fermentation is a well-established technology, but making it economically feasible remains challenging for the food industry. To develop more cost-effective downstream processing methods, it is important to first characterise the recombinant proteins and the nature of non-protein components from the host cell in the crude material to understand how to best separate them.

Methods: Crude fractions of recombinant β-lactoglobulin (BLG), recombinant α-casein, and recombinant lactoferrin (LF) secreted from K. phaffii were collected after microfiltration and diafiltration to remove the host and small impurity molecules, respectively. Several characterisation methods were used to determine the overall composition of proteins and non-protein components in the crude materials, as well as more in-depth characterisation of the recombinant protein properties compared to their animal-based counterparts. Three simple separation methods, 1) isoelectric precipitation, 2) dead-end membrane separation, and 3) anion exchange chromatography (AEX), were compared for their efficiency in separating the target proteins from impurities.

Results: Irrespective of the type of milk protein, all crude recombinant fractions contained similar amounts of mannan (52-66% d.b.) as the main impurity after diafiltration, with 75-87% of the carbohydrates in mannan comprised of mannose residues ranging from 2 to 299 kDa. Regardless, the target milk proteins showed a good resemblance to the animal-derived references (~95%) in their secondary protein conformation. Although the recombinant milk proteins showed differences from their animal-derived counterpart with respect to post-translational modifications and glycation, their overall solubility profile was not significantly altered by the structural deviations, and also not by the presence of mannan. Consequently, isoelectric precipitation was a valid option for casein separation, but not for BLG or LF. Achieving effective membrane separation of proteins and mannans was challenging, due to similarities in the molecular weight of the mannans and the target proteins. The various recombinant proteins and mannan were effectively separated by AEX, but the low protein recovery and dry matter yield of only 32-37% and 12-18%, respectively, makes this method less viable for food processing.

Conclusions: The presented results demonstrate that mannans are the main impurity in the recombinant production of different milk proteins in K. phaffii. This makes size-based separation challenging, while charge-based separation is less affected. Future research should focus on further strategies for effectively removing mannan when a separation is desired.

[O.5] LEVERAGING MACHINE LEARNING TO EXPLORE AND IMPROVE BEER FLAVOR

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Introduction: The perception and appreciation of food flavor depends on many interacting chemical compounds and external factors, and therefore proves challenging to understand and predict. As a result, the food industry relies on humans to evaluate flavor. We believe modern machine learning can support or complement humans at this task.

Methods: We combine chemical and sensory analyses of 250 different beers to train machine learning models that predict flavor and consumer appreciation. We measured over 200 chemical properties for each beer, performed quantitative descriptive sensory analysis with a trained tasting panel, and mapped data from over 180,000 consumer reviews to train 10 different machine learning models.

Results & Conclusions: Machine learning models significantly outperform predictions based on conventional statistics and accurately predict food features and consumer appreciation from chemical profiles. Examination of the models revealed specific and unexpected compounds as drivers of beer flavor and appreciation. Adding these compounds to commercial alcoholic and non-alcoholic beers resulted in improved consumer appreciation. These results highlight the value of a machine learning strategy to improve products and gain a deeper understanding of flavor, and reveal new approaches to further improve the flavor profile of foods.

[O.6] RUMEN VIROME TRANSFER CAN TRANSIENTLY REDUCE RUMEN METHANE PRODUCTION

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Abstract

Introduction: Methane produced by ruminants significantly contributes to worldwide human greenhouse gas emissions. Methane is generated by archaeal methanogens during the digestive processes of ruminants. We hypothesized that rumen viruses influence the ruminant microbiota, hence affecting their methane emission potential.

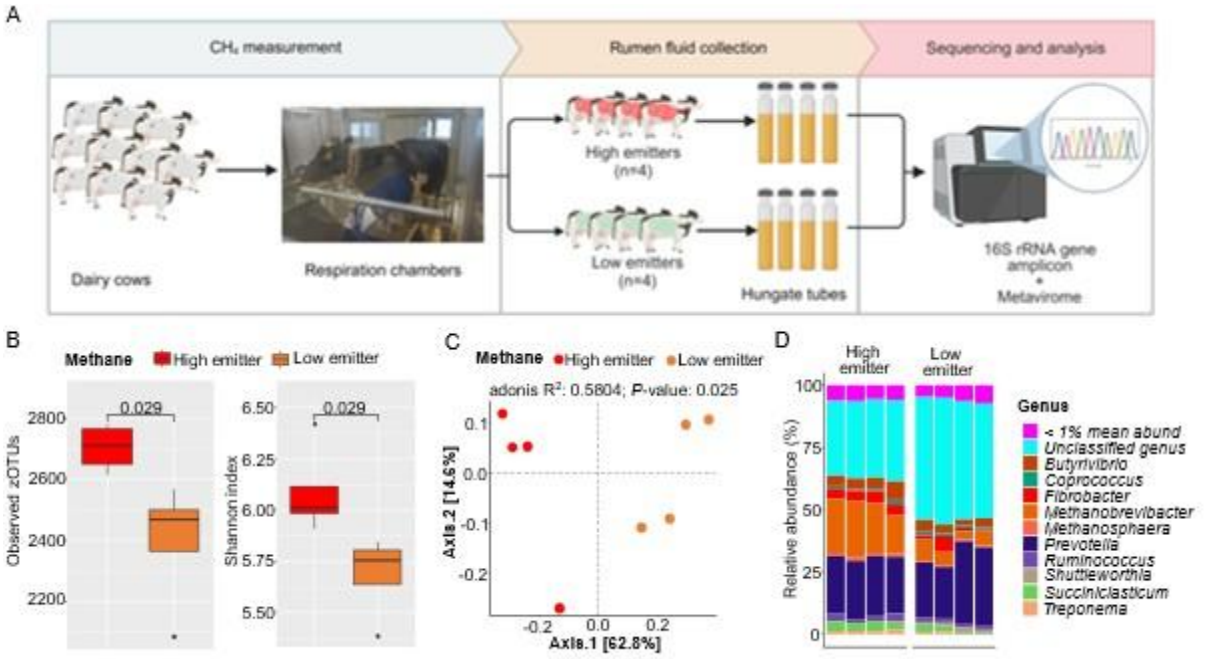
Methods: To evaluate this, we isolated the virome from the rumen fluid of cows with low methane emissions and evaluated its efficacy in mitigating methane production from high methane emitters using in vitro and in vivo virome transfer. Methane production and microbiota were analyzed during the studies.

Results: We uncover different prokaryotic microbiome and virome profiles from pre-identified naturally high and low methane-emitting dairy cows, with methanogens present at three times greater levels in high methane emitters. Low methane emitters have ruminant microbiomes characterized by a greater prevalence of viruses absent in high emitters. The in vitro viruses exhibited a significant decrease in methane production approximately 6 hours following the introduction of the virome combination from low-methane emitters to the rumen fluid of high-methane emitters; however, this reduction was not sustained over an extended period. Unlike the in vitro onservation, in vivo viruses from low methane emitters to high methane emitters can diminish methane emissions compared to the non-viral control group, although not significantly during the initial two days, after which methane production levels return to baseline. The alterations in specific microbe taxa correspond to the treatments, with some experiencing a decline quickly after viral transfer, while others exhibit a spike; nonetheless, they generally seem to partially recover in high emitters. Furthermore, the virome transfer does not directly engage methanogens for methane generation; rather, it influences their bacterial syntrophic associations. Nonetheless, the efficacy differed among rumen fluids, highlighting the unique characteristics of microbial populations.

Conclusions: Our findings indicate that the virome's role in diminishing ruminant methane production is moderate and remains complex, necessitating additional strategies for microbiome engineering to reduce methane emissions in ruminants.

Keywords: Methane production, rumen microbiome, microbiome engineering, virome transfer

Supporting image 1



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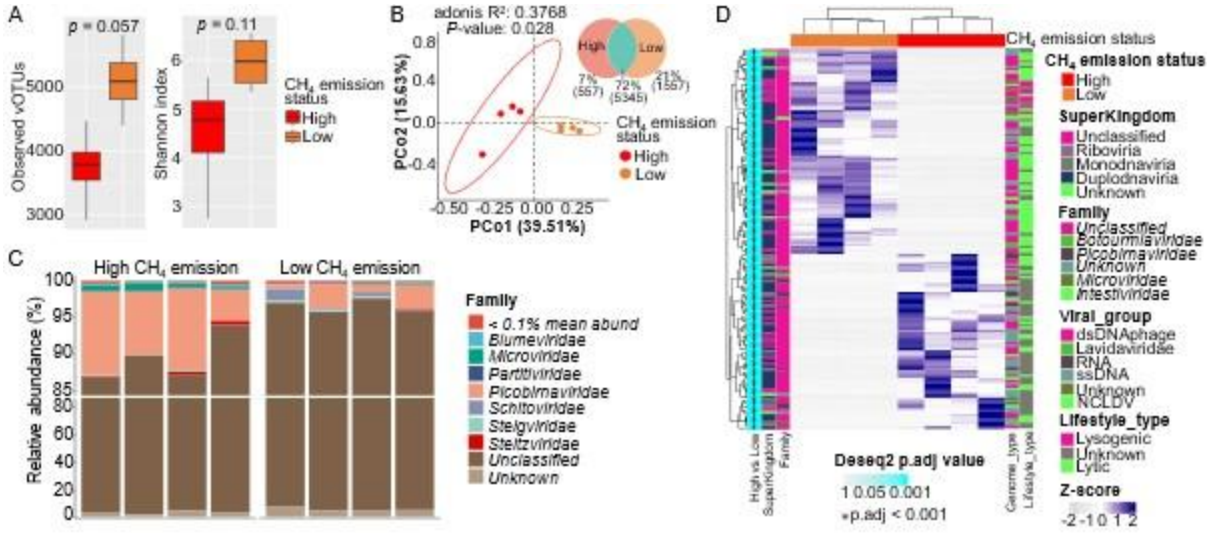
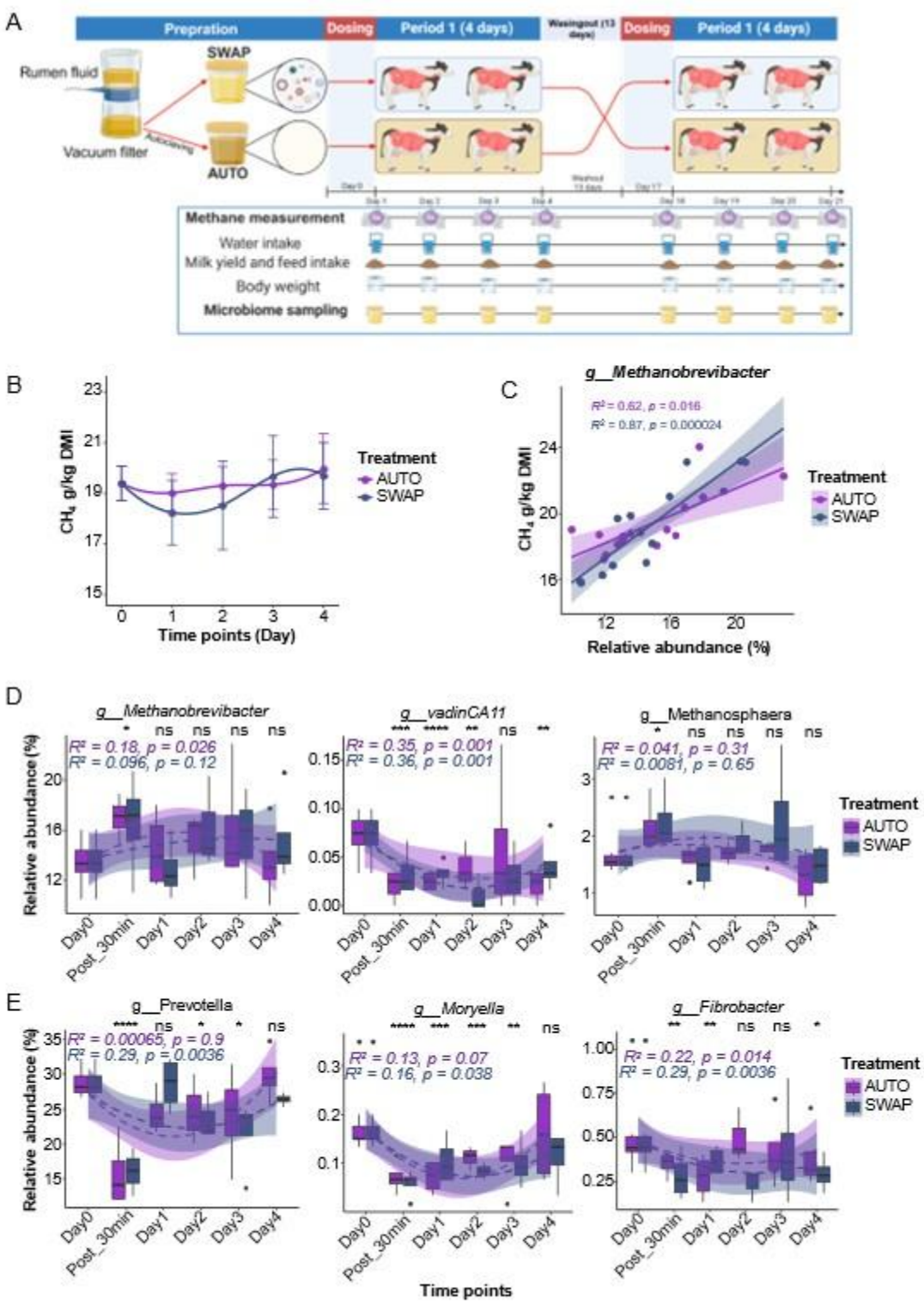


Supporting image 3



[O.7] SAFERMENT– EXPLORING FOOD SAFETY ASPECTS IN PRECISION FERMENTATION PRODUCTION CHAINS FOR DAIRY PROTEINS

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Introduction: Precision fermentation makes use of genetically engineered microbial hosts for the recombinant production of food and feed ingredients that are traditionally derived from animals or plants. In particular, the animal-free production of dairy proteins by precision fermentation has seen many technological developments in recent years, with products approved for marketing in the USA, Israel and Singapore. When developing these novel food products, it is important to identify and address the potential (new) food safety hazards in the precision fermentation production chains, so they can be tackled in an early phase of product development. Such hazards may arise from the use of specific or novel production hosts and recombinant production methods, introduced during the upstream and downstream processing methods or by the use of specific (bio)chemical aids. The SAFERMENT project is a Public Private Partnership that aims to identify and analyse the potential food safety hazards in production chains of dairy protein analogues produced using precision fermentation, from substrate to the end-product and consumer.

Methods: In the SAFERMENT project specific food safety aspects are addressed using different approaches:
(i) Allergenic properties of recombinant dairy proteins are assessed using biochemical methods (IgE-binding immunoblotting) and in vitro assays (basophil degranulation tests);
(ii) Biochemical differences between recombinant and conventional dairy protein ingredients and their implications for allergenicity and food authenticity are analysed using mass spectrometry methods;
(iii) Safety of precision fermentation production chains for dairy protein analogues is assessed via hazard analysis and critical control point (HACCP) analysis followed by risk analysis.

Results: Preliminary outcomes of the SAFERMENT project will be discussed. Initial results for the biochemical and allergenic properties of recombinant dairy proteins (beta-lactoglobulin, caseins, lactoferrin), indicate differences in post-translational modification between the recombinant and conventional dairy protein ingredient. Hazard analysis indicates that most hazards can be prevented through pre-requisite programs, such as hygienic equipment design and Good Microbiology Practices. Critical control points identified in the selected precision fermentation productions chains were mainly related to microbiological hazards and would require monitoring of pH, temperature, pressure and water content at different stages of production. In addition, monitoring the presence of recombinant DNA in the final product is important for marketing in several jurisdictions.

Conclusions: Outcomes generated from the SAFERMENT project will fill knowledge gaps on food safety aspects of recombinant dairy protein production, thereby contributing to the development of safe production chains and successful commercialization of cell-based dairy proteins as food ingredients.

[O.8] EFFECT OF A HIGH PROTEIN DIET VS. GUIDELINE-BASED DIET ON GUT MICROBIOME, METABOLISM, AND IMMUNE RESPONSES IN ENDURANCE ATHLETES

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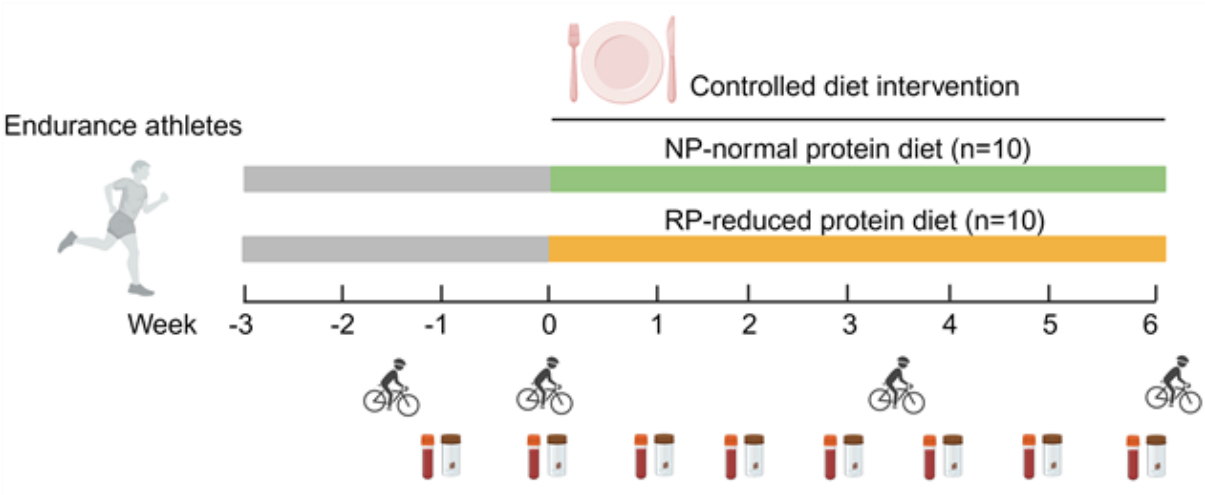
Introduction: Endurance athletes display a high energy turnover and with a diet often high in protein. It remains uncertain if the amount of dietary protein influences the gut microbiome, metabolism, and systemic immune response.

Methods: A six-week dietary intervention study was conducted on 20 well-trained cyclists and triathletes in Denmark. After a three-week run-in period, participants were assigned to either a diet with a protein intake corresponding to their habitual levels (NP, n=10, 2.49±0.31 g/kg/d) or a diet with protein intake aligned with WHO recommendations (RP, n=10, 1.04±0.07 g/kg/d). Throughout the intervention, fecal pH was assessed, and gut microbiome shifts were analyzed through shotgun sequencing of fecal samples. Targeted metabolomics was used to determine short-chain fatty acid (SCFA) and amino acid-derived acid profiles in fecal and plasma samples, and plasma cytokine and hormone levels were measured to evaluate systemic effects.

Results: The fecal pH level increased over time in RP group, although with considerable inter-individual variation and week-to-week fluctuations. The gut microbiome composition was notably altered in weeks 5 and 6, with RP promoting the abundance of species like Clostridium leptum, Parabacteroides distasonis, Roseburia inulinivorans, Roseburia hominis, and Bacteroides ovatus which among others are involved in SCFA production. NP group experienced an increase in Faecalibacterium prausnitzii, a known butyrate producer. Functional profiling revealed an upregulation of key metabolic pathways in RP group compared to NP group, particularly those involved in propionate and butyrate metabolism. Correspondingly, fecal metabolite analysis showed a reduction in succinic acid, caproic acid, and heptanoic acid but increased isocaproic acid concentrations in RP group compared to NP which also showed an increasing trend in isocaproic acid. Plasma metabolite analysis further indicated a diet-dependent shift in systemic metabolites, with RP leading to a reduction in 2-hydroxybutyric acid but an increase in indole-3-propionic acid as opposed to increased propionic acid in NP. This metabolic shift was accompanied by changes in plasma immune responses: RP led to a reduction in plasma concentrations of IL-6 and IL-15, alongside increased leptin concentrations over time, whereas NP resulted in reduced IL-15 and elevated IL-1β concentrations.

Conclusions: These findings suggest that when endurance athletes reduce their protein intake to 1 g/kg/d, it has potential benefits for the systemic immune response. These results provide valuable evidence for the protein needs of endurance athletes and emphasize the important role of microbiome and host interactions in regulating immune parameters in endurance athletes.

Supporting image 1



[O.9] CRICKET-FORTIFIED FOOD: STUDY THE GUT HEALTH IN POST-WEANING PIGLETS

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Introduction: Alternative proteins source plays a major role in shaping the gut microbiome. Since edible insects can be a new source of proteins and unexplored bioactive compounds in the near future, deep investigation in animal models should be conducted. In this study the effects of dietary cricket (Acheta domesticus) meal on growth performance, general health status and gut microbiome was assessed.

Methods: A total of three experimental diets were formulated: standard feed containing soybean meal (Control diet, CD); standard feed with the inclusion of cricket flour as partial replacement of soybean meal (Classic A. domesticus, CAD); commercial feed with the inclusion of bioprocessed cricket meal to improve the nutritional profiles as partial replacement of soybean meal (Hydrolized A. domesticus, HAD). A total of 48 post-weaning piglets were reared for 60 days under controlled microclimatic and light conditions. At the end of the feeding trial, 3 pigs/pen were regularly slaughtered. The intestinal content was collected for microbiome analysis. Shotgun meta-genomics was carried out focusing on microbiome composition.

Results & Conclusions: The genome-based analysis was useful to highlight specific microbial strains. In details, piglet fed with A. domesticus meal showed significant different in the microbiota composition if compared to the standard diet. Lactobacillus amylovorus showed higher frequency in CAD and HAD diet. While pathobionts taxa as Clostridium sp, Collinsella aerofaciens, Turicibacter sanguinis, and Streptococcus hyointestinalis were associated with standard diet. At the same time, also Dorea longicatena, known for its positive impact on gut health, showed an high presence in the standard diet. CAD meal was associated with the abundance of Lactobacillus johnsonii and Prevotella. Phylogenetic analysis of metagenome assembled genomes (MAGs) belonging to Clostridium sp, and Lactobacillus amylovorus showed a strain selection due to the A. domesticus inclusion with potential effect of the health status of the piglets.
A. domesticus used as partial replacement of the main protein sources in the standard diet provided significant variation in the gut microbiome structure of piglets, increasing the presence of potentially beneficial bacteria belonging to the genus Lactobacillus. A further exploration of the microbial function will be assessed.

[O.10] A BACILLUS-BASED DIET AS A PROBIOTIC ALTERNATIVE TO ANTIBIOTIC GROWTH PROMOTERS IN BROILERS IN GHANA.

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Introduction: The overuse of antibiotics as growth promoters in poultry production has contributed to rising antimicrobial resistance (AMR). As alternatives, probiotic-based feed additives have been explored for their potential to mitigate this effect. This study investigated the effect of probiotic-based diets on broilers overall growth performance, feed efficiency and gut microbiota.

Methods: A total of 210 one-day-old broiler chicks were assigned to seven dietary treatment groups (G1–G7) over seven weeks. (G1control - poultry basal diet only), (G2 – with unfermented kapok seeds), (G3 - with commercial probiotic), (G4, G5, G6 - supplemented with 1%, 2%, and 3% Bacillus fermented kapok seeds) and (G7 – with antibiotic-supplemented feed). Growth performance and gut microbiome were analyzed weekly. A three-day pathogen challenge with Klebsiella pneumoniae ST101 was carried out ethically (ECBAS 105/22-23). A survey of 100 commercial poultry farmers in Ghana was conducted to assess their knowledge of AMR, antibiotic usage, and probiotic awareness.

Results: About 75% of farmers lacked knowledge of AMR, 100% reported antibiotic use while 95% were unfamiliar with probiotics. Growth performance showed no significant differences in weekly feed intake, except in Week 6 ($p < 0.05$), where G2 had the highest intake (11,862.67 ± 155.09 g), followed by G4 (11,074.00 ± 1,042.98 g) and G5 (8,508.00 ± 4,059.54 g). G6 had the lowest intake (9,451.33 ± 1,958.28 g). Weight gain differed significantly in Week 5 ($p < 0.05$), with G2 recording the highest (458.33 ± 90.40 g) compared to G7 (51.84 ± 109.20 g). The feed conversion ratio (FCR) was comparable in Week 1 (4.00 ± 0.00) but varied significantly thereafter. G7 exhibited the most efficient feed utilization in Week 2 (FCR: 3.67 ± 0.58), while G2 peaked in inefficiency in Week 4 (FCR: 8.33 ± 0.58, $p < 0.05$). Microbiome analysis revealed a resilient core microbiome dominated by Bacillaceae and Lactobacillaceae families, with probiotic-supplemented groups demonstrating stable microbial compositions even after pathogen challenge.

Conclusion: This study demonstrates the potential of probiotic-based feed additives to enhance poultry growth performance while maintaining gut microbiome stability, offering a sustainable alternative to antibiotic growth promoters. These findings contribute to addressing antimicrobial resistance in poultry farming and highlights the need for greater awareness and adoption of probiotics for improved animal health and productivity.

[O.11] ASSEMBLING MICROBIAL CONSORTIA FOR THE DEVELOPMENT OF NEW PLANT BASED FERMENTED PRODUCTS: THE COCONUT PROJECT

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Introduction: The growing demand for plant-based sustainable foods has primed innovation in fermentation technologies, aiming towards enhanced sensory, nutritional, and safety profiles to alternative products. Recognizing that traditional fermentations harness diverse microbial consortia, this research explores whether microbial communities derived from established food fermentations can be reconstructed and tailored for plant-based substrates.

Methods: A comprehensive collection of over 250 food-origin strains, including Lactic Acid Bacteria (LAB) and coagulase-negative staphylococci from the University of Bologna and University of Parma culture collections (UPCCO), was employed. Genetic diversity was assessed using genotyping techniques, such as Amplified Fragment Length Polymorphism (AFLP). Safety evaluations involved antibiotic susceptibility testing and screening for biogenic amine production, ensuring the selection of strains devoid of harmful traits. Antimicrobial properties were examined using agar overlay and cell-free supernatant assays. Metabolic profiling was conducted with Biolog GENIII MicroPlates, measuring the ability of each strain to utilize 71 carbon sources, thereby highlighting distinct substrate assimilation patterns. Based on these evaluations, selected strains were combined into consortia and their performance assessed on plant-based substrates, specifically nuts and legumes.

Results: Genotypic analysis revealed significant biodiversity among isolates, and the combination with safety screenings identified 28 LAB and 15 staphylococci strains suitable for further development. Metabolic profiling clustered strains into groups with complementary substrate utilization capabilities; for instance, certain isolates efficiently converted sucrose, fructose, and pectin, whereas others were adept at metabolizing maltose, lactose, and raffinose. Notably, LAB strains demonstrated robust antimicrobial activity against common foodborne pathogens, primarily via acidification, with select isolates suggesting the production of non-acidic bioactive compounds. The integration of these data enabled the formulation of microbial consortia that exhibited promising growth and persistence on both nut and legume substrates, laying the groundwork for developing a novel fermented food prototype.

Conclusions: By integrating genetic, safety, and metabolic assessments, this study successfully developed targeted microbial consortia for plant-based fermentation. The approach not only valorizes the microbial biodiversity of traditional fermentations but also paves the way for innovative, sustainable food prototypes with potential industrial applications. These findings contribute to expanding the portfolio of alternative foods and support the transition toward a more sustainable food system. Financed by the European Union - NextGenerationEU through the Italian Ministry of University and Research under PNRR - Mission 4 Component 2, Investment 1.1 " miCrObial COnsortia for New plant-based fermented prodUcTs- COCONUT" (project code MUR P2022SPCRW – CUP: D53D23022040001).

[O.12] ADVANCING LIVESTOCK PRODUCTION THROUGH LONG-READ METAGENOMIC SEQUENCING AND INTEGRATED OMICS APPROACHES

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Introduction: Understanding the microbiome's role in livestock production systems is important for optimizing animal health, performance, and sustainability across terrestrial and marine systems. Recent advancements in long-read metagenomic sequencing, particularly with Oxford Nanopore Technology, have provided unprecedented insights into microbial communities, enabling genome-resolved metagenomics with strain-level resolution, functional potential, and antibiotic resistance gene profiling. Integrating this approach with targeted and untargeted metabolomics or proteomics further elucidates host-microbe and microbe-microbe interactions, offering actionable insights for industry stakeholders.

Methods: We propose a framework leveraging metagenomics and integrated multi-omics analyses to investigate the microbiome in production animal systems.

Results: Using metagenomics, we identify key microbial taxa that influence growth, disease susceptibility, and feed conversion efficiency. Additionally, untargeted metabolomics reveals metabolic pathways linked to microbial activity and host physiology, providing a comprehensive understanding of the microbiome's functional contributions to features of interest within livestock production.

Conclusions: Our framework demonstrates how these technologies can help livestock producers mitigate disease outbreaks, improve animal welfare, and enhance productivity. By transitioning from descriptive to predictive microbiome research, we aim to foster data-driven decision-making in production systems. These advancements showcase the potential of integrated omics strategies to revolutionize animal agriculture by promoting sustainable and efficient practices.

[O.13] EXPLORING THE GUT MICROBIOTA'S IMPACT ON SOW PERFORMANCE: LINKS TO REPRODUCTIVE CYCLE AND KEY FACTORS IN A EUROPEAN SOW STUDY

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The gut microbiota (GM) significantly impacts sow health and performance. Recent developments within machine-learning show that it might be possible to predict future sow health and performance based on GM data. However, factors like geography, diet, and reproductive stage may limit the predictive power of the machine learning based approaches.

The objectives of this study were to determine if an European sow GM can be defined and show associations between the GM and various sow characteristics, including reproductive stage, parity, use of probiotics, and performance. This would indicate that it is possible to predict performance based on GM data from conventional farms.

Fecal samples were collected from over 200 multiparous sows across major swine-producing countries in Europe (Denmark, France, Germany, Netherlands, Spain and UK) on day 109 of gestation and day 21 of lactation representing a total of 16 farms (min. 12 sows per farm). Six farms administered probiotics. Samples were analyzed using 16S rRNA gene long-read amplicon sequencing.

The reproductive stage was more associated with the sow gut microbiota than country, as shown by microbiota composition by comparing robust Aitchison distances followed by PERMANOVA testing. Probiotics and performance (based on weaned pigs per sow per year) also modulated the microbiota, while parity had a smaller, yet still significant impact.

During the reproductive stage, a core microbiota was identified (prevalence above 90%, abundance > 0.5% of total reads), comprising 41 species on day 109 of gestation and 31 species on day 21 of lactation, with an overlap of 20 shared species. Notably, most species unique to each stage exhibited differentially abundant levels between the two sampling periods (26 out of 31 species), while 8 of the shared species also showed differentially abundant levels between the two sampling periods.

In conclusion, the reproductive stage of European sows has a greater impact on GM composition than country or parity, with distinct core microbiota at each stage indicating a European sow microbiota. Furthermore, GM composition is strongly correlated with probiotics and performance.

[O.14] FREEZING AND FREEZE-DRYING STRATEGIES TO PRESERVE MICROBIOMES OF NATURAL WHEY STARTERS

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Introduction: Microbial consortia (MC) play a fundamental role in many fermented foods, and maintaining their composition and performances is crucial for the development of high-quality products. Although the preservation techniques of axenic cultures are now well-established, the storage of MC is still challenging, because of several constraints that may compromise viability and functionality.
In the Project Micro4ever (PRIN-PNRR 2022, n. P2022RJYCN), we investigated the effectiveness of cryopreservation (CrP) and freeze-drying (FD) protocols on the survival, composition and functionalities of MC from natural whey cultures (NWC), to identify the best operative conditions that provide the maximum protection to all microbial fractions of MC.

Methods: Five NWC (from Basilicata and Apulia regions, Italy) underwent to CrP and FD processes, using different protective agents (glycerol or DMSO for CrP; skimmed milk or sucrose solution for FD). After 4 months of storage (-135°C for Crp; -20°C for FD) the survival of MC was evaluated with both plate counting (differential media) and fluorescence microscopy (live/dead and membrane integrity staining), while the composition and relative abundance was evaluated with Amplicon-Targeted High-Throughput Sequencing, using the V5-V6 region of 16S rRNA (prokaryotes) and ITS2 (eukaryotes) as targets. Functionalities of MC was estimated with a meta-phenomic approach by using the EcoPlates® and OmniLog® Phenotype MicroArrays System.

Results: A wide microbial diversity (mesophilic and thermophilic LAB, yeasts) was found among the unprocessed NWCs. The survival of microbial groups depended on the type of preservation technique and protective agent. High viability levels were kept after 4 months of storage, in both CrP and FD samples; overall, thermophilic LAB exhibited a significant robustness to preservation processes, while yeasts were the most sensitive group. The staining protocols (Syto 9/PI; PI/FDA) were effective and rapid in assessing MC viability, and the results were consistent with those obtained by plate counting.
Metataxonomic and meta-phenomic analyses confirmed that microbial composition and functionalities of preserved NWCs were correlated to the storage process and cryo-/lyo-protectants.

Conclusions: The results confirm the effectiveness of the CrP and FD in the mid-term storage of NWC. Further studies are ongoing to evaluate the survival and functionality of MC even after a long-term period and to verify the reuse and the performances of preserved MC in food matrices.

Acknowledgment: Financed by the European Union - NextGenerationEU – Project PRIN 2022 PNRR - Mission 4 Component 2, Investment 1.1; project title “Fermenting metacommunities of food interest: an invaluable biodiversity asset to be maintained overtime in its wholeness and functionality”; Project code P2022RJYCN, CUP: H53D23010720001

[O.15] A NOVEL MICROBIAL CONSORTIUM FOR THE PRODUCTION OF ANTIMICROBIAL FERMENTATES CONTAINING CAPROATE

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Introduction: The history of fermentation dates back to 10,000 years ago and fermentation remains a powerful technology for the precision production of food components. Caproate is naturally presented in goat cheese and is a promising candidate to replace chemical preservatives since it confers antimicrobial activity at pH 4.5 to 6.5, which are common in many food products. This study aimed to develop minimal synthetic microbial consortia for caproate production from glucose and fructose, and to validate the antimicrobial efficacy of fermentates towards food spoilers and pathogens in vitro and in a food system.

Methods: Anaerobic bioreactor runs were conducted with yeast casitone broth that supplemented with glucose and fructose. We designed a bacterial consortium (BC) composed of the heterofermentative Limosilactobacillus reuteri and Clostridium kluyveri, which forms caproate from acetate and ethanol; and a multi-kingdom consortium (MKC) that included the yeast Saccharomyces cerevisiae. Substrate utilization and fermentation metabolites were detected by liquid chromatography, while cell counts were determined by quantitative PCR. Antimicrobial activity of lactate, caproate, and fermentates was carried out using broth dilution assay against foodborne bacteria and yeast. Caproate and/or lactate were added to commercial minced pork meat, before artificial inoculation with/without Salmonella enterica. Microbial profiles were analyzed with the plate count method during the storage at 7℃.

Results: BC produced a total of 63.9 mM caproate after 22 days. When ethanol was added, 28.9 mM of caproate was formed by the BC during a 12-day incubation, while the MKC produced 37.9 mM of caproate without any additional ethanol. We observed the formation of acetate from fructose and mannitol by L. reuteri, and ethanol from glucose by S. cerevisiae, before butyrate and caproate were produced by C. kluyveri. Lactate (106-147 mM) and caproate were the major metabolites in fermentates. Caproate reduced the final cell density to 50 % (MIC50) at concentrations that were 10 times lower than lactate towards S. enterica, Listeria innocua, Staphylococcus aureus, and Candida albican at both pH 4.5 and 6.5. The combination of caproate and lactate decreased the total bacteria count of minced meat more than caproate/lactate alone during the 7-day storage, with no significant change on the pH of the products.

Conclusion: This study demonstrates a novel approach to producing caproate from simple sugars using microbial cross-feeding, and the feasibility of fermentates containing antimicrobial caproate and lactate for food biopreservatives.

[O.16] RIBOSWITCH-MEDIATED IMPROVEMENT OF LYSINE PRODUCTION IN BACILLUS AND PRIESTIA SPECIES

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Introduction: Lysine is an essential amino acid, often present in low amounts in plant-based ingredients. Fermentation with Bacillus and Priestia species has potential to improve the protein quality of plant-based sources through de novo synthesis of lysine. A limiting step of lysine biosynthesis in Bacillus and Priestia species is the expression of aspartokinase lysC, which is under riboswitch control. Binding of the antibacterial lysine analogue aminoethylcysteine (AEC) to the lysC riboswitch represses transcription of lysC gene, which can deplete the bacterial cell from lysine. Through exposure to AEC, we aimed to induce mutations in the lysC riboswitch and limit the riboswitch control of lysine biosynthesis, to develop lysine overproducing strains.

Methods: Eleven strains from six different Bacillus and Priestia species were selected based on their differences in lysC riboswitch structures. Strains were grown on a chemically defined medium including AEC, but not containing lysine. Mutants were selected for whole-genome sequencing based on recurrent growth in AEC-supplemented media. Genomes of the wild-type and mutants strains were compared through analysis of single nucleotide polymorphisms (SNP), insertions and deletions. Selected mutants were cultivated at 30°C on four different defined media: with AEC (no lysine), without lysine, with lysine, and with a higher concentration of threonine. After 22-23 hours of cultivation, free lysine production was quantified using Ultra-high Performance Liquid Chromatography.

Results: A total of 22 AEC-resistant mutants were selected for whole-genome sequencing. Comparative genomics revealed mutations in the lysC riboswitch element for three strains. Two AEC mutants of Bacillus and Priestia were particularly effective in increasing lysine concentration when cultivated on a medium containing lysine, resulting in a higher lysine content compared to their wild-type. One of the AEC mutants was successful in overproducing lysine in all tested media, including a significant increase of lysine in lysine-deprived medium.

Conclusion: Mutations in the lysC riboswitch likely resulted in reduced riboswitch binding affinity to both AEC and lysine, and consequently less repression of lysC transcription even in the presence of lysine. Bacillus and Priestia mutants demonstrated medium-dependent increases in lysine concentration, which holds relevance for fermentation in food matrices with various amino acid profiles. AEC-induced mutagenesis of lysC riboswitches can therefore be an efficient way to improve the lysine production of Bacillus and Priestia species.

[P.1] THE POTENTIAL OF REDUCING ENTERIC METHANE PRODUCTION THROUGH THE ADDITION OF METHANOTROPHIC BACTERIA TO CATTLE FEED

NANNA JENSEN

Introduction: Methane (CH_4) emissions from ruminants contribute significantly to global greenhouse gases, with cows producing methane through microbial fermentation in the rumen. Methanotrophic bacteria, which oxidize methane into less potent compounds, offer a promising strategy to mitigate these emissions. This study explores the feasibility of introducing methanotrophic bacteria into the rumen microbiome to reduce methane release while maintaining rumen health. Due to safety concerns with methane in lab settings, methanol was used as a substitute to cultivate and evaluate bacterial activity. Additionally, a stable dry powder additive containing these bacteria was developed for easy incorporation into cattle diets.

Methods: Methanotrophic bacterial cultures were cultivated in media with varying methanol concentrations to determine optimal conditions for growth and metabolic activity. Bacterial proliferation was monitored through optical density measurements at 600 nm (OD_{600}), while methanol consumption, serving as an indicator of metabolic activity, was quantified using headspace gas chromatography (HS-GC). To develop a stable feed additive, bacterial cultures underwent different drying processes, optimizing key parameters such as temperature and protective agents to minimize log reduction and enhance survival under high-heat exposure. The resulting dry powder was evaluated for long-term stability and viability, ensuring its functional integrity when incorporated into conventional cattle feed.

Results: HS-GC analysis confirmed significant methanol consumption across all concentrations, with optimal conditions identified for supporting bacterial growth and activity. OD_{600} measurements showed a positive correlation between bacterial growth and methanol availability, while excessive concentrations had inhibitory effects, emphasizing the need for controlled substrate levels.
In the drying process, pre-experimental studies established extreme conditions for key parameters, including temperature, flow rate, pump speed, and protective agents, providing critical insights for subsequent Design of Experiments (DoE) optimization. These preliminary findings guided the determination of optimal drying conditions that minimized bacterial log reduction while maintaining viability. Additionally, reducing water activity was explored as a strategy to improve long-term storage stability. These results highlight the potential of methanotrophic bacteria for methane bioconversion and support the feasibility of developing a stable feed additive with minimal loss of bacterial functionality post-drying.

Conclusions: This study highlights the potential of methanotrophic bacteria to utilize methanol, supporting their application in methane reduction strategies for ruminants. Post-drying viability assays and sequencing analyses confirmed bacterial survival, demonstrating the feasibility of developing a stable feed additive. Further research will focus on optimizing bacterial activity for direct methane consumption, assessing their impact in simulated rumen environments, and refining drying parameters to enhance viability and long-term stability.

[P.2] A COMPREHENSIVE APPROACH TO THE CHEMICAL ANALYSIS OF FUNGAL BIOMASS – THE PITFALLS OF NUTRITIONAL STANDARDIZATION

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Introduction: Since current protein production systems are reaching maximum capacity, alternatives like microbial biomass, particularly fungal mycelium, are gaining interest due to their lower environmental impact and favorable amino acid profile. Nevertheless, defining the compositional analysis of fungal biomass, which is required for use as a food ingredient, is not yet standardized. Current chemical analysis of fungal biomass relies on standardized methods for plant biomass or animal protein, often leading to inaccurate and incomplete results. Here, we highlight consequences of nitrogen-based protein quantification methods on the incomplete quantification of protein and fibers. To standardize fungal biomass analysis, this review provides a novel approach circumventing incomplete data, enabling accurate quantification of the main constituents such as protein, lipid, fiber, and RNA.

Methods: Standardized methods used for food products were evaluated for their applicability to fungal biomass. These include established AOAC methodology, often gravimetrically based. We mapped the challenges of these methods when applied to fungal biomass and suggest an approach to arrive at standardized compositions of fungal biomass for food products.

Results: A crucial pitfall of the standardization of fungal biomass analysis is the overestimation of crude protein. The crude protein content is calculated by total nitrogen*6.25. Overestimation occurs due to the presence of RNA and chitin, necessitating amino acid analysis or incorporation of these non-protein nitrogen components in crude protein calculations. Crude protein also affects gravimetric fiber quantification, where the fiber fraction is corrected for residual crude protein, and does not take into account the in this fraction accumulating chitin (N-acetyl-glucosamine polymer) from fungal cell walls. Acid hydrolysis methods followed by monomer analysis generate more accurate fiber contents. Another primary macronutrient, lipid, exists in fungi primarily as phospholipids in membranes, although triglycerides are often present in smaller quantities. Conventional fat extraction methods make use of apolar solvents, majorly excluding phospholipids, hence, other more polar solvents must be considered here.

Conclusion: A novel approach for the complete chemical analysis of fungal biomass is proposed, considering non-protein-nitrogen, the difficulty of fiber quantification, the solubility of lipids/phospholipids, and provides a strategy for elemental insights into possible gaps (Figure 1). Our proposed strategy can be expected to apply not only to fungal biomass but also to microbial or single-cell biomass. Such compositional understanding is essential to further unlock and implement these novel food ingredients, contributing to a more resilient and sustainable food system.

Supporting image 1

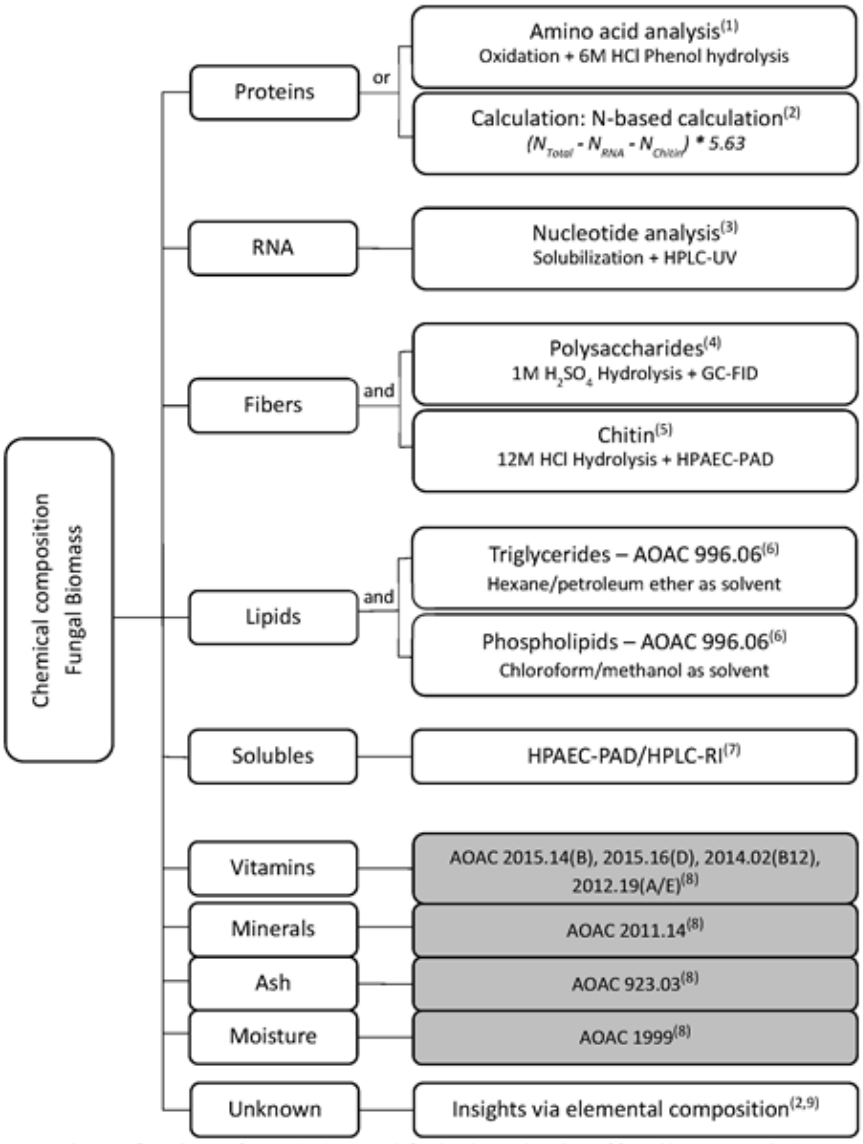

Figure 3 Schematic flow chart – of a standard approach for the chemical analysis of fungal biomass. Methods in grey boxes are according to standard procedures [1]Rutherfurd & Gilani (2009), [2]this paper, [3]Azarani & Hecker (2000), [4]Englyst & Curnmings (1984); Kouzounis et al. (2021), [5]Dolgopyatova et al. (2013), [6]Srigley & Mossoba (2017), [7]Hounsell et al. 2009, [8]Latimer (2023), [9]Kuveke et al. (2022)

[P.3] HOW TO ADDRESS QUESTIONS ON APPLICATIONS TO EFSA

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[1]The European Food Safety Authority (EFSA)

The European Food Safety Authority (EFSA) has published specific administrative and scientific guidance documents for each food domain, aimed at enhancing transparency and understanding of the application procedure and requirements.
If applicants have inquiries for EFSA during the preparation of their applications and prior to submission, they may address their questions through the CONNECT.EFSA portal via two distinct services: General Pre-Submission Advice, which covers questions regarding the content and requirements of an application, and Ask a Question, which addresses queries about procedural steps, the use of IT tools, or the status of an application.
The poster outlines the characteristics of each service in detail, helping EFSA applicants to choose the most suitable option for their needs

[P.4] PHENOTYPIC AND GENOTYPIC CHARACTERISATION OF WATER KEFIR ISOLATES AND GENOME-SCALE MODELLING FOR CONSORTIA PROTOTYPE DEVELOPMENT.

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Introduction: Water Kefir (WK) is a traditional fermented beverage and has been widely consumed across the globe for millennia. While various health claims have been linked to its consumption, there is limited scientific evidence relating to the positive impact of WK and its associated microbiome on human health. Genotypic and phenotypic characterization of water kefir-derived microbes is crucial for understanding their potential health benefits, optimizing fermentation processes, developing probiotic and postbiotic products, and ultimately contributing to the development of a novel plant-based kefir beverage. This research aimed to characterize WK-derived microbes and develop optimized microbial consortia for the fermentation of novel water kefir.

Methods: In this study, thirty bacterial isolates and four yeast isolates were identified by Sanger sequencing and phenotypically characterized using various cultivation-based assays. For genotypic characterization, DNA from the isolates was sequenced using Oxford Nanopore long-read and Illumina short-read technologies, followed by hybrid genome assembly. The genomes were screened for genes related to antimicrobial resistance, pathogenicity, vitamin biosynthesis, and genes relevant to WK fermentation. Although traditional substrates of WK are figs and sucrose water, this study tested fermentation with waste streams as alternative substrates. The metagenomic DNA of the fermentate was extracted and sequenced using Illumina NovaSeq technology. Metagenomics data and annotated genomes of isolates were utilized for genome-scale modeling and consortia development.

Results: Thirty-four isolates were identified including sixteen lactic acid bacteria (LAB), thirteen acetic acid bacteria (AAB), and four yeasts. LAB showed exopolysaccharide production, proteolytic activity, and higher tolerance to NaCl and phenol, while AAB showed higher bile salt tolerance. AMR testing revealed variable resistance patterns between LAB and AAB, and gene screening showed the presence of chromosome-associated bla, ampC, and ArsN1 genes in AAB, indicating intrinsic resistance. Genomics analysis revealed the presence of genes for complex carbohydrate degradation in LAB and vitamin B biosynthesis in AAB. Additionally, GSM successfully developed six consortia for novel WK fermentation with two sustainable substrates.

Conclusion: Overall, this research advances a novel water kefir fermentation via microbial characterisation and consortia prototype development.

[P.5] INNOVATIVE FERMENTATION NUTRIENTS: ENHANCING PROBIOTIC BACTERIA PRODUCTION WITH A BALANCED PROFILE OF RNA AND DNA PRECURSORS.

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Introduction: Probiotic bacteria have gained popularity over the past two decades due to growing scientific data pointing to their positive benefits on human health. Lactobacillus reuteri, Lactobacillus rhamnosus, Bifidobacterium, Bacillus coagulans, Lactobacillus casei, the Lactobacillus acidophilus group, Escherichia coli, and various enterococci, are some of the more prevalent probiotic bacteria. Probiotic lactobacilli are nutritionally fastidious organisms, meaning their growth activity and viability are commonly influenced by growth factors such as medium formulations, pH, temperature, and others. Understanding their nutritional needs is essential for maximizing production and viability of probiotic biomass. Lallemand Bio-Ingredients in collaboration with AS TFTAK have investigated the specific needs of probiotic lactobacilli especially for nucleotides, nucleosides, and nucleobases.

Methods: Three model bacterial strains, Lactobacillus rhamnosus, Pediococcus acidilactici, and Lactobacillus acidophilus, were cultured in 2L bioreactors with an industrial complete media supplemented with three distinct nitrogen sources: yeast peptone rich in nucleotides, yeast peptone containing nucleosides and nucleobases, and standard yeast extract. The consumption of nucleotides, nucleosides, and nucleobases was quantified using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Design of experiments and microbioreactors Biotector XT system were utilized to identify the optimal synergy between a yeast peptone rich in nucleotides and a yeast peptone containing nucleosides and nucleobases.

Results & Conclusions: Most bacteria are able to produce nucleotides de novo, while others, including some lactic acid bacteria require addition of purines and/or pyrimidines to growth medium. These auxotrophic bacteria utilize salvage pathways for conversion of the required nucleobases or nucleosides to nucleotides. Based on this observation, Lallemand bio-ingredients and AS TFTAK studied the consumption of DNA and RNA precursors, highlighting the absence or variable capability to metabolize nucleotides for P. acidilactici, L. rhamnosus, L. acidophilus. A balanced combination of RNA and DNA precursors significantly enhanced the growth of Lactobacillus rhamnosus. The yeast extract containing nucleosides and nucleobases reduced the lag phase duration by 50%, and boosted the total viable cell count by a factor of 6. This finding highlights a promising opportunity to synergistically combine various yeast-based nutrients, through Design of Experiments, to optimize the effects of RNA and DNA precursors.
Lallemand Bio-Ingredient has developed a rational design for next-generation fermentation nutrients, enabling probiotic producers to create optimal culture media. This approach should be extended to more challenging species to produce as Bifidobacterium and microbiome bacteria.

[P.6] 16S RDNA SEQUENCE-BASED BACTERIAL GENOTYPING FROM SELECTED GHANAIAN SPONTANEOUSLY FERMENTED CEREALS, LEGUMES, ROOTS AND TUBERS CROPS

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Introduction: Spontaneous fermentation has been the foundation of traditional food processing, transforming raw food materials into safe, nutritious, and flavourful products. However, the microbial consortium driving these transformative processes remains unresolved, particularly in indigenous African substrates like cereals, legumes, roots, and tubers. This study examined the bacterial communities in these fermentations, to uncover their dynamics and diversity.

Methods: Cereals (orange maize, pearl millet, and sorghum), roots (orange-fleshed sweetpotato), and tubers (cassava) plantain from Ghana were subjected to spontaneous fermentation over 48 hours in Ghana. Samples were collected at 6-hour intervals and air-dried under ambient conditions. Unfermented samples, representing the 0-hour time point, were used as controls. DNA was extracted using culture-independent methods with ZymoBIOMICSTM (Zymo Research Europe GmbH, Freiburg Germany) kits, followed by amplification of bacterial full-length 16S rDNA with barcoded primers. The subsequent preparation of DNA libraries and polymerase-SMRTbell complexes was carried out according to the manufacturer's instructions using SMRTbell prep kit 3.0 and Binding kit 3.1 (Pacific Biosciences Inc., Menlo Park, CA, USA) and then 16S microbiome sequencing on Sequel IIe platform (Pacific Biosciences). Sequenced data were processed using DADA2 and taxonomic classification was performed against the SILVA database v138.1. Analysis was done using MicrobiomeAnalysts 2.0, and alpha diversity and relative abundance metrics were calculated.

Results: Distinct microbial dynamics were observed across the substrates. The fermented cereals (millet, maize, and sorghum) showed increasing bacterial diversity over time, dominated by Weissella confusa, Enterococcus hirae and Pediococcus acidilactici. Soybean fermentation exhibited distinct bacterial communities with fluctuating diversity throughout the fermentation process. The dominant bacterial species closely resembled those observed in the fermented cereals. The starch-rich roots and tubers (sweetpotato and cassava) supported the growth of Leuconostoc mesenteroides, Weissella confusa, Gluconobacter frateurii and Pediococcus pentosaceus. Plantain fermentation displayed its microbial dynamics, with a sharp decline in microbial diversity after 18 h, then followed by a fluctuating trend, influenced by the fruit's biochemical properties. Weissella confusa and Fructobacillus fructosus were the dominant taxa during the spontaneous fermentation of plantain.

Conclusion: This study highlights the microbial diversity and dynamics in spontaneous fermentations, particularly for complementary food ingredients. The findings enhance our understanding of microbial ecosystems, supporting improvements in food safety, consistency, and nutritional quality. Probiotic species such as Weissella confusa, Pediococcus pentosaceus, and Fructobacillus fructosus demonstrate potential as beneficial ingredients for gut health. Integrating traditional fermentation practices with molecular tools promotes innovation while preserving the cultural and nutritional value of complementary food ingredients.

[P.7] HIGH PROTEINOUS BIOMASS FROM RANDOM MUTATED MICROALGAE CHLORELLA VULGARIS FOR FOOD APPLICATIONS

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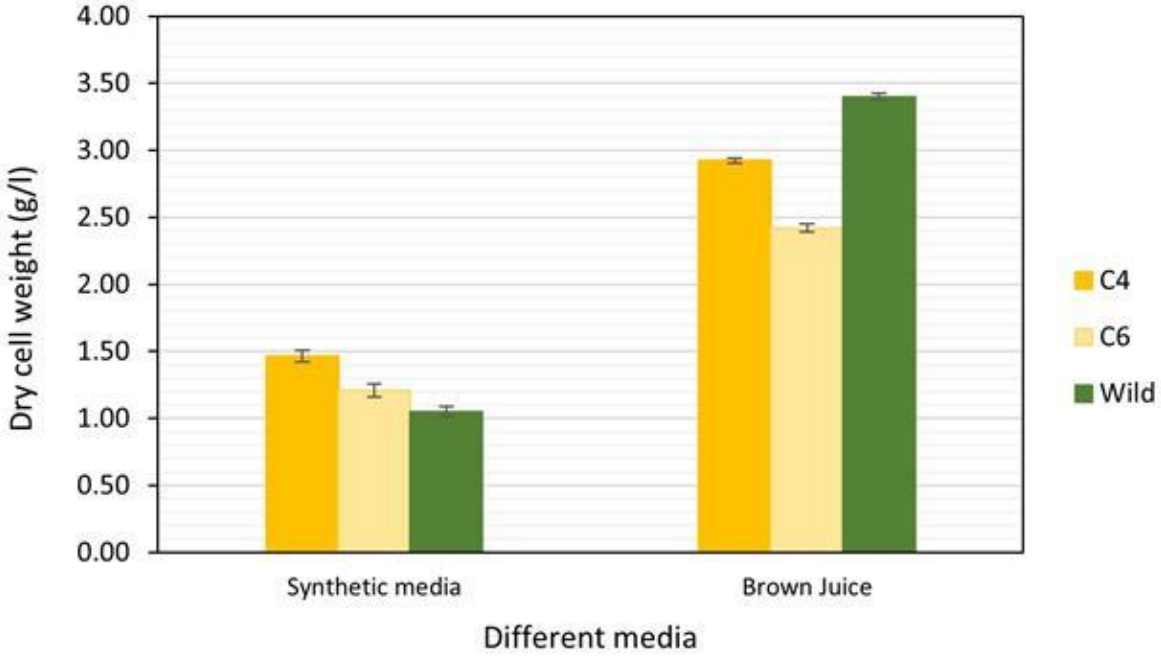
Introduction: As the world moves toward a sustainable and healthy diet, people have started to replace animal protein in their diets with alternative protein sources such as algae, fermented, insect, and plant-based protein. In particular, microalgae have been considered an alternative protein source since they provide protein that is either equal to or of higher quality than many current protein sources (meat, eggs, soybeans, milk, etc.). Furthermore, its biomass is valued for other biocompounds such as polysaccharides, vitamins, minerals, and polyunsaturated fatty acids (PUFAs). Hence, they could become a major alternative food supply for the growing population if the organoleptic features (grassy taste, smell, and green color) of the microalgae biomass improve, to be more acceptable for the final consumers.

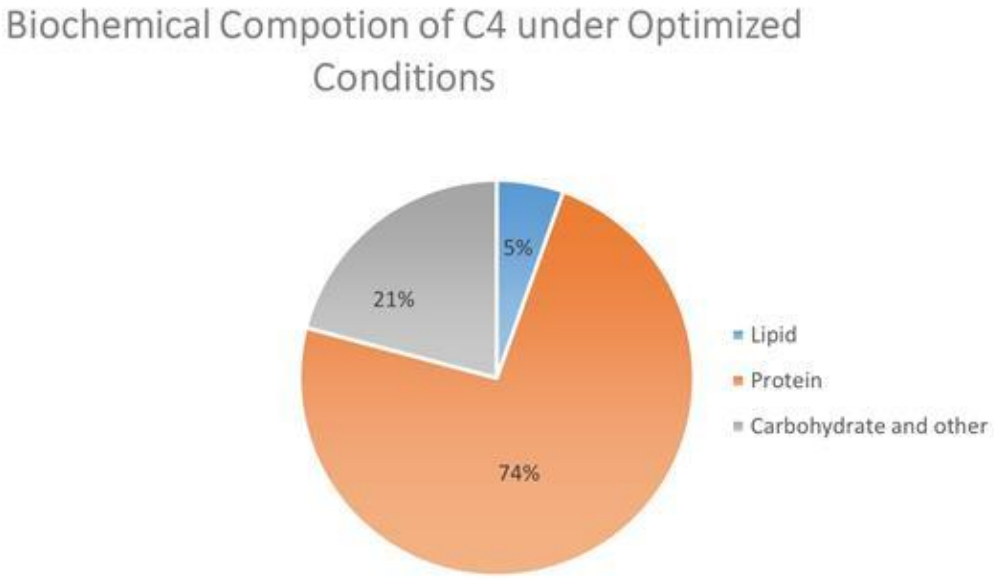
Methods: In this study by using random mutation (UV-irradiation) of Chlorella vulgaris (C. vulgaris) as the target microalgae and produced mutants of C. vulgaris devoid of chlorophyll. Besides, Brown Juice (BJ) as an alternative and cheap substrate was selected for the mutant cultivation. BJ was obtained from the grass protein production process, after heating the green juice and separating the protein concentrate. Then, based on the initial characterization of the BJ, the nutrients e.g., nitrate, phosphate, and glucose concentrations were adjusted and optimized accordingly, for the most robust growth of the strains. The growth profile of wild and mutants was monitored and measured daily using the synergy microplate reader. The total protein content of the mutants (C4 and C6) and wild C. vulgaris was measured at the end of cultivation.

Results & Conclusions: The results demonstrated by the cultivation of mutants in BJ under optimized nutrient concentrations (Nitrate, Phosphate, and Glucose), biomass production increased from 1 to 2.9 g. L-1 for mutants C4. Besides, protein content of C4 increased from 23 % in synthetic medium to 73.6% in the optimized BJ based on dried C4 biomass. Their rapid growth rates (5 days) and high protein yields make them an efficient source of nutrition, potentially surpassing conventional crops and a promising resource for sustainable food. Furthermore, microalgae biomass offers a complete nutritional profile due to its abundance of essential amino acids, vitamins, and minerals. In addition to their nutritional benefits, mutants are useful in wastewater treatment and carbon sequestration through alternative, low-cost side streams. All things considered, using high-protein microalgae biomass is a renewable and sustainable way to satisfy the growing demand for protein across the world.

Supporting image 1



Supporting image 2



[P.8] EXPLORING THE APPLICATION OF YEAST-BASED NUTRIENTS FOR ENHANCING THE PRODUCTION EFFICIENCY OF PROBIOTIC STRAINS

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Probiotic strains are well known for conferring a health benefit after administration. Key challenges of producing probiotic products are the complex nutritional requirements of probiotic strains as well as the requirement for effective delivery of viable cultures in high concentrations. Optimizing the production processes for high yields, productivity and reproducibility is crucial to ensure the functionality and cost-efficiency of probiotic products.

In this study, various yeast-based nutrients have been screened in lab-scale fermentations for their application in production processes of selected probiotic strains.

When looking at the cultivation process, the medium composition is known to highly influence growth and the physiology of the produced strains. Industrial media commonly contain yeast-based nutrients that consist of a mixture of peptides, free amino acids, carbohydrates, vitamins and trace elements and thus provide readily available building blocks for microbial biosynthesis. Selecting the optimum yeast extract is essential to support optimal growth and metabolic activity of the produced strains. Besides their application as medium component, yeast-based nutrients can also serve as ingredients of lyoprotectants during cryopreservation of the probiotic strains.

We present exemplificative cases that demonstrate the importance of optimal yeast extract selection for effective production of probiotic strains.

[P.9] DECIPHERING THE ROLE OF THE GUT MICROBIOME IN INFLAMMATORY BOWEL DISEASE (IBD) DEVELOPMENT IN MONOZYGOTIC TWINS DISCORDANT FOR IBD

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Introduction: Inflammatory Bowel Diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory disorders of the gastrointestinal tract with rising global prevalence. While genetic susceptibility plays a key role in IBD pathogenesis, environmental factors, particularly gut microbiota dysbiosis, are increasingly recognized as critical contributors. However, distinguishing microbial changes as either a cause or consequence of disease remains challenging. To address this, we conducted a comprehensive gut microbiome analysis in 40 monozygotic twin pairs discordant for IBD, leveraging their shared genetics and early-life environment to isolate microbial contributions to disease risk.

Methods: Faecal samples from 40 monozygotic twin pairs discordant for IBD were collected in a mobile laboratory, stored at -80°C within one hour of collection, and preserved until DNA extraction using the DNeasy PowerSoil Pro Kit. 16S rRNA gene amplicon sequencing was performed on the GridION X5 platform, and shotgun metagenomics sequencing was conducted on the Illumina NovaSeq 6000 platform. Statistical analyses were performed using R with packages such as Vegan, Phyloseq, and DESeq2 for differential species analysis.

Results: The number of observed species was reduced in IBD patients compared to their healthy twin counterparts ($p<0.05$ for both 16S and metagenomic data). The gut microbial composition of the IBD patients also differed significantly from their healthy counterparts (Permutational multivariate analysis of variance, PERMANOVA, $p<0.05$). Interestingly, the Bray-Curtis dissimilarity distance between twin pairs was smaller ($p<0.05$) than the distance between IBD group samples, with also a trend towards smaller distances compared to the healthy control group. In terms of specific taxa, the relative abundances of Actinobacteriaceae members ($p<0.01$), Akkermansia muciniphila ($p<0.05$), and Gemmiger formicilis ($p<0.01$) were reduced in IBD patients compared to their healthy counterparts based on metagenomic data.

Conclusions: IBD is associated with alterations in gut microbiota composition also among closely related subjects, as evidenced by reduced the number of observed species and distinct gut microbial composition. While genetic and early-life environmental factors contribute to gut microbiota similarity among twins, substantial microbial alterations persist in IBD. The reduced abundance of beneficial taxa in IBD patients highlights potential microbial contributors to disease pathogenesis.

[P.10] VITAMIN K2 PRODUCTION BY STAPHYLOCOCCI: FROM METHOD DEVELOPMENT TO APPLICATION

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Introduction: Menaquinones (MK-n; vitamin K2) are a vitamin K form produced from microbial metabolism and made available either in the gut or in fermented foods. Because of its multiple health-related benefits that overpass its role in blood coagulation, its microbiological production is of interest. Among the MK-n forms, which differentiate based on the number of isoprenyl units (4-13), MK-7 exhibits the highest bioavailability (Bonaldo & Leroy, 2024). While lactic acid bacteria and the genus Bacillus have been explored with respect to their vitamin K2-producing capability, less is known about food-grade staphylococci.

Methods: A procedure to extract vitamin K2 from staphylococci was developed together with a rapid (10-min) analytical method based on ultra-high performance liquid chromatography with diode array detection (UPLC-DAD) to quantify MK-7. The method was validated according to ICH guideline M10 (ICH, 2022) on the following strains: Staphylococcus equorum IMDO-S257, S. carnosus IMDO-S10 and S. shinii IMDO-S216. Moreover, the applicability of the method was tested at two levels. First, the method was used to screen MK-7 production across the three strains. Secondly, S. shinii IMDO-S216 was selected to monitor its MK-7 production profile during a controlled fermentation experiment at high oxygen exposure.

Results: The developed UPLC-DAD method was successfully validated for linearity and intra/inter-day accuracy and precision. The screening revealed that S. carnosus IMDO-S10 was the best MK-7 producer with concentrations equal to 1688 nmol/g cell dry weight which corresponded to substantially higher concentrations than those reported in literature for S. xylosus (Seel et al., 2020). Production of MK-7 in S. shinii IMDO-S216, a recently discovered species that bears a close genetic resemblance to S. xylosus (Sosa-Fajardo et al., 2024), could be monitored over the course of the fermentation experiment. The MK-7 concentrations were in line with the values obtained during the screening. The concentrations of MK-7 decreased from 665 nmol/g cell dry weight after 8 h to 7 nmol/g cell dry weight after 26 h, possibly because of prolonged exposure to high oxygen levels.

Conclusions: A rapid UPLC-DAD method was successfully developed and validated for MK-7 quantification in staphylococcal biomass. The method showed applicability in screening MK-7 production by different staphylococci as well as monitoring a MK-7 production profile during a controlled fermentation experiment.

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ICH (2022). https://www.ema.europa.eu/en/ich-m10-bioanalytical-method-validation-scientific-guideline.
Seel, W. et al. (2020). Sci. Rep., 10, 330. Sosa-Fajardo, A., et al. (2024). BMC Genomics, 25, 575.

[P.11] LACTIC ACID BACTERIA CULTURE MEDIUM OPTIMIZATION FOR ENHANCED METABOLIC ACTIVITY IN MILK FERMENTATION APPLICATION

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Introduction: The dairy industry faces challenges in optimizing fermentation to improve product quality and consistency. Lactic acid bacteria, used as starter cultures, are crucial for milk acidification, a vital step for achieving desired textures and flavors in products like yogurt. Maintaining high cell viability and strong acidifying power in these cultures is a persistent issue. Starter cultures need precise nutritional support, with B vitamins, proteins, and alpha-amino nitrogen being key for their metabolic activities, directly impacting their growth and acidification capabilities. Traditional media often lack the optimal nutrient profile, leading to suboptimal fermentation performance. Our research addresses this by developing Lalmedia™ VITALITY VP, an optimized yeast extract enriched with a B-vitamin profile, high protein, and alpha-amino nitrogen content. This innovative solution enhances the growth and viability of yogurt production strains, improving acidification and ensuring more consistent ferments in dairy products.

Methods: Two model strains, Lactobacillus bulgaricus and Streptococcus thermophilus, were cultured in microplates and shake flasks using a minimized medium with yeast-based nutrients, with or without an optimized B-vitamin profile. Lag phase duration, growth rate, and optical density were measured. Cell density and viability were assessed using traditional plate count methods and flow cytometry with IP and SYTO 9 staining. Milk acidification was studied in shake flasks with 100 g of sterile milk inoculated with 2.5 x 10^9 cells of each strain, cultured in media containing the respective yeast nutrients. Peptidomic analysis was conducted using a TimsTOF Pro mass spectrometer and NanoElute chromatographic system to profile peptides in the yeast-based nutrients. Alpha-amino nitrogen content was measured spectrophotometrically using 2% ninhydrin reagent and glutamine standards.

Results & Conclusions: The growth of Lactobacillus bulgaricus and Streptococcus thermophilus was significantly enhanced in a medium with Lalmedia™ VITALITY VP, featuring an optimized B-vitamin profile. Viability improved by 20-40%, positively impacting milk acidification. Peptidomic analysis and alpha-amino nitrogen measurements showed high levels of small peptides and free amino acids (over 60%) and alpha-amino nitrogen (4.9%). Our study demonstrates that optimized yeast extract with enhanced B-vitamins significantly improves the growth and viability of these strains, supporting more efficient fermentation and addressing key challenges in the dairy industry.

[P.12] POLYPHENOL NON-COVALENT BINDING TO PLANT CELL WALLS TRANSIENTLY MODULATES GUT MICROBIAL METABOLISM AND THE PREBIOTIC EFFECT OF PLANT CELL WALLS

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Polyphenols (PPs) can interact with Plant Cell Wall (PCW) polysaccharides, with most PPs in fruits and vegetables being bound to the PCW when plant cells rupture either during consumption or processing. Thus, PCWs play an important role in transporting PPs to various sites of action during gastrointestinal digestion, as well as to the proximal colon, where microorganisms can release and metabolize PCW polysaccharides and PPs. Yet, it is still elusive whether PP-PCW interactions cause sustained changes in microbiota composition and activity and affect the prebiotic potential of dietary fibers. Due to the high diversity of PPs and PCWs, studies on the three-way interaction among PPs, PCW polysaccharides and gut microorganisms are often highly reduced in complexity. In this work, we synthesized multicomponent cellulose hydrogels using Komagataeibacter xylinus, incorporating both pectin and xyloglucan to improve our understanding of PP-PCW interactions by imitating the real food system apple. The dynamics and partitioning profiles of PP binding during soaking in a PP extract from apple were studied via UHPLC-ESI-QTOF-MS/MS, identifying 45 PPs. Flavonol glycosides were overall most strongly retained by the PCWs. In vitro fermentations using fecal slurries as inoculum were performed on selected three-component PCWs, with and without bound PPs. Microbiota composition and fermentation activity were monitored by 16S rRNA gene sequencing and short chain fatty acid analysis. We detected 14 PPs, including three aglycones, at various fermentation timepoints. PPs that were exclusively detected in fermentations treated with PP-soaked PCWs rapidly decreased during the early phase of fermentation with complete depletion after 5 h. The formation of SCFAs was transiently influenced by PCW-bound PPs in comparison to control fermentations. Moreover, the presence of PCW polysaccharides and PPs impacted the abundance of various microbial families. Whether these compositional changes were facilitated more by PCW polysaccharides than PPs and vice versa was shown to be highly dependent on the initial microbial community before treatment. This work not only provides novel insights into the microbial response to PPs non-covalently bound to food polysaccharide matrices, but also gives new insights into the role of food composition in transporting dietary PPs to the colon, laying the basis for finetuning prebiotic formulations with PPs non-covalently bound to dietary fibers.

[P.13] THE ROLE OF FERMENTATION IN THE PRODUCTION OF PLANT-BASED PROTEIN INGREDIENTS

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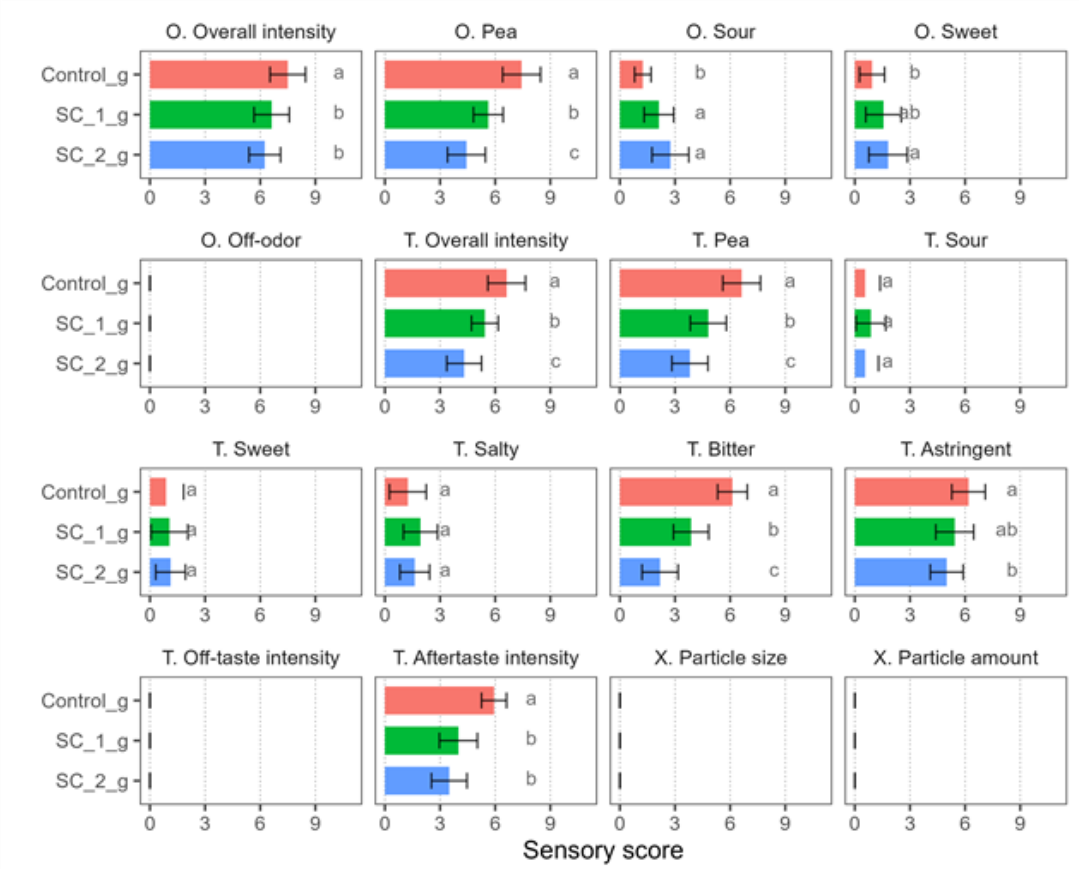
Introduction: Plant proteins have a huge potential to play in transforming food system towards a more sustainable path. Pea protein is increasingly used to produce plant-based meat and dairy alternatives, but its use is limited due to the high off-flavors. There are different technologies applied to improve the sensory properties of plant proteins, but the current market situation needs still improvement. Fermentation is a promising technique to improve sensory properties of plant proteins. Besides, it could act as a technological aid for precipitation and improve nutrition via antinutrient reduction and improving the digestibility. The objective of current study was to see the effect of the fermentation on the sensory properties of pea protein, performance in protein precipitation and the effect on techno-functional properties of the protein.

Methods: During the study tree protein isolates were produced: 1) control with chemical precipitation, 2) fermented with a starter culture that had a fast acidification rate, 3) fermented with a slower starter culture but with a stronger ability to reduce the pea off-flavor, as determined in preliminary experiments. The samples were assessed using the following analyses: sensory analysis, volatile with GC-MS, molecular weight distribution of proteins, free amino acids, and solubility.

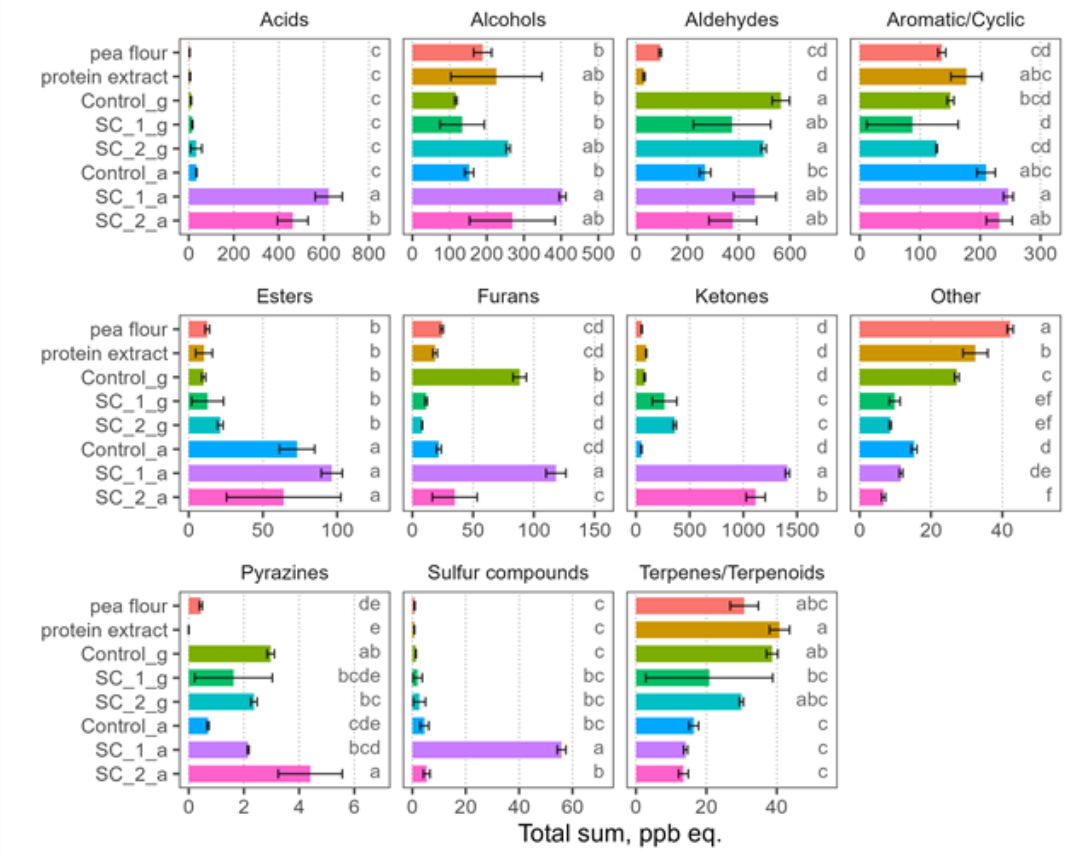
Results & conclusions: Sample 2 completed fermentation in four hours and moderately improved sensory properties, but Sample 3 required two more hours and had the strongest pea flavor reduction. Importantly, it decreased bitterness 3 times from 6.1 to 2.2 (sensory scale 0–9). Protein recovery was the same in all samples, but fermented samples had more peptides, free amino acids, and protein aggregates. Fermentation decreased powder solubility.

In conclusion, we showed that fermentation with lactic acid bacteria can be used for pea protein precipitation after alkaline solubilization and can greatly reduce typical undesirable flavors. The changes in the sensory and techno-functional properties depended on the starter culture.

Supporting image 1



Supporting image 2



[P.14] ASSESSING THE CAPACITY FOR FOOD FERMENTATION RELEVANT BACILLUS SPP. TO PRODUCE BIOGENIC AMINES

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Introduction: Fermentation with Bacillus spp. has the potential to enhance the nutritional value of plant-based foods, particularly by improving protein digestibility and protein quality. However, safety concerns related to biogenic amine (BA) formation require thorough screening of strains before being used in food fermentation. Biogenic amines, such as histamine and tyramine, can accumulate in fermented foods and pose health risks, including food poisoning and hypertensive crises. This study focuses on assessing the BA formation capacity of food fermentation relevant Bacillus spp. with the aim of identifying strains with minimal BA production potential.

Methods: To assess safety, Whole Genome Sequencing (WGS) was used to screen Bacillus strains for toxin- and biogenic amine-encoding genes. Ultra Performance Liquid Chromatography (UPLC) quantifies biogenic amines in fermented oat and pea matrices.

Results: Preliminary genomic analysis indicated that certain Bacillus strains lacked hdcA and tdcA genes, suggesting a lower possibility of histamine and tyramine production. However, some strains carried speC, cadA, speA, and speE, indicating potential production of putrescine, cadaverine, agmatine, and spermidine. These findings inform strain selection for fermentation to minimize safety risks.

Conclusions: Initial results suggest that strain selection based on genomic screening can help mitigate BA formation in fermented plant-based foods.

[P.15] COMPARISON OF VOLATILE COMPOUNDS AND SENSORY QUALITY AMONG VINEGARS MADE FROM FINNISH APPLE JUICE AND POMACE

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Introduction: Apple pomace, a significant by-product of apple juice processing, presents opportunities for value-added applications. This study investigates its utilization in vinegar production using pomace from three Finnish cultivars, focusing on the chemical composition and sensory quality of both the alcoholic beverages and their resulting vinegars.

Methods: Three local, underutilized Finnish apple cultivars were selected for the study. Fermentations were carried out using juice, pomace (from juice extraction), or their combination with Saccharomyces cerevisiae to produce alcoholic beverages. These beverages were then further fermented into vinegars using unpasteurized commercial vinegar. Our ongoing experiments focus on the analysis of organic acids with 1H nuclear magnetic resonance (NMR), volatile compounds with headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS), ethanol and acetic acid with gas chromatography flame ionization detector (GC-FID). In addition, the sensory quality of selected cider and vinegar products is evaluated by a trained sensory panel.

Results & Conclusions: Results indicate that the alcoholic fermentation of pomace requires a longer fermentation time but ultimately achieves a higher ethanol concentration compared to juice fermentation, despite all samples from the same cultivar being adjusted to the same ºBrix level before fermentation. These findings highlight that the fermentation products differ depending on the raw material used. This study contributes to the valorization of apple processing side streams in vinegar production, demonstrating that fermenting apple pomace presents a promising and sustainable approach to enhancing the value of agricultural by-products.

[P.16] BUILDING HYBRID ASSEMBLIES TO INVESTIGATE METABOLIC DIVERSITY IN LACTICASEIBACILLUS STRAINS

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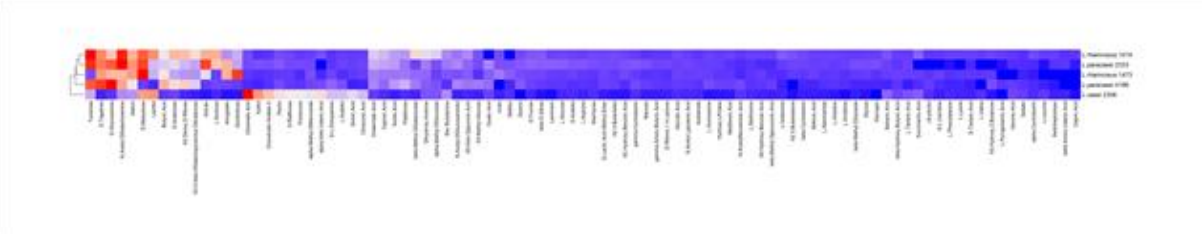
Background: Microbial communities in long-ripened hard cheeses, primarily composed of Lactic Acid Bacteria (LAB), play a central role in ripening through acidification, proteolysis, and other metabolic activities that contribute to texture and flavor development. Accurate identification and characterization of these bacteria are crucial for understanding their metabolic potential and predicting interactions occurring during cheese maturation. Short-read Whole Genome Sequencing (WGS) is a powerful tool for taxonomic and functional profiling but often results in fragmented genome assemblies. Conversely, long-read sequencing technologies enhance genome contiguity but exhibit higher error rates. Hybrid assembly, which integrates short-read and long-read sequencing data, combines the strengths of both methods, enabling high-accuracy and near-complete LAB genome reconstruction. Coupled with functional assays like Phenotype Microarrays, this approach facilitates a comprehensive assessment of microbial metabolic capabilities. This study investigates the metabolic potential of five Lacticaseibacillus strains using hybrid sequencing and phenotypic data.

Methods: Five Lacticaseibacillus strains, L. rhamnosus (1019, 1473), L. paracasei (2333, 4186), and L. casei 2306, were previously isolated from dairy matrices. Following DNA extraction, Illumina and Nanopore sequencing reads were generated, enabling subsequent genome reconstruction and annotation. Phenotypic assays using Biolog Phenotype Microarrays assessed substrate utilization, providing insights into metabolic capabilities.

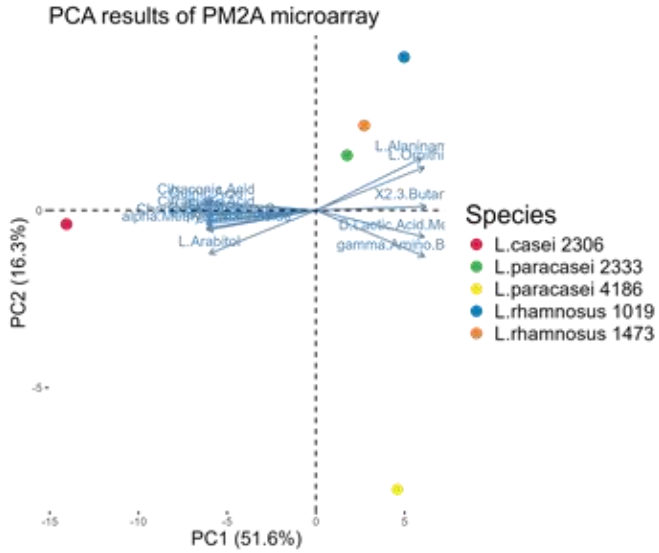
Results: Substrate utilization analysis revealed both inter- and intra-specific metabolic diversity (Figure 1). While a core set of substrates was metabolized by almost all strains, strain-specific substrates highlighted metabolic specialization. As shown in Figure 2, clusters of strains were observed by doing a Principal Component Analysis (PCA). Genomic annotations integrated with phenotypic data identified genes possibly associated with substrate metabolism, including strain-specific pathways.

Conclusions: Hybrid sequencing enables precise genome reconstruction, supporting detailed functional analyses of LAB strains and their roles in cheese ripening. The integration of genomic and phenotypic data facilitates a comprehensive understanding of metabolic pathways, providing insights into strain-specific adaptations that influence ripening processes such as flavor and texture development. These findings highlight the value of hybrid sequencing in elucidating microbial contributions to dairy fermentation. Moreover, correlating genomic features with functional phenotypes aids the rational selection of LAB strains for industrial applications. Optimizing bacterial consortia based on metabolic profiles may enhance fermentation efficiency, improve consistency in cheese production, and support the development of dairy products with tailored sensory attributes.

Supporting image 1



Supporting image 2



[P.17] SAFETY OF HUMAN-IDENTICAL MILK OLIGOSACCHARIDES PRODUCED BY A SAFE STRAIN LINEAGE OF ESCHERICHIA COLI K-12 DH1 MDO

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Introduction: Human milk oligosaccharides (HMOs) are key bioactive components present in breast milk. Their industrial counterparts, human-identical milk oligosaccharides (HiMOs), can be manufactured through precision fermentation using genetically modified microorganisms (GMMs) of Escherichia coli K-12. While E. coli K-12 derivatives are well-established production hosts, they cannot gain qualified presumption of safety (QPS) status in the EU due to taxonomic proximity to pathogenic strains, resulting in more stringent regulatory requirements for HiMOs safety evaluation.
In regulatory approval process of feed or food enzymes, the safe strain lineage (SSL) concept leverages historical safety data of well-documented GMM production strains to optimize safety assessments and reduce toxicological testing requirements for the enzymes manufactured with closely related newly developed GMMs using established production processes. This study evaluates the safety of nine HiMO ingredients produced by E. coli K-12 DH1 MDO-derived GMMs and proposes the application of the SSL concept to streamline future safety assessments.

Methods: Safety assessment was conducted on crystallized HiMOs: 2'-fucosyllactose (2'-FL) and lacto-N-neotetraose (LNnT); and non-crystallized HiMOs: 2'-FL, 2'-FL/difucosyllactose (2’-FL/DFL), 3-fucosyllactose (3-FL), lacto-N-fucopentaose I/2'-FL (LNFP-I/2'-FL), lacto-N-tetraose (LNT), 3'-sialylactose (3'-SL) and 6'-sialylactose (6'-SL) sodium salts. Comprehensive evaluation included bioinformatic analysis of whole genome sequences, phenotypic antimicrobial susceptibility testing, and Average Nucleotide Identity (ANI) analysis comparing host strain and HiMO production strains with non-pathogenic (n=11) and pathogenic (n=8) E. coli strains. Toxicological assessment following EFSA's Tier 1 approach included AMES test, in vitro micronucleus test, and 90-day oral toxicity studies in neonatal rats.

Results: Bioinformatic and phenotypic analyses confirmed non-toxigenicity, non-pathogenicity, and absence of allergenicity in all HiMO production strains. No acquired antimicrobial resistance or virulence genes were detected, and all GMMs were susceptible to tested antimicrobials. ANI analysis demonstrated clear genomic distinction between production (>0.999 similarity) and pathogenic strains. In the 90-day toxicity studies, the no observed adverse effect level (NOAEL) was 4000-5000 mg/kg bw/day, the highest dose tested. All HiMOs were non-genotoxic, regardless of used carbon source, production strain, their metabolic pathways, purity levels, or whether a crystallization step was part of the manufacturing process.

Conclusions: We establish E. coli K-12 DH1 MDO derivatives as an SSL for HiMO production, supported by comprehensive genomic, genotypic assessment and toxicological evidence for HiMO ingredients. The SSL concept could reduce animal testing requirements for future HiMO authorizations when strain modifications raise no safety concerns.

[P.18] BIOPURIFICATION IMPROVES PLANT PROTEIN INGREDIENT FLAVOR WITHOUT AFFECTING PROTEIN FUNCTIONALITY AND IN VITRO DIGESTIBILITY

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Securing a sustainable global food supply in the decades to come requires a shift toward a more plant-based diet. As a result, plant-based ingredients such as protein concentrates and isolates are increasingly used, but their application is limited by unpleasant sensory-active compounds. Current processing solutions, including traditional fermentation, effectively remove off-flavors but are sometimes associated with substantial decline of (techno-)functionality of proteins or require lengthy incubation where spoilage microbes can thrive. Here, we systematically screened about 100 food-grade microorganisms for their potential to remove off-flavors in almond, oat, pea and potato proteins. To produce a purified rather than fermented ingredient carbon sources were limited which prevented production of organic acids and ethanol. Despite the limited energy supply for the cell various Lactic Acid Bacteria (LAB) and yeasts were able to remove “green” volatiles belonging to aldehydes and ketones which was confirmed using sensory evaluation. Following process optimization, such conversions can be achieved in less than an hour without product acidification. Meanwhile, protein solubility, emulsification, foaming abilities, and digestibility remained unaltered. This biopurification process allows for the use of microbes to produce more neutral tasting ingredients.

Reference: Nugroho, A.D.W., van Schalkwijk, S., Cebeci, S. et al. Biopurification using non-growing microorganisms to improve plant protein ingredients. npj Sci Food 8, 48 (2024).

[P.19] BACTERIAL–FUNGAL INTERACTIONS IN KOJI FERMENTATION: IMPROVING FLAVOR AND CONSUMER APPEAL

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Introduction: Bacterial–fungal interactions play a pivotal role in naturally fermented foods by contributing to both ecosystem stability and the development of complex, rich flavors. This study elucidates the interplay between the ancient koji mold Aspergillus oryzae (AO) and Bacillus subtilis var. natto (BsN), focusing on their combined influence on flavor development and consumer perception.

Methods: A series of experiments were conducted to analyze the dynamics of the bacterial–fungal interaction and inhibition patterns. Koji barley fermentations were performed in monoculture and co-culture conditions using AO and BsN. The resulting products were evaluated by an extensive tasting panel, while flavor and aroma profiles were characterized using liquid chromatography (LC) and gas chromatography–mass spectrometry (GC–MS).

Results: An amensalistic relationship was observed, with AO consistently inhibiting BsN. This suppression was not caused by a metabolite produced by AO, but rather resulted from nutrient depletion and a lowering of the medium's pH. Of the koji fermentations, the co-cultured product exhibited a unique flavor profile, higher scores in fruity and sweet flavours for both sensory data and LC- and GC-MS data. Furthermore co-cultures significantly exibited the lowest ratings for strange aromas and flavours, thereby enhancing sensory appeal.

Conclusions: Despite the observed ammensalic bacterial–fungal relationship, co-culturing AO together with BsN improved the flavor profile and consumer acceptance of a fermented barley product compared to monocultures. This study underscores the potential of co-cultures to optimise sensory characteristics in naturally fermented foods.

[P.20] MODULATION OF UREASE ACTIVITY IN STREPTOCOCCUS THERMOPHILUS BIOMASS BY FERMENTATION PARAMETERS OPTIMIZATION

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Introduction: In Streptococcus thermophilus urease activity increases its catabolic efficiency by modulating cell bioenergetics, while participating in important biosynthetic pathways. More recently, it was reported that the probiotic S. thermophilus BT01 was able to modulate urease activity in fecal samples from healthy subjects based on the level of urease activity of the biomass administered to the subjects. Therefore, it was hypothesized that modulating urease activity during the biomass production process could be a key aspect in producing a more effective probiotic formulation. This study aimed to investigate the effect of culture conditions on urease activity, fermentation metabolites and cell viability of S. thermophilus BT01 to be used in probiotic formulations.

Methods: S. thermophilus BT01 biomass production was performed in 1 L bench-scale bioreactors at controlled pH (pH 6.0, pH 6.5 and pH 6.8). Growth kinetic was measured through flow cytometry analysis and by buffering agent tracking. Lactose, galactose, glucose, and L-lactic acid have been quantified enzymatically. Urease activity was quantified spectrophotometrically by phenol-red assay.

Results: Comparison of biomasses collected after growth at pH 6.0 and pH 6.5 revealed no significant changes in growth kinetic, lactose catabolism, or L-lactic acid production.
However, urease activity was 31.33 ± 5.20 and 11.08 ± 2.07 mOD555nm/min*mgprotein pH 6.0 and 6.5, respectively. In contrast, growth at pH 6.8 resulted in a marked reduction of the urease activity (0.48 ± 0.33 mOD555nm/min*mgprotein), which was accompanied by a significant decline in cell viability, lactose utilization, and the other metabolites.

Conclusion: This study revealed how the pH control during the biomass production process can impact on S. thermophilus urease activity and cell viability, allowing to set the most appropriate fermentation conditions for biomass production to be used in probiotic formulations where urease activity is recognized to have a key role in improving host health.

[P.21] BEYOND LACTATE: EXPLORING MICROBIAL CROSS-FEEDING TO IMPROVE ANTIMICROBIAL ACTIVITIES

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Introduction: Fermentation by lactic acid bacteria (LAB) results in a mixture of short-chain carboxylic acids (SCCA), with the specific composition depending on the microbial species involved. While lactate is typically the dominant fermentation product, its antimicrobial effect is limited compared to other organic acids [1]. In addition, recent studies have suggested synergy effects between individual SCCA or even a negative influence on the antimicrobial effect [2]. Therefore, the preservation potential of fermented foods might be improved by converting excess lactate into propionate or other SCCA with stronger antimicrobial properties. In this study, we examine whether lactate-utilizers can facilitate this transformation.

Methods: We established co-cultures of a lactate-producing LAB strain, Liquorilactobacillus ghanensis DSM 18630 or Latilactobacillus fuchuensis DSM 14340, with potential lactate-utilizing bacteria. Ultra performance liquid chromatography with refractive index detector was used to quantify metabolite production, with additional genome analysis to identify potential metabolic pathways involved in lactate conversion.

Results & Conclusions: This study aims to determine whether certain bacteria can efficiently metabolize lactate to propionate, thereby improving the antimicrobial potential of fermented foods. If successful, this approach could offer a natural strategy to enhance food preservation and modulate the metabolic profiles of LAB fermentations. By utilising microbial interactions in fermentation, we explore a potential solution to reduce lactate accumulation while enhancing propionate or other SCCA with antimicrobial activity. This work provides new insights how microbial cross-feeding can be exploited and may be implicated in food biotechnology.

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[P.22] MINIMIZING RISK RELATED TO TYRAMINE FORMATION BY APPLYING CONDITIONS RELATED TO CHEESE MANUFACTURE

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Introduction: Members of the non-starter lactic acid bacteria (NSLAB) can produce biogenic amines such as tyramine, one of the main issues compromising safety and quality of long-ripened cheeses. The increasing reports on new species from the NSLAB, with ability for production of biogenic amines, bring new challenges to the dairy industry. Therefore, alternatives to deal with such challenges are in demand. This study evaluates how key cheese-making parameters such as salt levels, ripening temperatures, and initial pH, affect the tyramine production ability of Lentilactobacillus otakiensis, a newly identified member of the NSLAB from aged Cheddar cheese.

Methods: Two L. otakiensis strains were inoculated at low levels (about 2.0 log cfu/mL) in MRS minimal media, with 0.2% tyrosine added. After a 14-day incubation at 10°C, 15°C, or 30°C, the tyrosine/tyramine ratio was measured to assess the impact of initial pH (4.9 or 5.4), NaCl levels (0%, 3.5%, or 5.0%), and temperature on tyramine production by the strains. The two independent experiments were conducted in duplicate, and the results were analyzed using ANOVA followed by Tukey's HSD test at a significance level of 0.05%. The results are shown as the minimum and maximum averaged values for each temperature and/or parameter tested.

Results & Conclusions: The results indicated an individual and a synergistic effect of the tested parameters. The levels of the amino acid tyrosine after incubation at 10°C (5.971-6.539 mM) did not differed significantly from the levels before incubation (6.772-6.802 mM). At 10°C, very low levels of tyramine were formed, and only when NaCl was not added (0.258-0.511 mM). On the other hand, at 30°C tyrosine was completely depleted, and the maximum levels of tyramine were produced (6.247-6.460 mM). Therefore, the effect of salt and start pH could not be evaluated at 30°C. Interestingly, at 15°C the levels of tyramine in 3.5 % NaCl were higher than in the absence of NaCl, particularly at start pH 5.4 (6.099 mM). At 15°C, the lowest level of tyramine was detected when 5.0 % NaCl was added (4.144 mM). In conclusion, incubation at the lowest tested temperature (10°C), with the highest salt concentration (5.0% NaCl added) and lowest start pH (4.9) generated the lowest level of tyramine after 14 days of incubation. The results indicated the relevance of applying controlled parameters in cheese production to minimize the risk related to tyramine formation by L. otakiensis.

[P.23] THE EFFECT OF DIFFERENT MILK PRETREATMENT METHODS ON MICROBIOME COMMUNITY DEVELOPMENT DURING HERRGÅRDSOST CHEESE FORMATION

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One of the biggest challenges for dairy producers is the substantial variability in final product properties caused by changes in the production environment. In cheese production, this variation is influenced by several factors, particularly the milk base and its pretreatment, which shape the microbiome throughout the process and ultimately affect the cheese's organoleptic characteristics. To examine the impact of three different pretreatments for pasteurised milk— microfiltration, protein fortification, and only pasteurisation (control)— on microbiome dynamics, we generated metagenome sequencing data from 14 cheese production steps across these three production trials at a Danish dairy factory. We constructed three metagenomic co-assemblies, identifying nine high-quality metagenome-assembled genomes (MAGs). Our analysis revealed that a specific strain of Lactococcus lactis dominates the process, while other minor bacterial species persist at very low abundances (<1%), contributing non-negligibly to product properties. Notably, Clostridium tyrobutyricum, a known dairy spoilage bacterium, was present at low levels in pasteurised-only and protein-fortified milk trials but was nearly absent in microfiltered milk. To enhance our analyses, we implemented KHILL, a novel k-mer-based method applied directly to raw sequencing reads, which facilitates metagenomic co-assembly and enables early detection of unwanted microorganisms. Our findings provide industrial dairy producers with a comprehensive view of microbial dynamics during cheese production, offering insights to improve process consistency and product quality.

[P.24] IMPACT OF FERMENTATION CONDITIONS ON BLUEBERRY POMACE SENSORY AND AROMA PROFILE

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Introduction: Berry pomaces are valuable nutrient-rich byproducts that are often underutilized – discarded, used as animal feed or in biogas production. Fermentation offers a promising strategy to enhance the nutritional profile, extend the shelf-life, or create a unique flavor profile. However, fermentation is highly dependent on the composition of berry pomaces, including their sugar content, fiber structure, polyphenolic content, total acidity etc. Majority of the studies have focused on the fermentation of fresh berries or berry juices and the changes in bioactive molecules, whereas less is known how different fermentation conditions affect the outcome, especially volatile composition and sensory profile, in berry pomaces.

Methods: In this study the aim is to optimize lactic acid bacteria (LAB) fermentation conditions in blueberry pomace by investigating the effect of pH and carbohydrate content on the flavor profile. The changes during fermentation are screened with multichannel pH-meter, the microbial activity is detected with isothermal microcalorimetry, and the fermented samples are characterized by sensory evaluation as well as using GC-MS analysis for volatile compounds. Additionally, the changes in sugar content, total acidity, and organic acid profile are determined in parallel with microbial composition.

Results & Conclusions: The results of this study will provide an overview of the changes in the flavor profile, give input for further process optimization as well as for screening of different LAB. A deeper understanding of different compositional factors of berry pomaces and the influence of their adjustment is crucial to maximize the benefits of fermentation and to promote the sustainable utilization of berry byproducts as food ingredients.

[P.25] CONSTRUCTION OF SYNTHETIC MICROBIAL COMMUNITIES FOR THE OPTIMIZATION OF BACTERIAL CELLULOSE PRODUCTION

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Introduction: Bacterial cellulose (BC) is a biopolymer boasting potential applications in food and biomedical fields. Although acetic acid bacteria (AAB) are recognized as high BC-producers, several factors may affect the efficiency yield, making the BC synthesis variable and strain-dependent. In SynBioCell project (PRIN2022 n. 20228Z34PF), we hypothesized the construction of Synthetic Microbial Communities (SMCs) composed by AAB as core and lactic acid bacteria (LAB) and yeasts (Y) as helper members, to boost BC production in different substrates (including agri-food wastes), compared to the single strains.

Methods: Komagataeibacter xylinus K1G4, K2G30 wildtype, K2G30 gdh-deficient, K. rhaeticus K2G14, Lacticaseibacillus casei N87, Lactiplantibacillus pentosus O17, Levilactobacillus brevis LB12, Brettanomyces bruxellensis KCO149, Kluyveromyces lactis UMCC701, Saccharomyces cerevisiae UMCC165 were used for SMC construction. Twelve SMCs were assembled using a Plackett-Burman design to select the best strain combinations, based on BC yield (microaerophilic static cultivation in HS medium). The best (SMC2, SMC9, SMC11) and worst (SMC7) communities were re-assembled to verify the dynamics and compartmentalization (free cells in liquid fraction, FC; weakly adherent cells to BC interface, AC; BC-entrapped cells, BEC) of AAB, LAB and Y during BC production (differential plate counting and BC yield). The members of SMC2 were differently re-combined to evaluate the effect of LAB on BC production (plate counting, BC yield, 16S rRNA-based qRT-PCR). Single AAB strains were used as control.

Results: Regression analysis on Plackett-Burman results highlighted that SMC2 (wtK2G30, K1G4, O17, N87, LB12), SMC9 (wtK2G30, mK2G30, K1G4, O17, KCO149, UMCC701) and SMC11 (mK2G30, K1G4, K2G4, N87, UMCC701) produced a higher BC yield than single AAB strains. SMC7 (mK2G30, N87, O17, UMCC165, KCO149) was the worst combination. Ratio between FC, AC and BEC of AAB were dependent on SMC combinations. LAB were able to grow in HS and migrate into the BC layer, while Y developed to a lesser extent in all compartments. SMC2 was the best BC-producing consortium. O17 and LB12 were the best growing LAB, providing a higher stimulatory effect on BC production compared to N87. The abundance of 16S rRNA confirmed the occurrence and compartmentalization of AAB and LAB during BC synthesis.

Conclusions: A suitable SMC was selected to boost BC production in different conditions and media. The interaction with LAB significantly increased the BC production efficiency of AAB, suggesting the effective role of LAB as helper cultures. However, metabolic and transcriptomic studies are needed to elucidate interactions and labour division of SMC members during BC production.

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[P.26] REGULATORY CONSIDERATIONS AFFECTING THE DEVELOPMENT OF FERMENTATION PRODUCTS

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Fermentation is used in food production for a broad range of purposes, e.g.,

- For food processing, thereby transforming conventional and unconventional sources (e.g., plant-based foodstuffs or -side streams from food production)
- To produce biomass for direct human consumption (e.g., non-animal cheese- or meat alternatives)
- For the manufacturing of specific food ingredients, food enzymes, or flavourings, with or without genetically engineering the microorganisms

The regulatory route to the EU market of a target compound is determined by the

- Manufacturing of the used production strain (in particular genetically modified microorganisms (GMM), qualified presumption of safety (QPS))
- The regulatory status of the raw materials for the fermentation
- Positioning and marketing of the finished product as food ingredient or food improvement agents (food additive, -enzyme, -flavouring)

Strategic considerations for product development will be illustrated by recent authorizations and applications for the authorization of food ingredients and food improvement agents in the EU.

[P.27] OPTIMIZED ANTIFUNGAL PROPIONATE PRODUCTION BY LOIGOLACTOBACILLUS CORYNIFORMIS FOR FOOD PRESERVATION

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Introduction: Propionate is widely utilized as a preservative in bakery goods, cheeses, and animal feed due to its antifungal properties. Traditionally, its production relies on chemical synthesis; however, microbial production represents a promising alternative that could offer a "clean label" preservative solution. Recently, it was shown that the deoxyhexose L-rhamnose can be metabolized into propionate Loigolactobacillus coryniformis subsp. coryniformis DSM 20001 via the intermediate 1,2-propanediol. In this study, we optimized propionate production by L. coryniformis and tested the antifungal properties of propionate against common bread and fruit spoilage molds in vitro and in a food system.

Methods: Fermentation of L-rhamnose by L. coryniformis was performed in small-scale, anaerobically run bioreactors at 30°C and pH 6.5 for 72 h. Metabolite production and deoxyhexose consumption were quantified using high-performance liquid chromatography with a refractive index detector. Fermentates were collected, and the antifungal activity of fermentates and synthetic fermentate mixtures containing the major short-chain carboxylic acids (SCCA) alone and in combination were tested against Aspergillus niger, Penicillium purpurogenum, and Penicillium roqueforti in vitro, and as a biopreservative for snack carrots.

Results: The optimized fermentation yielded up to 16 mM propionate, along with lactate (26 mM), formate (2 mM), and acetate (4 mM) from 32 mM L-rhamnose. Fermentates reduced the growth of molds. Among the SCCA present in fermentations, propionate completely inhibited P. roqueforti, A. niger, and P. purpurogenum at higher (12.5-50 mM), and reduced mold growth at lower concentrations (6.25–12.5 mM). Notably, propionate delayed spore formation of A. niger. Acetate and formate were only inhibitory at high concentrations (25-50 mM), and lactate did not affect mold growth. We used synthetic fermentates and a leave-one-out approach, and identified propionate as the primary inhibitory compound of the fermentates. When applied on snack carrots, with propionate significantly delayed mold development compared to untreated controls. Synthetic fermentate mixtures had no effect on mold growth possible due to the presence of lactate.

Conclusions: L. coryniformis produced propionate-rich fermentates from L-rhamnose, which have the potential to inhibit the tested molds. Findings highlight the promise of propionate as a highly effective biopreservative, offering a sustainable alternative to chemical preservatives in the food and feed industries.

[P.28] CHARACTERIZATION OF KOMBUCHAS PRODUCED AND MARKETED IN BRAZIL: CONTRIBUTIONS TO THE FIRST SPECIFIC LEGISLATION OF THE BEVERAGE IN THE WORLD

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Introduction: Kombucha is a slightly sweet and acidic beverage produced from fermented sweetened tea. It is widely marketed as a non-alcoholic, healthy alternative to soft drinks. However, little was known about the microbial and physicochemical characteristics of commercial kombucha in Brazil. Given the market growth and the need for proper labeling, this study aimed to characterize the microbiological and physicochemical profiles of kombuchas produced and marketed in Brazil.

Methods: Six kombucha brands, including flavored and unflavored varieties with no alcohol content indication on the label, were analyzed. Microbial communities were identified through sequencing of 16S rRNA (bacteria) and ITS (fungi) genes. Physicochemical parameters (pH, total titratable acidity, and alcohol content) were assessed. Statistical analyses were conducted to compare physicochemical variations among brands.

Results: The dominant bacterial species were Liquorilactobacillus nagelii, Oenococcus oeni, Komagataeibacter rhaeticus, and Gluconobacter oxydans, while fungi were mainly Brettanomyces bruxellensis, Brettanomyces anomala, Saccharomyces cerevisiae and Lachancea fermentati. The pH ranged from 2.88 to 3.43, total titratable acidity from 1.80 to 4.86 g/L, and alcohol content from 1.03% to 2.54%, exceeding the 0.5% legal limit for non-alcoholic beverages in Brazil. These findings indicate significant variability among brands, influenced by fermentation conditions.

Conclusions: This study was the first to apply high-throughput sequencing to analyze the microbiome of Brazilian kombuchas. The results highlight substantial differences in microbial composition and physicochemical properties between brands. Notably, all samples exceeded the 0.5% ethanol threshold, reinforcing the need for proper labeling and quality control. In collaboration with the Ministry of Agriculture, Livestock, and Food Supply (MAPA), the findings contributed to the development of Normative Instruction 41/2019, establishing the first specific kombucha legislation in Brazil and worldwide. While regulatory adjustments are needed, this legislation represents an important step in ensuring product safety and serves as a reference for other countries.

[P.29] GENOME-GUIDED SCREENING OF LACTIC ACID BACTERIA FOR ENHANCED FABA BEAN (VICIA FABA) FERMENTATION

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Introduction: Plant-based fermentation offers a promising strategy to enhance food nutrition. Here, we investigate Faba beans (Vicia faba), a legume of interest due to the growing demand for plant-based proteins and its adaptability to cultivation under a wider range of geographic conditions than soybeans. However, its nutritional potential is limited by antinutrients such as vicine and phytic acid. Vicine can trigger a acute hemolytic syndrome in susceptible individuals, while phytic acid binds minerals, reducing their bioavailability. Lactic acid bacteria (LAB) have demonstrated the ability to degrade these antinutrients, thereby improving nutrients and safety. Given the known strain-specific variability in enzyme presence, we aimed to use a bioinformatics approach to screen a diverse range of LAB for their potential in Faba beans (Vicia faba) fermentation.

Methods: We employed nanopore-based long-read sequencing to obtain high-quality, complete genomes of LAB isolated from various fermentations. Genomes were annotated using the tool “Bakta” and subsequently compared using the pangenome analysis tool “Roary”. Based on a literature review, we compiled a list of the primary carbohydrates and antinutrients present in Faba beans (Vicia faba). Enzymes involved in the degradation of these substrates were then identified using databases such as BRENDA, MetaCyc, and KEGG, supplemented by additional literature on carbohydrate and antinutrient degradation. This process yielded a comprehensive list of enzymes potentially relevant for our fermentation process.

Results: Using our enzyme list—which includes enzymes capable of degrading cellulose, starch, hemicellulose, lignin, phytic acid, and vicine—we observed that only a subset of these enzyme-encoding genes was detected across the LAB species analyzed. Furthermore, diversity was observed in both the number and types of enzymes seen among the strains. This variability highlights the distinct enzymatic profiles of our selected strains and reflects their different isolation sources. It also fit with our enzyme list being tailored specifically for the degradation of Faba beans (Vicia faba) rather than being species-specific, allowing for its application across a broader range of LAB. Based on the presence and absence of these enzyme genes, we were able to group strains and select representatives that maximized genetic diversity.

Conclusions: Whole-genome sequencing enabled the identification of candidate LAB strains predicted to efficiently degrade both carbohydrates and antinutrients in Faba beans (Vicia faba). However, experimental validation is required to support our selection strategy and further refine our enzyme list. If successful, this approach could expedite the identification of effective fermentative strains for other plant-based substrates.

[P.30] PRODUCTION OF XYLITOL AND ETHANOL FROM PINEAPPLE WASTE THROUGH FERMENTATION AND HYDROLYSIS PROCESSES

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Pineapple is a promising alternative raw material for the production of bioethanol and xylitol, as Indonesia ranks among the top 10 pineapple-producing countries in the world, generating approximately 0.62 million tons of pineapple waste annually. Producing ethanol and xylitol from lignocellulosic materials, such as pineapple cores, involves hydrolysis and fermentation processes. The hydrolysis process can be conducted enzymatically or with acid, while the fermentation process utilizes microorganisms. This research aims to identify the best yeast for producing ethanol and xylitol with the highest concentration among Saccharomyces cerevisiae, Debaryomyces hansenii, and Candida tropicalis. Characterization of pineapple cores using the van Soest method revealed hemicellulose content of 36.06%, cellulose content of 14.20%, and lignin content of 10.05%. The relatively high hemicellulose and cellulose content indicates that pineapple cores are a potential raw material for ethanol and xylitol production. The production process employs Simultaneous Saccharification and Fermentation (SSF) for ethanol and separated hydrolysis fermentation (SHF) with enzymatic and acid hydrolysis for xylitol. The purification process is carried out through a two-stage distillation method. In ethanol production, the use of D. hansenii yeast and a single purification process resulted in the highest ethanol concentration of 4.32 g/L. However, double purification reduced the ethanol concentration, making it unsuitable as an alternative method in ethanol production. In xylitol production, C. tropicalis yeast proved to be the most effective microorganism for converting xylose into xylitol, yielding a xylitol concentration of 4.29 g/L.

[P.31] INSIGHTS INTO THE METABOLIC PROFILES OF B. CEREUS AND B. SUBTILIS IN DIVERSE FOOD MATRICES

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Introduction: The genus Bacillus contains both pathogens (Bacillus cereus clade) and microorganisms used for food fermentation (Bacillus subtilis clade). Monitoring these species in food matrices is crucial to ensure food quality and safety. This study integrates isothermal calorimetry and metabolomics to investigate the microbial activity of different Bacillus clades cultivated on various nutrient sources.

Methods: Six B. cereus and six B. subtilis strains were cultivated at 30°C in four food matrices: oat drink, whole milk, 5% pea protein hydrolysate (PPH) in water solution, and oat drink supplemented with 5% PPH. Metabolic heat was analyzed for 24 hours to derive calorimetric lag time, calorimetric growth rate, and accumulated heat. Sugars, organic acids, and free amino acids (FAA) were quantified at two time points, right before the metabolic peak of growth (8h for B. cereus, 11h for B. subtilis) and after metabolism had slowed down (24h).

Results: Cultivation in combined oat drink with PPH led to a notable increase in maximum calorimetric growth rate for both B. cereus and B. subtilis. Compared to cultivation in the individual oat and pea matrices, Bacillus metabolism in the combined oat and PPH matrix resulted in a lower total sugar content and higher organic acid content. FAA profiles were influenced by Bacillus clade, matrix, and sampling time-point. FAA increased significantly after 11h of Bacillus subtilis fermentation on oat, but not in pea or combined oat and PPH matrices. Bacillus cereus predominantly increased FAA concentrations after 24 hours of fermentation in pea.

Conclusion: Organic acids, sugars, and free amino acid composition revealed overall distinct metabolic profiles both between B. cereus and B. subtilis clades and the matrices in which these species were cultivated. B. cereus generally had a shorter calorimetric lag time compared to B. subtilis across all food matrices. Major metabolic changes by B. cereus (organic acids and FAA production) were predominantly visible at the later time-point (24h). Although B. subtilis generally started growth later than B. cereus, high FAA concentrations in PPH were already noticeable before reaching its metabolic peak (11h). This suggests a difference in matrix preference (carbohydrate-rich versus protein-rich matrix) and timing of amino acid metabolism between the Bacillus clades. These findings enhance our understanding of clade-specific and matrix-dependent effects on microbial metabolism, offering potential applications in food safety management and fermentation practices.

[P.32] SUSTAINABLE MICROBIAL PROTEIN PRODUCTION FROM BIOGAS

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With the increasing demand for animal feed and human food production, there is a need to develop new alternatives to animal and plant-based protein by relying on fermentation technology for microbial protein production that is more environmentally friendly. Bacterial biomass can contain up to 80% of protein, with all essential amino acids being a good source of nutritious animal feed. The use of low-value organic waste streams for bacterial fermentation and microbial protein production creates a sustainable alternative for feed and food production in the future. Biogas produced through anaerobic digestion of organic wastes is a promising carbon source, in the form of methane and carbon dioxide, that can be converted into microbial protein by specific bacteria capable of gas fermentation. Methane can be used as an energy and carbon source by methanotrophic bacteria (MOB), while carbon dioxide can be assimilated for biomass growth by hydrogenotrophic bacteria (HOB), which generate energy through hydrogen oxidation. Both of these bacteria types can be used as proteinous animal feed, creating a potential for their cocultivation for biogas to microbial protein conversion, combining methane and carbon dioxide utilization in one reactor. In this process, HOB could assimilate carbon dioxide from the biogas and the additional CO_2 generated by MOB during methane metabolism. In addition, hydrogen and oxygen required for the bacteria activity could be acquired from water electrodialysis powered by solar or wind energy to make the process sustainable.

This study has explored the potential for utilizing biogas as a carbon source for the cocultivation of MOB and HOB cultures. MOB and HOB mixed cultures were enriched from landfill biocover soil during a series of culture transfers under conditions favoring either MOB or HOB growth. Enriched cultures showed good growth over batch cultivation, reaching stationary phase after 48h, final biomass concentration of 0.23 g/L and maximum growth rate of 0.125 ± 0.01 h-1 and 0.131 ± 0.04 h-1, for MOB and HOB, respectively. The enriched MOB and HOB cultures were then used to co-inoculate a new culture cultivated in the presence of hydrogen, oxygen and biogas. The combined MOB+HOB culture showed good growth under applied conditions, which was retained in the consecutive transfers, showing a maximum growth rate of 0.092 ± 0.003 h-1. Observed culture growth shows the promise of the use of mixed MOB+HOB culture for the development of biogas to the microbial protein conversion process.

[P.33] FERMENTATION OF FABA BEAN STARCH-RICH FRACTION: EFFECTS ON MICRO-STRUCTURE, RHEOLOGY, AND APPEARANCE OF VEGAN CREAM SPREADS

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Introduction: The starch-rich fraction, known as the coarse fraction, derived from the protein extraction process of legumes, represents an underutilized byproduct with a significant potential in the food industry. However, its application is constrained by taste and odor, which may be mitigated through fermentation. This study examines the fermentability of faba bean starch-rich fraction to develop a vegan alternative to cream cheese (cream spreads, CS). It investigates the effects of starch and protein content alongside process parameter modifications on appearance, microstructure, and rheological properties. This work contributes to the biovalorization of agro-food side streams from legume protein extraction, for future circular food systems.

Methodology: Three setups were employed to produce cream spreads using a combination of two lactic acid bacteria: Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophiles (Vega Classic, Chr. Hansen A/S):

1) CS with varying concentrations of faba bean starch-rich fraction (6.2%–10.0%).
2) CS produced by modified processing parameters: Increased hydration temperature (from 40°C to 65°C), pre-hydration salt addition instead of post-fermentation, and extended pasteurization (from 3 min to 5 min).
3) CS enriched with faba bean or pea protein isolates to improve nutrition.

Analysis: pH behavior during fermentation, microstructural assessment using confocal laser scanning microscopy, rheological analysis (amplitude sweep), and visual appearance.

Results: The hydrated faba bean starch-rich fraction is fermentable with lactic acid bacteria. Decreasing starch concentration improved texture by reducing graininess and enhancing fat droplet distribution. Furthermore, the fermentation affects the fat domains and the continuous starch-protein network surrounding the fat droplets, enhancing structural continuity for starch and protein.
Additionally, modified process parameters, especially increased hydration temperature influences microstructural properties and rheology by increasing storage modulus, while fat domains are reduced to a continuous network. The incorporation of faba bean or pea protein isolates negatively affected fermentation efficiency, as the final pH values only reached 5.3 and 5.6, respectively, despite an intended target of 4.8. Rheological analysis indicated that substituting protein isolate with faba bean starch led to a decrease in both the storage and loss modulus, affecting the textural properties.

Conclusion: Faba bean starch-rich fraction can undergo fermentation using lactic acid bacteria and influences the microstructure and rheology of CS. Process modifications, particularly an increased hydration temperature of 65°C, further impact these properties. However, adding faba bean or pea protein isolates compromises fermentation efficiency. These findings highlight the need to balance fermentation potential with nutritional enhancement when incorporating protein isolates into legumes starch-based fermented products.

[P.34] BREWER'S SPENT GRAIN-BASED FERMENTATION PROCESS FOR PRODUCTION OF AMINO ACIDS AS A FISH FEED PROTEIN SUPPLEMENT

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Introduction: Protein is the main and most expensive ingredient in fish feeds. Many industrially produced protein sources, such as fishmeal and soyabean meal, are costly and more sustainable alternative protein sources need to be developed [1]. Brewer's spent grain (BSG), due to its high and stable availability, low market price, and chemical composition, constitutes a potential resource as a feedstock to produce many industrially valuable compounds. Even after the extraction of protein and lignin from BSG, it still contains high cellulose and hemicellulose levels [1]. The valorization of BSG based complex sugars using fermentation process to produce protein source for fish feed in the form of amino acids (AA) has been explored and reported in this study. AA produced by this process could be a potential good supplementary source of valuable high-quality protein for the food and feed industries. This is the first demonstration of AA production through fermentation using BSG as a feedstock.

Methods: BSG was supplied by Carlsberg, Denmark and underwent pretreatment process. BSG hydrolysate was produced by enzymatic hydrolysis of micronized BSG fraction using commercial enzyme mixture. Different microorganisms were screened for their growth on BSG hydrolysate. Based on the results, Saccharomyces (S) cerevisiae and Corynebacterium (C) glutamicum were selected and further investigated for AA production by cultivation in shake flasks and bioreactor.

Results: A fermentation process was developed at 3.5 L scale for the cultivation of selected micro-organisms using BSG hydrolysate as a substrate and the production of AA was evaluated. C. glutamicum produced alanine, proline, valine, and glycine in shake flasks and bioreactor. Highest alanine production was found in shake flasks whereas production of proline, valine, and glycine was highest in bioreactor. During fed-batch fermentation using C. glutamicum, no other AAs were produced except glycine.
S. cerevisiae produced alanine, proline, valine, and glutamic acid in shake flask but not in the bioreactor. Highest production of alanine, proline, and valine was obtained after 50 h while glutamic acid production peaked after 60 h [2].

Conclusion: The use of BSG-derived hydrolysate as a fermentation substrate for AA production using C. glutamicum and S. cerevisiae was demonstrated. Cultivation mode and growth phase affected amino acid production in both microorganisms. A variety of AA were produced by both microorganisms in shake flask. However, in the bioreactor, only C. glutamicum showed production of AA. To improve the productivity, further process optimization is needed.

References:
[1] https://doi.org/10.1016/j.jclepro.2022.131986
[2] https://doi.org/10.1016/j.bej.2023.109059

[P.35] MICROBIAL CASEINS AND THE IMPORTANCE OF PHOSPHORYLATION FOR THE ASSEMBLY OF CASEIN MICELLES

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Precision fermentation is a promising technology for synthesizing bovine milk proteins with a low climate footprint. Using microbially produced bovine milk proteins has a high potential in dairy alternatives and hybrid products. When native caseins are synthesized in the mammary gland, they undergo post-translational modifications (PTMs), including disulfide bonds, phosphorylations, and glycosylations. These PTMs give bovine caseins special functional properties, including calcium binding and their ability to form casein micelles, which are essential in yogurt- and cheese production. However, generating these PTMs can be challenging, depending on the microbial host. The present study aimed to investigate the presence of phosphorylation of microbially-produced caseins and optimize conditions for the formation of artificial casein micelles during reconstitution to increase the knowledge of the use of caseins in reassembly of casein micelles system (RCMs). Initially, the phosphorylation level of bovine αs1-casein variant B and β-casein variant A2 expressed in E. coli was compared to those naturally present in caseins from bovine milk. The mass and retention times were compared with native bovine caseins using reverse-phase LC-ESI/MS Single Q. The results showed that the identified masses of the microbial αs1- and β-caseins corresponded to the expected masses of non-phosphorylated variants when the mass of the His-tag was subtracted. For the optimization study of the RCMs, sodium caseinate was used with varying amounts of added minerals (calcium and phosphate). The study showed a positive correlation between calcium concentration at constant phosphate level and resulting RCM diameter (Zetasizer). However, at lower calcium concentrations, a greater proportion of caseins appeared to integrate into micelles, as indicated by the reduced amount of non-micellar pellet observed after mild centrifugation (4000 × g, 20 min, 22°C). This suggests that both micellar diameter and optimal mass balance between micellar and freely suspended casein are mainly determined by the amount of calcium during reconstitution. These results offer insights into the properties of αs1- and β-caseins produced in E. coli, as well as the optimal conditions for RCMs, which is relevant for the potential application of microbial caseins in future food production.

[P.36] TARGETING A REGULATORY STRUCTURE IN BACILLUS SPP. TO SELECT FOR THIAMINE OVERPRODUCING STRAINS

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Introduction: Thiamine is not only a vitamin, but also an important flavor precursor in food contributing to the formation of meaty and savory aromas upon heat-induced degradation. Increasing the thiamine content of plant-based foods could thus improve palatability. We aimed to develop food relevant thiamine-overproducing Bacillus strains to boost flavor precursor production during novel plant-based fermentations. Strain improvement focused on altering the functionality of a structure regulating thiamine biosynthetic genes.

Methods: A thiamine analog and TPP, the active form of vitamin B1, both bind to a regulatory structure element in bacterial cells, to stop transcription of downstream genes. In this experiment, exposure of the thiamine analog was used to induce mutations in the regulatory structure. We hypothesize the mutations of the regulatory structure would alter the binding affinity for thiamine analog and consequently also TPP itself, allowing the cell to overproduce thiamine.
We first determined the sub-inhibitory concentration of the thiamine analog for the bacterial strain using isothermal microcalorimetry (IMC). The bacterial strain was then cultured on chemically defined medium lacking TPP and supplemented with the thiamine analog. Colonies were isolated and subjected to whole-genome sequencing, followed by single nucleotide polymorphisms (SNP) analysis to identify mutations.

Results: A total of 38 single colonies were isolated. IMC was then used for verification of three random mutants, when grown in a chemically defined medium depleted of thiamine. Whole genome sequencing of all 38 PT mutants revealed mutations across the genome including the regulatory element when compared to the wild-type strain.

Conclusion: This study suggests that the regulation of the thiamine de novo biosynthesis pathway can be bypassed, making a regulatory structure a potential target for developing thiamine-overproducing strains.

[P.37] SACCHAROMYCES CEREVISIAE POSTBIOTIC INFLUENCES CALF GROWTH, METABOLISM, GUT MICROBIOTA AND IMMUNE STATUS

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Introduction: The weaning phase of calves is very critical, and the transition from liquid to solid diet can be a source of stress for the animal, affecting both its growth and health. One possible strategy for improving calf performance and immune system function is the use of postbiotics. Thus, the aim of this study was to evaluate the effects of supplementing Saccharomyces cerevisiae fermentation products on the performance, gut microbiota, metabolism and immune status of calves.

Material and methods: The research was conducted in accordance with Italian laws on animal experimentation and ethics (Italian Health Ministry Authorization No. 130/2022-PR in agreement with D. Lgs.no. 26, 04/03/2014). Eighteen female Holstein calves were randomly assigned to either a control (CTR, no supplementation; n = 9) or treatment (SCFP, 1 g/d of SmartCare® in milk replacer until weaning, along with 5 g/d of NutriTek®; n = 9) group until 70 d old. Calves were weaned at 60 d, feed intake was recorded until 70 d, and body measurements measured until 160 d. Blood samples were collected to assess metabolic profile, volatile fatty acids, and blood leukocyte gene expression after ex-vivo stimulation with lipopolysaccharides (LPS). Rumen fluid and fecal samples were collected for volatile fatty acid (VFA) and metagenomic analysis of the microbiota.

Results and conclusions: No differences were detected between the two groups for feed intake before and after weaning. The SCFP calves had higher average daily gain and body weight from 70 to 160 d compared with CTR. Further, SCFP calves had higher plasma acetate, propionate and β-hydroxybutyrate (60 d). Inclusion of SCFP to diet increased rumen activity and plasma VFA concentration, suggesting an improved nutrient absorption and a lower weaning stress. In addition, SCFP calves exhibited a better immune response due to gene expression of leukocytes following stimulation with LPS. As indicated by metagenomic analysis, SFCP induced a few significant changes in microbiota at 7, 21 and 42 d. Higher levels of Streptococcaceae, Erysepelotrichaceae and Ruminococcus gnavus group were found in SCFP vs CTR calves at 21 d, and lower abundance of Bifidobacterium pseudolongum, whereas no relevant variations were observed at 60 and 70 d. In conclusion, early supplementation with SCFP supported rumen development and improved metabolic and immune function in dairy calves. However, it remains to be elucidated if and to what extent these changes correlate with shifts in gut microbiota populations.

[P.38] AN IN VITRO COLON MODEL FOR EFFICIENT GUT MICROBIOME INTERVENTION SCREENING: CASE STUDY WITH ENGINEERED ANTI-TNF- PRODUCING ESCHERICHIA COLI

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Introduction: Mouse studies are widely used to investigate the gut microbiome's impact on disease. However, ethical standards in science are crucial, and in vitro gut models can reduce the need for animals. In this context, we introduce the SNIPR-gut, an economical in vitro colon model for rapid screening in microbiome research.
Tumor necrosis factor- (TNF-) is a pro-inflammatory cytokine and anti-TNF- is used in the treatment of e.g. inflammatory bowel disease.
To validate the SNIPR-gut as a pre-clinical screening tool, an Escherichia coli strain was engineered to produce anti-TNF- and its production and colonization ability were tested both in a mouse study and in the SNIPR-gut to confirm accurate replications of results.

Methods: The SNIPR-gut comprises of 12 parallel anaerobic reactors (10 mL working volume) operating in batch or semi-continuous fermentation modes. Fermentations are temperature and pH-controlled and contain a mucin-microcosm mimicking the intestinal mucus. Both the SNIPR-gut and mouse experiments included the anti-TNF-α-producing E. coli strain (b8050) and control strains. In the SNIPR-gut, each strain was inoculated to 10^6 CFU/mL in a colon medium with 0.1% feces and run in batch fermentation mode for 22 hours. Feces from three donors (n=3 per strain) were used. To assess colonization, samples were collected at multiple time points. Anti-TNF-α levels were measured using ELISA. In vivo, streptomycin-treated mice were dosed with 109 CFU E. coli bidaily on study days 1, 2, and 3, and fecal pellets were collected on days 2 and 3 for CFU quantification and ELISA analysis.

Results: The E. coli strains colonized well in both the SNIPR-gut and mice. E. coli fecal levels ranged between 10^8 and 10^{10} CFU/g in mice and 107 and 108 CFU/mL in vitro. The highest anti-TNF-α concentration was detected in mouse feces on Day 2, with levels on Day 3 aligning closer with those in SNIPR-gut. To assess translatability, anti-TNF-α production was normalized per 10^9 b8050 CFUs and statistical analysis revealed no significant differences ($p = 0.14$).

Conclusions: The SNIPR-gut supported the colonization of an E. coli strain engineered to produce anti-TNF-α. Anti-TNF-α production was comparable between in vitro and in vivo conditions, confirming the SNIPR-gut as a reliable pre-clinical screening tool.

[P.39] SOME FUNCTIONAL AND STRUCTURAL CHANGES IN SUNFLOWER MEAL EXTRACT PROTEINS FERMENTED BY LACTIC ACID BACTERIA

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Introduction: Sunflower meal (SM) is an extremely valuable raw material due to its high protein content and amino acid composition although generally used for animal feed purposes. To enhance nutritional quality and support health benefits of plant-based raw materials, fermentation appears as a promising processing method. In this study, the fermentation of SM extract obtained through alkaline extraction was conducted by Lactobacillus helveticus. The changes in microbial growth, pH, soluble protein content, and protein profiles (SDS-PAGE) were investigated over 48 h to evaluate the effect of fermentation on SM proteins.

Methods: Sunflower meal protein extract was prepared by alkaline extraction process at pH 9.0 and 35°C. The extract was sterilized at 121°C for 15 min and inoculated with 5% (v:v) of L. helveticus. The fermentation was conducted at 30°C for 48 h. Microbial growth was checked via viable cell counts (log CFU/mL) at 0, 24, and 48 h. The change in pH and soluble protein content (mg/mL) were assessed at the same intervals. SDS-PAGE was used to visualize the protein degradation and structural changes throughout the fermentation. Effect of fermentation on some techno-functional properties (water holding capacity, oil holding capacity, and emulsifying activity and stability index) of SM proteins were analyzed.

Results: Microbial growth increased from 8.26 ± 0.05 log CFU/mL at 0 h to 9.30 ± 0.18 log CFU/mL at 48 h, while pH decreased from 5.61 ± 0.03 to 4.66 ± 0.02, indicating formation of some organic acids. At the end of fermentation, the pH of the medium was adjusted to pH 8. Soluble protein concentration, which was 12.70 ± 0.21 mg/mL at the beginning, decreased to 7.97 ± 0.06 mg/mL after 48 h of fermentation. However, SDS-PAGE analysis showed no effect of fermentation on molecular weight distribution of proteins. Additionally, fermentation reduced oil holding capacity of proteins (0.73 g oil/g fermented sample and 3.73 g oil/g unfermented sample) but had no impact on the water-holding capacity. Emulsifying activity index and stability index also declined from 210.03 g/m² and 91.32 min to 203.89 g/m² and 51.81 min, respectively.

Conclusions: The study confirms that SM supports the growth of lactic acid bacteria, leading to acidification and protein modification. Further optimization could improve its utilization for such biotechnological applications and enhance its other techno-functional properties.

[P.40] ENGINEERING THE NEXT GENERATION OF FERMENTED DAIRY-PLANT BASED HYBRID FOODS

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With the growing demand for nutritious and sustainable foods, replacing animal-based foods with plant-based alternatives is essential. This transition remains slow due to low consumer acceptance, and the nutritional and cultural importance of animal-based products. Hybrid fermented foods, which may combine conventional animal-based with plant-based sources, in combination with fermentation, which could enhance flavor and nutritional value, offer an intermediate solution. Several knowledge gaps remain for their development, which include ingredient compatibility, optimal conditions, plant protein diversity, finding compatible and preferably industrially viable strains, and improving both flavor and nutrition.

As part of a large collaboration between Chalmers University of Technology, Arla Foods and Lantmännen, this PhD project will bridge food technology and fermentation-biotechnology to develop the next generation of hybrid foods. The project aims to understand the effect of different plant protein sources (pea protein isolate (PPI) and oat protein concentrate (OPC)) and dairy protein sources (casein and skim milk powder (SMP)) and their mixing ratios on functionality and flavor of their hybrid foods. Their impact on solubility, emulsification, foaming, rheology, water binding, flavor profile, and tribology will be assessed. In the next step, the most promising mixtures will be used to develop model emulsions and gels to investigate the molecular and microstructural mechanisms behind positive and negative outcomes for further optimization the hybrid products. To further improve the digestibility, texture, flavor, and nutrition of hybrid foods, fermentation technology will be integrated into the project. Relationships between microbial composition, fermentation conditions, and ingredients quality will be identified. The best-performing strains will be optimized to develop a fermentation toolbox that enables tailoring functional and sensorial properties of hybrid foods by investigating microbial metabolism, interactions, and the effects of pre-treatments or extra processing steps.

The results of the PhD will provide a deeper understanding of the role of plant and dairy protein hybridization conditions and sources in combination with a tailored and compatible microbial starter culture and fermentation processes for improving the functionality, flavor, and nutritional properties of hybrid foods. An overview of the PhD project and its possible latest results will be presented as a poster in the conference.

[P.41] FOOD-CHAIN APPLICATIONS - WORKFLOW AND IT PLATFORMS

IRENE BARATTO

The European Food Safety Authority (EFSA) is responsible for assessing the risk of food regulated products in the EU market, while the European Commission, European Parliament, and Member States make the regulatory decisions.
The application process includes several steps: pre-submission, submission, risk assessment, and post-adoption phases. The procedure differs for each food area, according to specific legislation and applicable guidance documents. Specialized IT platforms such as IUCLID, ESFC, Connect.EFSA, and Open.EFSA assist during different stages of the application process.
In summary, EFSA's application process is designed to ensure the safety of food products before they are authorized for use in the European Union. The dedicated IT platforms and specific workflows facilitate the application procedure, while the public consultation and risk assessment phases add transparency and scientific rigor to the process.

[P.42] PROBIOTICS AS SUSTAINABLE DISEASE CONTROL IN AQUACULTURE

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Introduction: Rainbow trout is a fish species of great economic importance in Denmark, and worldwide, and along with other salmonid species it has been affected by the globally distributed bacterial pathogen Flavobacterium psychrophilum. This pathogen is the causative agent of Rainbow Trout Fry Syndrome and Bacterial Cold Water Disease, detrimentally affecting fry and adult fish respectively. With rising antimicrobial resistance and limited vaccine efficacy, sustainable preventive biocontrol measures are necessary. To address this, we hypothesize that re-introducing native bacterial strains as probiotics would increase the likelihood of ecological compatibility and their potential for long-term establishment, representing a more systemic approach for healthy microbiome management.

Methods: This study aimed to identify and characterize bacteria with probiotic potential against F. psychrophilum, sourced from within the rainbow trout aquaculture environments, focusing on recirculating aquaculture units (RAS). For that, a screening assay was developed using the F. psychrophilum strains 950106-1/1 and FPS-S11A of weak and moderate virulence respectively. Each strain was embedded in TYES-A agar at optimal concentrations, and bacterial colonies from plated samples from rainbow trout rearing tank water, skin, gill, and RAS biofilter units were screened for inhibitory activity using replica plating. Candidate strains were identified through 16S rRNA gene sequencing, while Whole Genome Sequencing (WGS) was employed for further characterization. AntiSMASH analysis was used to identify biosynthetic gene clusters (BGCs) potentially responsible for antimicrobial activity.

Results: Thirty-nine candidate strains exhibiting clear inhibition zones, and consistent in-vitro inhibitory activity against F. psychrophilum were identified using 16S rRNA gene sequencing, shown to belong to the genera Pseudomonas, Janthinobacterium, Bacillus, Acidibacter, Rhodococcus, Stenotrophomonas, Serratia and Shewanella. WGS of three selected strains revealed an unknown Pseudomonas strain, a Pseudomonas yamanorum strain and a Janthinobacterium tructae strain, while AntiSMASH analysis showed multiple BGCs, such as siderophores and lipopeptides. Specifically, high-similarity clusters (>40%) in the Pseudomonas strains included a Non Ribosomal Peptide Synthetase (NRPS) cluster with 80% similarity to pyoverdine SMX-1 in one strain, and an NRPS cluster with 62% similarity to viscosinamide A/pseudodesmin A in the other. The J. tructae strain showed Ribosomally Synthesized and Post-Translationally Modified Peptide (RiPP)-like, terpene, and thioamide-NRP clusters.

Conclusions: This study combines traditional microbiological screening with genomic approaches to identify and characterize native probiotic candidates in rainbow trout aquaculture. Future work will assess the impact of candidate probiont application on hatchery system viability, including egg survival and fry development, and its influence on microbiome dynamics during critical feeding stages. Additionally, we seek to elucidate the potential inhibitory mechanisms against F. psychrophilum. Overall, this research could contribute to developing sustainable disease management strategies for rainbow trout aquaculture.

[P.43] IMPROVEMENT OF NUTRITIONAL PROPERTIES OF FABA BEAN PROTEIN THROUGH FERMENTATION WITH LACTIC ACID BACTERIA AND BACILLUS SPP.

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Introduction: Faba beans are highly nutritious legumes, rich in proteins and dietary fibers. However, their consumption is limited by undesirable beany and bitter flavors, as well as the presence of anti-nutritional factors. This study aimed to evaluate the effects of fermentation with selected lactic acid bacteria (LAB) and Bacillus spp. on the production of volatile aroma compounds, organic acids, free amino acids, kokumi peptides, and the reduction of vicine-convicine in faba bean protein.

Methods: Fermentation medium (FBM) was prepared by sterilization of faba bean protein concentrate (F65X, Vestkorn A/S) in water (8% w/v). FBM was fermented using Lactiplantibacillus paraplantarum, Limosilactobacillus fermentum, Bacillus subtilis, and Bacillus velezensis. Bacterial species were identified using nanopore-based long-read sequencing of native DNA. Samples were collected after 8, 24, and 72 hours fermentation and analyzed for volatile organic compounds (VOCs) using GC-MS, organic acids by HPLC, free amino acids and γ-glutamyl peptides by UHPLC-MS, and vicine-convicine levels by LC-MS.

Results: All strains effectively reduced aldehydes associated with off-flavors, including hexanal, butanal, and pentanal. Species-specific differences were observed in VOC production: LAB, particularly L. fermentum, promoted alcohol formation (e.g., ethanol, methylbutanol, butanol, isopropyl alcohol); B. velezensis showed the highest production of ketones such as diacetyl and acetoin, while B. subtilis produced the highest levels of esters (e.g., methyl esters of acetic, butanoic, and propanoic acids). LAB and B. subtilis increased acetic and lactic acid concentrations, whereas fermentations with B. velezensis resulted in higher production of propionic acid. Fermentations with Bacillus spp. led to the most significant increase in free amino acids, including essential amino acids. Several kokumi dipeptides were detected, including glu-ala produced by B. subtilis, glu-gln produced by Bacillus spp., and glu-phe generated by L. fermentum. Notably, L. paraplantarum and B. subtilis reduced vicine-convicine levels by 3–4 fold at the end of fermentation.

Conclusions: Fermentation of faba bean protein enhances its nutritional and sensory properties by reducing off-flavors, generating desirable aroma compounds, increasing essential amino acids, and decreasing vicine-convicine levels. These effects are strain- and time-dependent, emphasizing the importance of selecting appropriate bacterial cultures for optimal improvement. Further sensory evaluation is required to confirm the impact on overall flavor perception.

[P.44] A COMPREHENSIVE METAGENOMIC APPROACH FOR IDENTIFYING BIOLOGICAL IMPURITIES IN DIRECT INTAKE FERMENTATION FOOD PRODUCTS

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Introduction: Fermentation food products (FFP) with direct intake, such as vitamins and food supplements (FS), are widely consumed, rendering their purity highly relevant. Unexpected biological impurities (BI), including genetically modified microorganisms (GMMs) harboring antimicrobial resistance (AMR) genes, allergens, or pathogens, have previously been detected in commercial FFPs, posing potential health risks. Current detection methods rely on PCR-based approaches, which are limited to known targets and require case-by-case development. To improve the detection, a more open strategy is necessary. This study aims to develop an untargeted metagenomic approach for the comprehensive screening of BI in direct intake FFP.

Methods: A shotgun metagenomics approach combining short- and long-read sequencing was developed to monitor BI in commercial FFP, including vitamins. This involved the optimization of a DNA extraction protocol suitable for a diverse range of vitamin formulations (tablets, capsules, liquids, powders) and the development of a tailored bioinformatic workflow for analysis of sequencing data, integrating taxonomic classification, GMM screening and allergenic ingredient detection. To evaluate the method's performance, vitamin products were artificially spiked with DNA from two GMM strains (Bacillus subtilis host and Bacillus velezensis host), Aspergillus niger (a potential mycotoxin-producing species) and soybean (an allergenic ingredient). Additionally, the developed workflow was applied to two commercial vitamin products with known contamination to evaluate its applicability for real-case impurity detection and validate its effectiveness.

Results: The study demonstrated that DNA extractability varies significantly across vitamin matrices, with liquid and soft-gel capsules yielding the highest DNA recovery. A DNA extraction protocol was selected and optimized to enhance method performance. Metagenomic sequencing, combined with the newly developed bioinformatics workflow, successfully detected high-level spiked contaminants, while sensitivity for low-abundance impurities remained variable. Furthermore, the metagenomic analysis of the two commercial vitamin products uncovered expected GMMs and unexpected bacterial and fungal species.

Conclusion: This study presents, a proof-of-concept for using shotgun metagenomics to detect BI in FFP. While challenges remain regarding DNA extraction efficiency in highly complex matrices and sensitivity for trace-level contaminants, this approach represents a first step towards a valuable tool for impurity surveillance in microbial FFPs. Furthermore, it could be extended to other types of fermentation products. Metagenomics, as an untargeted approach, offers significant added value over conventional PCR-based screening and could serve as a powerful complement to regulatory quality control strategies.
References: Buytaers et al 2021; D'aes et al 2022; SPECENZYM RT11/6242.
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[P45] USE OF FUNGAL SEX TO CREATE INNOVATIVE NEW STRAINS FOR THE FOOD INDUSTRY

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Fungal strains play a crucial role in the manufacture of cheese, fermented meats and other fermented foods such as soy sauce, mycoprotein and tempeh. In the last 40 years, no new Penicillium roqueforti and camemberti fungal strains have been introduced within the blue and white mould cheese market. It is their fermentation process which provides the characteristic and unique flavours, appearance and features of these foods.

Penicillium roqueforti and camemberti are mainly characterized by different properties, such as colour, proteolytic (degradation of proteins) and lipolytic (degradation of fats) capacity. These determine the characteristics of the cheese at the end of the aging process. However, the current strains that are sold are from historic strain banks. Typically, these species reproduced asexually and with each generation these strains are subject to genetic drift, changing their characteristics and over time. This causes quality problems in manufacturing and acts as a barrier to innovation.

These barriers, if not addressed, may well see some popular products as we know them today become “Extinct”, and limit our ability to develop products that reduce the impact of climate change.

Myconeos Ltd discoveries are based on facilitating natural sexual breeding and control of their colour pathway. Indeed, our new technology platform led to the creation of new targeted strains with beneficial characteristics for the fermentation of food products, either for dairy, plant-based and meat industries.

This new technology allowed us to generate hundreds of new strains providing much needed novelty to the blue and white mould cheese industry, as well as in the cultivated-meat production. A selection of strains of interest have been patent protected and already introduced into the market for blue cheese production and other applications. Our Biobank allows the development of strains designed to specifically control key elements for development of quality cheese products.