Abstracts

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[1] ANT YOGURT: FERMENTATION AND COAGULATION OF MILK BY THE ANT HOLOBIONT

Verónica Ramos Viana[1], Veronica Sinotte[2], Diego Prado[3], Leonie Johanna Jahn[1], Nabila Rodríguez Valerón[3], Esther Merino Velasco[3], Sevgi Sirakova Mutlu[4], Rasmus Munk[3], Morten O. A. Sommer[1], Robert R. Dunn[5]

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Ant yogurt: fermentation and coagulation of milk by the ant holobiont

Milk fermentation has a rich ethnographic history by which culture, the environment, and microorganisms intersect. The cultural origins of fermentation practices and microbes inherent to them are often overlooked by industrialized food processes. Here, we use an interdisciplinary approach to characterize and apply a historical fermentation practice originating from Turkey and Bulgaria – ant yogurt. Traditionally, wood ants (***Formica***) have been used to start yogurt fermentation and have recently been applied in modern gastronomy for the same purpose. In this study, we examine the impact of the ant holobiont on the fermentation process, which consists of the ants and their microbes, whose acids and enzymes may facilitate fermentation. Additionally, we determine the effect of seasonality (spring and autumn) and different elaborations of ants (live, frozen, dehydrated) on the fermentation process. The analysis of lactic, acetic, and formic acids reveals that a lactic and acetic fermentation is occurring, and that formic acid plays a role in milk coagulation. The sequencing results indicate the presence of lactic acid bacteria from ants. Our ongoing experiments are focused on microbe metabarcoding of ant yogurts and the detection of proteases through enzymatic analysis and proteomics. Our interdisciplinary approach, combining anthropology, ecology, and gastronomy, will shed light on the group of mechanisms involved in milk coagulation by ants.

[2] SPECIFIC MICROBIOME SIGNATURES ALLOW TO TRACE PDO MOZZARELLA CHEESE GEOGRAPHICAL ORIGIN

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Introduction: Buffalo Mozzarella cheese is a traditional fermented food with unique flavour profile and texture that is produced following a detailed Protected Designation of Origin (PDO) regulation, based on a traditional fermentation process.

Methods: In this study we collected Mozzarella from ten different dairies producing PDO cheese and located in the area of Caserta and Salerno (Southern Italy). We assessed Volatile Organic Compounds (VOCs) by gas chromatography - mass spectrometry (GC-MS) and the microbiome by high-throughput shotgun metagenome sequencing.

Results: Hierarchical clustering of VOC profiles highlighted the separation of the samples according to producing area. A similar result was obtained when considering microbiome profiles. Indeed, the species-level taxonomic profiles were similar for all the samples, with ***Streptococcus thermophilus***, ***Lactococcus lactis***, ***Lactobacillus delbrueckii*** and ***Lactobacillus helveticus*** being the dominant taxa. However, strain-level profiles for the most abundant species allowed to differentiate the cheese origin, with a separation of Mozzarella cheese from Caserta and Salerno.

Conclusions: Microbiome can be considered part of the terroir that links typical products with the specific area of production, also contributing to the peculiar sensorial traits. Therefore, we highlighted the possibility of integrating VOC and microbiome profiles to trace the origin of PDO fermented foods, improving traceability and fraud protection.

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[3] GENOMIC CHARACTERIZATION OF LACTIC ACID BACTERIA STRAINS FOR THE USE AS POSTBIOTICS

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Introduction: Investigations of probiotic strains have led to the characterization of specific metabolic byproducts of probiotics called postbiotics defined as a preparation of inanimate microorganisms and/or their components that confers a health benefit to the host. Recent studies suggest that the viability of bacteria may not be necessary to achieve these health-promoting effects with the undoubted advantage of circumventing the problem of acquisition of antibiotic resistance genes and virulence factors, which may occur when the live strain is ingested. Observations in animal models have demonstrated the biological activity of inanimate bacteria, which offer significant formulation, safety, and regulatory advantages over their live counterparts. The use of postbiotics for human health is still at a preliminary stage, and in contrast to products containing live microbial strains, only a few products are on the market.

Methods: The growing volume of genomic information may facilitate systematic efforts to determine the metabolic pathways that may lead to obtain the desired postbiotic metabolites. Therefore, we aimed to screen Lactic Acid Bacteria genomes available in public repositories to identify the prevalence of genes possibly related to postbiotic activities. Several studies were selected from the available scientific literature identifying genes related to postbiotic activities.

Results and Conclusion: The results demonstrated the importance of computational biology tools for the rational discovery and identification of pathways leading to the production of bioactive molecules.

Acknowledgements: This work was partially funded by a grant from the Italian Ministry of Foreign Affairs and International Cooperation to the project FOODMICROHERITAGE–Quality and authenticity protection of artisanal fermented foods through the characterization and conservation of their microbial and genetic heritage 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).

[4] THE STRESSOSTAT: A NOVEL APPROACH IN ADAPTIVE LABORATORY EVOLUTION TO IMPROVE END-PRODUCT RESISTANCE

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Introduction: End-product inhibition is the major limiting factor in the production of lactic acid bacteria (LAB) biomass for the starter culture industry. Despite applying pH-control, bacterial growth is still inhibited, resulting in decreased biomass productivity, titer and yield. Adaptive laboratory evolution (ALE) is a powerful tool for phenotype optimisation over the past decades, but none of the existing ALE methods could select for improved end-product resistance.

Methods: We developed a novel ALE technology, which we coined the stressostat: STress Resistance Evolution in Substrate Surplus. This technology expands the use of chemostat cultivation in ALE from increasing substrate affinity to improving resistance towards end-products. In contrast to the classical chemostat, there is no substrate limitation in the stressostat. Instead, a constant inhibitory concentration of lactic acid is applied. During the fermentation, the lactic acid concentration increases ***in situ*** through LAB fermentation. In this study, we used ***Lactococcus lactis*** FM03P as a model organism.

Results: During 35 days of stressostat cultivations, we isolated 34 variants in which most of them could grow faster at higher initial lactic acid concentrations than the wild type. However, the variants grew slower than the wild type at control media without lactic acid indicating a possible evolutionary trade-off. In pH-controlled batch cultivations, which represent the starter culture production setup, some variants produced more biomass than the wild type.

Conclusions: Stressostat cultivation successfully generated ***L. lactis*** variants with improved end-product resistance. Further characterisation of those variants could improve our understanding of the underlying inhibition mechanism and discover possible relevant functionalities for application in the starter culture and food industry.

[5] IMPROVING SENSITIVITY OF DETECTION BY MONITORING OPTICAL DENSITY DURING MICROBIAL GROWTH IN RAW MATERIAL WITH MICROPLATE READER

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Introduction: ”Raw material for production of dietary products can be contaminated, at levels below the detection limit of traditional microbiological methods. This can compromise the safety and/or quality of the final products. Since raw materials can act as carriers of contaminations through production, there is a need for fast and sensitive methods, enabling the analysis of a wide range of contaminant levels, and has a high throughput. Monitoring the optical density (OD) during microbial growth in a microplate reader appears to be a good approach for these purposes.”

Methods: ”Here it was performed an evaluation of the suitability of such an approach for analysis of raw material samples. Samples that were non-contaminated, naturally contaminated at two different levels, as well as raw material samples intentionally contaminated with different contaminants at two different levels were tested. The raw material selected was the 2FL HMO (2’-Fucosylloctose Powder 4US number 1242) in CASO broth (+/- chloramphenicol), and the contaminants inoculated individually at approximately 2 and 3 log cfu/mL were: ***S. aureus***, ***P. aeruginosa***, ***B. subtilis*** (OD_{600nm}, aerobic incubation at 30-32.5°C/30-50h), ***C. albicans*** and ***A. brasiliensis*** (OD_{530nm}, aerobic incubation at 25°C/120h).”

Results: “The results in triplicates were compared with standard methods using agar plates. Low standard deviation between replicates, and a good correlation with the standard method was obtained.”

Conclusions: ”Since an enrichment-like step is included in the method using the microplate reader, this may be a promising alternative for investigation of low-level contaminations in the raw material.”

[6] ADVANCED BIOINFORMATICS ANALYSIS TO ENHANCE MICROBIOME DATA MINING

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Metagenomic data from microbiome samples generates great potential for deciphering the function and taxa involved in health and disease. Validated tools are required to mine the full potential of microbiome data.
At BaseClear we developed curated and validated databases with the aim to support advancements in integrating as well as interpreting microbiome data for health application areas. The advantage of such dedicated databases is taxonomic profiling to strain level, which is of high importance for monitoring the effects of e.g., probiotics on microbiome population dynamics. A second advantage is true detection of species at a very low abundance in combination with filtering out false positive hits – information crucial to advancements in understanding mode of actions. A specific case of high importance is low abundant pathogen detection in matrices such as the food chain, for ***in vitro*** models and production of Live Biotherapeutic Products, without ***a priori*** knowledge required.
The metagenome data and detected taxa can additionally undergo functional analysis for presence of virulence genes, resistome carryover, and other functional genes such as those involved in carbohydrate metabolism.
Advanced microbial bioinformatic and Machine Learning tools support researchers to identify key biomarkers in (pre)clinical trials. Such biomarkers give leads to development of diagnostic tools for early disease detection, personalized medicines and a better understanding of the mechanisms behind observed results in pre- and clinical trials.
This presentation takes you through the state-of-art validated bioinformatics technologies that aid the quest in understanding the role-mechanism of the microbiome for human health.

[7] MICROBIOME OF DAIRY PLANTS HARBOR POTENTIAL PROBIOTIC STRAINS AND CAN BE USED AS A MARKER OF CHEESE GEOGRAPHICAL ORIGIN

Francesca De Filippis[1], Vincenzo Valentino[2], Raúl Cabrera-Rubio[3], Giuseppina Sequino[2], Niccolò Carlino[4], José Cobo Díaz[5], Coral Barcenilla[5], Narciso Quijada[6], Carlos Sabater[7], Martin Wagner[8], Abelardo Margolles[7], Avelino Álvarez Ordóñez[5], Nicola Segata[4], Paul Cotter[3], Danilo Ercolini[2]

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Introduction: Facilities producing traditional dairy products are inhabited by a complex microbial community, that develop biofilms on the surfaces and it is not removed by routinary cleaning procedures. Besides pathogens and potentially spoiling microbes, food industry microbiome may represent a source of pro-technological and probiotics strains, that can be transferred to the product and actively participate to the production process.

Methods: Extensive microbiome mapping was performed in 78 dairy industries producing different types of cheeses (fresh, medium and long ripened) and located in 4 EU countries (Italy, Ireland, Spain, Austria). The investigation includes cheese samples (n= 524), raw materials (n=182) and environmental swabs collected from both food contact (n=308) and non-food contact (n=199) surfaces. Microbial DNA was extracted and shotgun metagenomics was carried out on all the samples.

Results: Dairy plants harbour a complex microbiome, that is characterized by high prevalence of genes involved in potentially probiotic activities and resistance to gastro-intestinal passage, suggesting that these microbes may potentially be transferred to the human gut microbiome. More than 6,100 high-quality Metagenome Assembled Genomes (MAGs) were reconstructed, including several Lactic Acid Bacteria species. Different strains from the same species (e.g., ***Lactococcus lactis***, ***Streptococcus thermophilus***, ***Lactobacillus helveticus***) were found in different facilities producing the same cheese type, with a specific impact in the production of flavours and sensorial traits. In addition, some of them represent clear microbial signatures of different facilities, highlighting the interesting potential of microbiome investigation for the traceability of cheese origin.

Conclusions: Microbiome mapping in food processing facilities may help to control the production process. In addition, the resident microbiome may represent an unexplored source of beneficial microbes.

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[8] A REPRODUCIBLE ENTERIC PHAGE COMMUNITY IMPROVES BLOOD GLUCOSE REGULATION IN AN OBESITY MOUSE MODEL

Xiaotian Mao1, Sabina Birgitte Larsen1, Line Sidsel Fisker Zachariassen2, Anders Brunse3, Signe Adamberg4, Josue Leonardo Castro Mejia1, Kaarel Adamberg4,5, Dennis Sandris Nielsen1, Axel Kornerup Hansen2, Camilla Hartmann Friis Hansen2, Torben Sølbeck Rasmussen1*

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Introduction: Recent evidences suggest a link between gut microbiome dysbiosis and metabolic syndrome, including type-2-diabetes, obesity, and non-alcoholic fatty liver disease (NAFLD). Fecal microbiota transplantation (FMT) has been explored as a way to restore a healthy gut microbiome in obese patients but poses safety concerns. The broad usage of FMT is limited by safety concerns over transferring the entire fecal microbiome from one individual to another. Fecal virome transplantation (FVT) is a safer alternative that transfers bacteriophages without bacterial transfer, but still carries the risk of eukaryotic virus infection. Therefore, a safer and effective tool for gut microbiome modulation is needed.

Methods: We explored the potential of implementing three alternative FVT techniques with increased safety established in a recent study (eukaryotic viruses were either eliminated or inactivated) to ameliorate symptoms associated with a diet-induced obesity mouse model. Male mice were fed with an ad libitum high-fat diet before being euthanized (23 weeks of age) and received the different FVT treatments twice with one week of interval. Body weight was measured, oral glucose tests were performed, feces were sampled frequently, and liver, fat pads, mesenteric lymph node, and blood serum were sampled at termination of study.

Results: FVT treatments had no effect on weight gain or the amount of epididymal white adipose tissue. Mice given regular untreated FVT (FVT-UnT) had a significant ($p < 0.05$) drop in the pathological score of their liver tissue when compared to HFD-control mice. Mice treated with a chemostat propagated fecal virome (FVT-ChP, eukaryotic viruses eliminated by dilution) improved their blood glucose regulation significantly ($p < 0.05$) compared to HFD-control mice. The analysis of the gut microbiome of both bacterial and viral components suggested that the gut microbiome could be modulated by bacteriophage mediating.

Conclusions: Bacteriophage-mediation could be a driving force for the observed effects, which could pave the way for the development of safer bacteriophage-based therapeutic tools for restoring the dysbiotic gut microbiome associated with metabolic syndrome.

[9] VERSATILE LACTIC ACID BACTERIA IMPROVE TEXTURE IN BOTH FERMENTED MILK AND SOYBEAN MATRICES

Vera Kuzina Poulsen[1], Elahe Ghanei Moghadam[2], Stjepan Kracun[3], Birgit Albrecht Svendsen[2], Wioleta Marta Nielsen[2], Gunnar Oregaard[2], Anders Krarup[2]

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Fermentation can aid in improving the sensory profiles, nutritional properties, texture, and microbial safety of plant-based dairy and meat alternatives whereby possibly eliminating the use of flavor masking and texturing ingredients. We investigated the texturing potential of lactic acid bacteria in plant-based fermentation by high-throughput screening of 1232 ***Lactococcus lactis*** strains for texture in milk and soybean drink.

We found that most strains with texturing abilities in fermented milk were also capable of enhancing the texture in fermented soybean, despite the large differences in composition of the two matrices.

Exocellular polysaccharide production is believed to contribute positively to fermented milk and plant-base texture. It appeared it was the properties of the polysaccharides rather than their protein interaction partners that were responsible for the enhanced texture in both matrices. The comparative genomics approach revealed 10 texturing strains with novel polysaccharide biosynthesis (***eps***) gene clusters.

[10] METABOLIC ENGINEERING OF RHODOTORULA TORULOIDES FOR ASTAXANTHIN PRODUCTION USING GOLDEN GATE ASSEMBLY PLATFORM

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Introduction: Astaxanthin is a red-colored keto-carotenoid widely used as a feed additive for aquaculture. Currently, majority of commercially available astaxanthin is produced using chemical synthesis. For development of circular economy, efforts have been directed to establish production of astaxanthin using microbial cell factories and upcycled substrates, such as biomass hydrolysates. ***Rhodotorula toruloides*** is a non-conventional yeast that naturally produces lipids and carotenoids growing on a wide range of carbon sources including lignocellulosic hydrolysates and pre-treated food waste. Diverse substrate consumption and tolerance to inhibitors make ***R. toruloides*** a suitable candidate for the metabolic engineering to obtain astaxanthin.

Methods: Astaxanthin is produced from β-carotene in two oxidation steps. To obtain desired final product a biosynthetic pathway was designed which contains β-carotene ketolase (crtW) and β-carotene hydroxylase (crtZ) from ***Haematococcus pluvialis***, ***Pantoea Ananatis*** and, ***Brevundimonas sp.*** SD212. The Golden Gate Assembly Platform (RtGGA) with modifications and additional steps was used to construct cassettes that were randomly integrated in six different combinations (bCrtW+bCrtZ; bCrtW+hpCrtZ; bCrtW+paCrtZ; hpCrtW+bCrtZ; hpCrtW+hpCrtZ; hpCrtW+paCrtZ).

Results: "All six constructs were successfully assembled in pGGA plasmid which was verified by sequencing and then randomly integrated in ***R. toruloides*** genome. Successful integration of astaxanthin pathway in ***R. toruloides*** was confirmed by PCR of extracted genomic DNA of transformants. The transformants were characterized in terms of growth, total carotenoids and astaxanthin detection.

Conclusions: Obtained knowledge is currently used as an input for the design of strategies to transform ***R. toruloides*** into future potential astaxanthin producer.

[11] SUITABILITY OF YARROWIA FOR FOOD APPLICATIONS.

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Abstract
Our current means of meat production are causing negative effects on animal welfare, the environment, and human health. The field of microbial biomass fermentation has received renewed interest, in particular fungi-based biomass referred to as mycoprotein. Here we are proposing the yeast genus ***Yarrowia*** as a potential future source of mycoprotein and evaluated nine different ***Yarrowia*** isolates. The evaluation consists of lipid accumulating ability, hyphae forming ability, metabolomic profile, and genomic analysis, including a pan-genomic comparison as well as screening for potentially allergenic proteins and production of toxic compounds. A high-performing strain was selected and was further analyzed for its nutritional profile. This study indicates that ***Yarrowia*** might be suitable for use as a food or for production of food ingredients.

[12] OPTIMIZATION OF CULTIVATION STRATEGIES TO ISOLATE NEXT GENERATION PROBIOTIC STRAINS FROM HUMAN GUT

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Introduction: The term Next-Generation Probiotics (NGPs) refers to microbial strains that can have a positive effect on human health, but do not belong to common probiotic species (e.g., lactic acid bacteria, ***Bifidobacterium***). However, several studies highlighted the unexplored source of potentially beneficial microbes in the gut microbiome. For this reason, academic and industrial research is focused on identifying and testing novel microbial strains of gut origin.

Methods: One of the main issues in NGP culturing was the selection of a suitable culture medium that allowed the growth of these high-demanding species. Consequently, we tested nine culture media with different formulations in terms of vitamins, minerals and fatty acids to study the culturable fraction of the gut microbiome. We collected bulk microbial cells grown on two plates of each medium and analyzed them by 16S rRNA sequencing of the V3-V4 regions. In addition, we sequenced amplicons obtained from the original fecal samples to identify differences among the media and which of them gives a more reliable picture of the gut microbiome.

Results: Results obtained allowed us to select four media that supported the growth of the highest number of putative NGP species. Samples were streaked on four media and incubated in aerobic and anaerobic conditions at 37°C to discard facultative anaerobes. To select strict anaerobes, we also tested a pre-enrichment step of fecal samples in different broths for 48 h in anaerobic conditions. Fifty-two bacterial colonies were isolated and identified, including the promising NGP candidate ***Bacteroides uniformis***.

Conclusions: Promising strains will be tested for the production of beneficial metabolites (e.g., short-chain fatty acids, urolithins); they will be tested in SHIME (Simulator of Human Intestinal Microbial Ecosystem) to verify their effect on health and the ability to modulate the gut microbiome.

Acknowledgement
A.E. PhD fellowship (Food Science, XXXVII cycle) was granted by the Italian Ministry of University within the Programme “PON R&I 2014-2020 – AZIONI IV.4 DOTTORATI E CONTRATTI DI RICERCA SU TEMATICHE DELL’INNOVAZIONE” (DOT1718749; CUP E65F21003630003)

[13] PLANT-BASED LACTIC ACID BACTERIA ISOLATION FROM SPONTANEOUSLY FERMENTED FOODS BY USING A NEWLY MODIFIED MEDIUM

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As a result of demanding variety of foods as well as ethical and environmental concerns, plant-based milk alternatives are increasingly popular in the world. Although the development of plant-based fermented dairy alternatives has attracted a great interest from both consumers and manufacturers, there are still bottlenecks to produce plant-based fermented products like dairy alternatives both in texture and nutritional profile. Considering that most industrial cultures and relevant knowledge have been set up for the dairy sector, more plant-based LAB need to be isolated and studied to develop better cultures for the next generation of plant-based dairy alternatives. In this study, we attempted to develop a new medium for LAB isolation, which was modified based on MRS and M17 formulas. Our results showed this medium support better growth for all the tested model LAB strains belonging to 21 species/subspecies, compared to the growth on M17 or MRS. By using the newly developed medium, a total of 136 LAB strains belonging to 23 species and 11 genera were isolated from 73 spontaneous fermented food samples after a rigorous de-replication process. In addition, assimilation of plant-based sugar such as galactose, sucrose, maltose, raffinose, xylose and arabinose were characterized. Further, we conducted a modified Voges–Proskauer test, in conjunction with the Biolog color analysis system, to determine the production of butter aroma compounds in fermented non-transparent commercial plant-based milks, including coconut, oat, rice, hemp, pea, hazelnut, and soy milk. Our results demonstrated significant variations in the performance of fermenting plant-based milk by using different strains. Notably, certain strains also exhibited different behaviors when fermenting different plant-based milks.

[14] MICROALGAE SCHIZOCHYTRIUM LIMACINUM AS A SOURCE OF OMEGA-3 FATTY ACIDS

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Introduction: It's important to consume omega-3 fatty acids. They are important components of cell membranes and required for synthesis of a number of signalling molecules. Recently we experience a lack of omega-3 in our diets due to lack of omega-3 in farmed fish and even wild fish because of climate change. For vegeterians and vegans, it is even more difficult to obtain the necessary amount without additional supplement.
Production of omega-3 fatty acid from fish and fish oil is complicated because of mercury pollution and is not environment friendly.
Fermentation of Schizochytrium algae as a source of omega-3 is a sustainable alternative.

Methods: Fermentation

Results and Conclusions: There are producers of omega-3 fatty acids based on fermentation of Schizochytrium, but we are looking for a no-waste technology using rest-overs of closely situated productions to grow the algae. We are at the very beginning of technology development, but as a stable and sustainable industry are ready to introduce the technology to life.
Our cooperation with vegan food producing company reveals possible applications of not actually purified omega-3 fatty acids, but already the inactive dry product rich in omega-3, which makes it cheaper and even more sustainable.

[15] IDENTIFYING RATIONAL STRATEGIES FOR REDUCING POST-ACIDIFICATION BY LACTOBACILLI THROUGH GENOME-SCALE METABOLIC MODELING

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Post-acidification by lactobacilli in fermented milk products can adversely affect the organoleptic properties of the final product. Cellular metabolism is the main driver of post-acidification and genome-scale metabolic models (GEMs) are therefore well suited for devising rationale-based strategies to reduce this process.
Here, the construction of a curated GEM for ***Lacticaseibacillus rhamnosus*** and the subsequent modeling of general metabolism, and proton metabolism in particular, could successfully identify metabolic strategies for significantly countering acid formation, through both the consumption and synthesis of metabolites.
Since many microbial traits of relevance to the food industry are metabolism-based, metabolic modeling approaches hold promise to aid the continued development of product strains with improved traits.

[16] FERMENTED FOODS AS A SOURCE OF BENEFICIAL MICROBES: A META-ANALYSIS

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Introduction: Fermentation is a traditional strategy to extend the shelf-life of perishable products. Furthermore, it is a sustainable process that increases the concentration of vitamins, essential amino acids and minerals, improving the nutritional value. Also, Fermented Foods (FF) can be a vehicle of beneficial microorganisms.
The purpose of this meta-analysis is to provide a map of the microbiota of traditional dairy- and non-dairy FFs and to investigate the potential beneficial outcomes for human health.

Methods: Seventy-two studies describing the microbiota of traditional FFs through metataxonomic approaches were selected. Metagenomic reads from each study were downloaded from the NCBI SRA database and denoised through DADA2, then taxonomy was inferred. Furthermore, we identified 30 studies focusing on the health effects of FFs and their potential in modulating the human gut microbiome.

Results: Our results indicate that traditional FF are a valuable source of beneficial microorganisms, mostly belonging to the Lactic Acid Bacteria group, with potential probiotic activities. In addition, we highlighted that most of the currently approved probiotic strains have been firstly isolated from traditional FFs, thus emphasizing the nutritional and beneficial value of these foods for human health. Observational and intervention studies found in literature suggest that probiotic strains from fermented foods might interact with the human gut microbiota, modulating its composition and promoting human health.

Conclusions: This meta-analysis highlights the wide distribution of traditional FFs worldwide, evidencing the cultural importance of fermentation. Also, our results indicate that traditional FFs are a valuable source of probiotic microorganisms, which might establish in the human gut and exert beneficial activities.

Acknowledgements: This work was partially funded by a grant from the Italian Ministry of Foreign Affairs and International Cooperation to the project FOODMICROHERITAGE–***Quality and authenticity protection of artisanal fermented foods through the characterization and conservation of their microbial and genetic heritage*** and by the project DOMINO-Harnessing the microbial potential of fermented foods for healthy and sustainable food systems, receiving funding from the European Union's Horizon Europe research and innovation programme under grant agreement n. 101060218.

[17] VALORIZATION OF MICROBIAL PROTEIN FERMENTATION THROUGH PROTEOMICS, BIOINFORMATICS, AND INTEGRATED, DATA-DRIVEN MEMBRANE PROCESS DESIGN FOR ISOLATION OF BIOACTIVE PROTEINS/ENZYMES.

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Introduction: The world population is projected to reach nearly 10 billion people by 2050 which puts pressure on current conventional food and feed systems. At the same time the EU has a strategy for becoming climate neutral by 2050 which requires all sectors, food, feed and especially energy to rethink their production with a circular approach. Enormous deposits of natural gas are found around the world and especially flaring, a biproduct of extracting oil from underground, needs an approach that is more climate friendly. In recent years, methanotrophs which are known for metabolizing methane and producing microbial biomass and single cell protein, can accommodate the demand for food and feed and at the same time use methane in a climate friendly way. Unibio, a Danish biotech company, utilizes methanotrophic bacteria to produce protein rich ingredients. Their product, Uniprotein®, is currently used as a protein ingredient in animal feed and in this study, we examine the possibilities of up-cycling a low-value waste stream from the production as a potential source of higher value functional proteins.

Methods: Proteomics analysis based on LC-MS/MS of their low-value waste stream and their downstream protein product has been used to identify potential protein candidates. A workflow was developed to determine protein function and properties, market value, and potential application to identify the proteins with high potential for commercialization. In-silico functional validation by structure and bioinformatics determined that proteins of interest have their predicted function.
Enrichment of the proteins of interest has been achieved by membrane filtration in lab-scale followed by protein isolation and ***in vitro*** functional validation.
Through determination of process parameters, up-scaling the process to pilot-scale was accomplished. These results facilitated techno-economic analysis to ultimately evaluate the profitability of targeting the protein candidates for isolation.

Conclusions: Through determination of process parameters, up-scaling the process to pilot-scale was accomplished. These results facilitated techno-economic analysis to ultimately evaluate the profitability of targeting the protein candidates for isolation.

[18] PLANT-BASED CHEESE ANALOGS: INSIGHTS ABOUT PRODUCTS ON THE MARKET

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In recent years, plant-based diets have become more widespread as a result of increased consumer attention to nutritional, ethical, cultural and sustainable concerns. Since dairy cheese is a food with a large impact on carbon emissions, there has been a growing movement to produce alternatives using plant-based substrates. To learn more about the products currently on the market, we investigated different plant-based cheeses commercialized in France, the Netherlands, and Brazil, by identifying their microbiota, chemical properties and fatty acid profiles. In total, we collected 20 samples, most of them produced with cashew nuts and using starter cultures or rejuvelac. We portioned them into rind and core, and we first applied metagenetic approaches to identify the microorganisms present. We observed that some French cheeses were dominated by ***Lactococcus lactis***, the traditional starter culture for dairy cheese, while others were abundant with ***Leuconostoc mesenteroides***, a species found in several plant-based fermented products. In the Brazilian and Dutch cheeses, we detected the dominance of ***Lactobacillus acidophilus***, species commonly incorporated into fermented dairy products worldwide. We also identified high abundances of ***Bifidobacterium animalis***, especially in the Dutch cheeses. These last two species have been used as probiotics. These results suggest that, in each country, producers used starter cultures from their dairy cheeses. Other abundant lactic acid bacteria detected were ***Weisella cibaria, Leuconostoc lactis, Latilactobacillus sakei, Enterococcus faecium, Lacticaseibacillus casei*** and ***Lactobacillus gasseri,*** as well ***Hafnia alvei***, the only Gram-negative bacterium used as a commercial ripening adjunct culture for cheesemaking. For fungi, since most of the samples collected were of a Camembert style, we detected high abundance of ***Geotrichum candidum*** in the rinds, but also ***Debaryomyces hansenii, Torulaspora delbrueckii*** and ***Penicillium roqueforti***. Furthermore, we measured the chemical compositions of the samples, where we detected a range of 16-35% moisture, 2-4% ash, 6-16% lipids, 10-14% proteins and 35-57% carbohydrates. The fatty acid profile showed a dominance of saturated fatty acids, such as palmitic and stearic, but also oleic acid (monounsaturated) and linoleic acid (polyunsaturated). These fatty acids are normally detected in cashew nuts and dairy cheeses, which may be one of the reasons why the majority of plant-based cheeses commercialized uses cashew nuts. This research can contribute to support future regulatory framework for these products, as well as add scientific knowledge about plant-based cheeses already on the market. These results may also provide guidelines for future research and for the development of novel fermented plant-based products.

[19] THE LAB SIMULATOR OF MILK FERMENTATION: CONTINUOUS MONITORING OF ACIDIFICATION DELAY CAUSED BY PHAGE CONTAMINATION

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Introduction: Bacteriophages (phages) are bacterial viruses which have the great potential to negatively influence milk acidification processes during e.g. cheese making. Conditions of heat treatment of milk used for making dairy products, especially cheeses, are not sufficient to inactivate all the phages found in the raw milk. Therefore, many bacteriophages can re-enter the cheese manufacturing process. Additionally, whey that remains in the vats between batches can lead to accumulation of the phages over time. To gain deeper knowledge of starter culture-phage interactions, we have investigated two commercial undefined DL-starter cultures and whey samples obtained from dairies using those cultures.

Methods: Simulation of dairy environment was designed in a way that mimics the different levels of cross-contamination (0.01, 0.1 and 1%) occurring with whey samples taken from early and late batches of cheese making process. The development of fermentations was monitored with iCinac system that allows continuous pH measurements. The delays in acidification were also monitored for each individual isolate against whey samples, with approximately 200 isolates per starter culture.

Results: The starter cultures that were challenged with 1% contamination levels of whey collected at the dairy from early and late batches of cheesemaking showed a significant delay in acidification. The time delay of acidification was higher in the samples obtained from last batches, indicating phage accumulation throughout the manufacturing process. Our results also show huge differences on phage sensitivity between the two tested starter cultures, both when the complete starter culture or individual strains are tested.

Conclusions: There was a significant interaction between the contamination level of whey and delays in acidification of the milk. Whey samples taken from the late vats caused acidification delays in a higher number of isolates compared with the early vats, which observed in isolates from the starter culture that is likely to be sensitive. Taken together, our data reveal the robustness of one of the starter culture to resist phage attacks, and the sensitivity of the other starter culture.

[20] HIGH-THROUGHPUT SCREENING: AN EFFICIENT TOOL TO REDESIGN BLENDS SUITABLE FOR PLANT-BASED FOODS

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Introduction: The consumption of plant-based foods is exponentially growing worldwide. This is linked to many reasons such as sustainability, life-style choices, diet preferences and animal welfare. Plant-based foods manufacturing is facing many challenges, mainly due to different sensorial and technological characteristics associated with plant-based ingredients compared to the animal-derived ones. In addition, while the microbiological quality and safety of animal-derived foods has been subject of many investigations, little is known on the microbiological risks and safety of plant-derived foods. Fermentation is a powerful tool to overcome all the above-mentioned hurdles in the manufacturing of new plant-based foods. In fact, it is well known that microorganisms may have an essential role for improving textural and sensorial properties of the final product, as well as to convey safety, prolonging the shelf-life.

Methods: Two legume- and cereal-based matrices were chemically characterized for their sugars, organic acids, amino acids and micronutrients compositions. The microbiological quality and safety of the substrates were investigated through classical microbiology and molecular methods. An High-Throughput Screening (HTS) with more than 100 lactic acid bacteria (LAB) isolated from food, insects and environmental sources was performed in order to test their ability to ferment plant-based matrices. Texture-improving strains were obtained selecting LAB able to produce exopolysaccharides (EPS), hence creating a stiff gel network. Moreover, LAB able to reduce green notes and off-flavor were identified. Finally, fast fermenting strains were selected for their ability to drive the fermentation process and to inhibit contaminants growth.

Results: Changing the traditional dairy application of LAB leads to changes of the strains behavior in terms of acidification profile, EPS production and flavor formation. Moreover, different LAB species may act differently in the diverse legume- and cereal-based matrices, but also strains belonging to the same species may have different properties. Due to the strains-specific behavior in these complex substrates, it is important to set up a massive HTS in order to select fast acidifying strains preventing contaminants growth and ensuring safe final products.

Conclusions: The application of fast and reliable HTS is fundamental to design starter culture optimized on specific plant-based substrates, which will lead to the best-fermented product.

Poster abstract

[21] PROMOTING INNOVATION OF FERMENTEDFOODS (PIMENTO) - COST ACTION CA20128

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Present in all European diets, fermented foods (FF) hold a strategic place due to the benefits they offer in terms of nutrition, sustainability, innovation, cultural heritage and consumer interest. The potential of FF for improving human health but also driving food innovation and local production in the next decades has become highly relevant. PIMENTO project, a COST Action CA20128 (Promoting Innovation of ferMENTed fOods; https://fermentedfoods.eu/), which started in November 2021, is supported by COST (European Cooperation in Science and Technology; www.cost.eu). The challenge of PIMENTO is to federate the scientific community and other key stakeholders working on FF. The long-term goal of PIMENTO is to place Europe at the spearhead of innovation on microbial foods, promoting health, regional diversity, and local production at different scales, contributing to economic and societal development as well as food sovereignty in order to promote multi-modal innovation and respond to the expectations of European communities.

The wide variety of stakeholders engaged will enable CA PIMENTO:
i) to tightly connect and clarify scientific knowledge on health aspects of FF
ii) to tackle technical, societal and legislative bottlenecks behind FF-based innovations
iii) to contribute to the establishment of long-term scientific workplaces
iv) to disseminate widely defined scientific knowledge on FF
v) to outline a strategic roadmap for future joint research.

PIMENTO will contribute to the European Green deal strategy "Farm to Fork" by enhancing research and innovation in fermentation-based solutions for food products and processes, improving nutritional, sensory and functional properties. This collaborative network of researchers that includes food scientists, innovators, entrepreneurs, microbiologists, biochemists, and nutritionists has a very broad geographical coverage with 396 partners from 283 institutions of 50 countries. This regional diversity will play an important role through considering a contrasted panel of FF in diets.

Poster abstract

[22] TITAN PROJECT - DIGITAL INNOVATION PILOTS FOR TRASFORMING THE FOOD SYSTEM: FOCUS ON3 PILOTS ON MICROBES

Antonio Del Casale[1], Isabelle Guelinckx [2], Vania Patrone [3], François Bourdichon [3], Argyro Vontzalidou [4], Andrea Colafranceschi [2], Edward Lucian Sliwinski [5]

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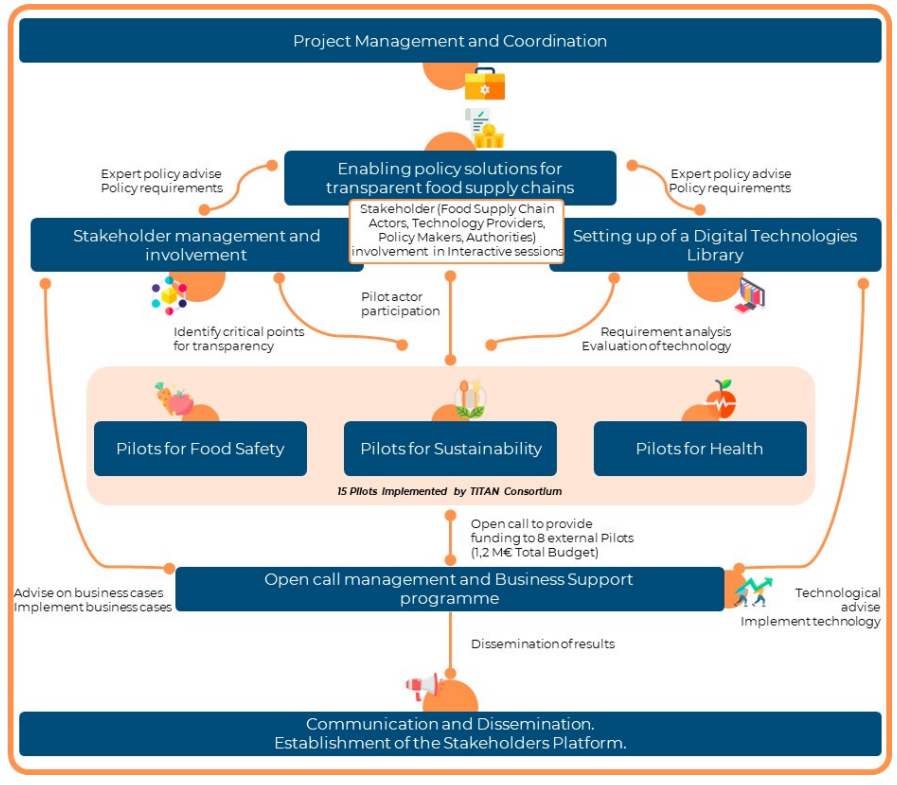
Transparency in Food System

Transparency is a critical component in modern food systems. Indeed, from an authentic Farm2Fork perspective, it is the key for linking all the actors of the system, informing about the origin, production method, ingredients, safety, ethical and sustainability aspects during the process of bringing food from production to distribution on the table.



TITAN in a nutshell

TITAN is a four-year project funded by the Horizon Europe. The overall aim of TITAN is to enhance food transparency in order to transform the food system into a demand-driven economy that provides consumers with healthy and sustainable food.

To achieve this, TITAN will demonstrate pre-identified technologies by deploying 15 pilots and new technologies – 8 pilots – that will be selected during the project with an open call. Innovations will involve the use of Blockchain, Internet of Things and Artificial Intelligence for enhance Healthy, Sustainable and Safe Food System.
It will also lay the foundations for an approach to meet future challenges, outlining a policy roadmap that puts transparency at the heart of the transforming food system.

Pilot on microbiology of fermented food products, safety demonstration of food cultures.
Next-Generation sequencing technologies will be exploited for the rapid characterization of the microbial ecology in fermenting foods, the formation of multi-strain cultures applied to foods for fermentation and bio-preservation and the composition food supplements based on live microorganisms.

Omics and molecular approaches for microbial and chemical quality of long shelf- life food products.

Third-generation sequencing technologies will be exploited for microbial DNA to will be exploited for microbial DNA to detect spore-forming pathogenic contaminants, antibiotic resistance genes and virulence factors. Results will be used for developing commercial PCR kits for detecting strains and/or genes of concern

Poster abstract

[23] SYNERGETIC EFFECT OF THE COCULTURE OF LEUCONOSTOC PSEUDOMESENTEROIDES AND LACTOCOCCUS LACTIS, ISOLATED FROM INSECTS, ON THE GENERATION OF PLANT-BASED DAIRY ALTERNATIVES BASED ON SOY, PEA, OAT AND POTATO DRINKS

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Introduction: The production of plant-based dairy alternatives has been investigated in the present study by testing four different plant-based raw materials: soy, pea, oat, and potato beverages (SPOP). The fermentation was performed through the mono- and co-culture of two LAB strains isolated from bees (***Apis mellifera***): ***Leuconostoc pseudomesenteroides*** NFICC 2004 and ***Lactococcus lactis*** NFICC 2005, based on their phenotypical capabilities. The metabolic, sensorial, and nutritional performance of the mono- and co-cultures fermenting SPOP was evaluated by observing their synergetic effect on each plant-based material.

Methods: Firstly, an extended genotypical analysis (Prokka, Blast, KEGG) of both strains based on their protein and amino acid degradation, sugar consumption, diacetyl and acetoin production, and antinutrient and off-flavour metabolisms were performed to characterize both strains in relation to their potential use as starter cultures in plant-based dairy alternative products. Secondly, the analysis of their co-metabolic performance demonstrated SPOP acidification, as well as lactic acid and mannitol production as the main secondary metabolites (HPLC and iCinac). Thirdly, volatiles and amino acid analysis was conducted using GC-MS and LC-MS to analyse off and dairy-like volatile compounds as well as the degradation of plant proteins and their complementary cometabolism.

Results: Combination of a proteolytic and a non-proteolytic strain was concluded by genotypical characterization. Production of mannitol by ***L. pseudomesenteroides*** was performed in mono and coculture, adding value to the fermented products. The removal of the main off-flavours found in SPOP, such as hexanal, 1-octen-3-ol, 2-pentylfuran, pentanal, octanal, heptanal, and nonanal, was achieved through their co-action, as well as the production of dairy-like flavours, such as diacetyl, acetoin, and 3-methyl-1-butanol. Lastly, their protein and amino acid synergy based on the ability of ***L.pseudomesenteroides*** to degrade SPOP plant proteins while ***L. lactis*** uptakes the released free amino acids, demonstrating their amensalistic and mutualistic interactions in SPOP.

Conclusions: The synergetic effect of ***L. lactis*** and ***L. pseudomesenteroides*** in coculture was demonstrated in soy, pea, oat, and potato beverage fermentations by enhancing growth, increasing acidification rate and lowering pH, removing off-flavours, and establishing a microbial interaction which favoured the potential proteolytic metabolism of ***L. pseudomesenteroides*** with the efficient amino acid metabolism of ***L. lactis***, useful for the production of dairy-like flavours. Their isolation source was the reason for the high adaptation of LP to produce mannitol, which could be used to enhance sweetness of PBDA, thus making them clean-label products, as well as to decrease their caloric index.

[24] MICROBIAL CONVERSION OF SYNGAS TO SINGLE CELL PROTEIN: THE ROLE OF CARBON MONOXIDE

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Introduction: ” Food security is a worldwide challenge in the 21st century due to population growth and climate change. Based on the challenges faced by conventional agriculture, it is necessary to find an alternative, sustainable, and efficient method to deal with the food crisis. Single-cell protein (SCP), a promising and environmentally friendly substitute for animal feed and human nutrition, has attracted more and more attention in recent years. Among the available SCPs producing microorganisms (e.g., fungi, yeast, algae, and bacteria), hydrogen-oxidizing bacteria (HOB) is a group of bacteria that could utilize hydrogen as the electron donor and oxygen as the electron acceptor to fix carbon dioxide into protein (up to 75% of cell content). In addition, syngas is a renewable gas including CO, H_2 and CO_2 has been widely utilized as substrate for microbial processes to produce various products. Thus, syngas could be an alternative substrate for HOB to produce SCP.”

Methods: ” This study used syngas as the substrate, first investigated the effect of different CO content on a model HOB strain, ***Cupriavidus necator*** H16 growth and SCP production during the process using H_2 as the energy source for CO_2 fixation.”

Results: “The growth of ***C. necator*** H16 was significantly restrained with the increase of CO partial pressure and almost completely ceased once the H_2/CO ratios were above 1. In addition, the influences of shaking frequency, inoculum size and gas/liquid ratio on H16 performance were also explored. The raw protein contents were around 50-60% regardless of CO concentration, and the amino acid profiles had no apparent differences.”

Conclusions: ” ***C. necator*** H16 can use H_2 and CO_2 but cannot metabolize CO.
Higher CO content can inhibit cell growth, but it did not affect the amino acid profiles.
High shaking frequency and big inoculum volume can facilitate SCP synthesis.
High biomass productivity was obtained with a high gas/liquid ratio and improved mass transfer capacity due to large interfacial areas.”

[25] MICROBIAL SYNTHESIS OF BOVINE GELATIN IN B. SUBTILIS FOR POTENTIAL FOOD APPLICATIONS

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Gelatin is a polymeric protein with unique functional properties, such as high water-binding capacity, gel-forming, and emulsifying abilities, making it a popular ingredient in the food, pharmaceutical, and biomedical industries. Traditionally, gelatin has been obtained from animal sources, but concerns over safety, batch-to-batch variability, high cost, and ethical issues have led to increased interest in developing alternative sources. Microbial cell factories offer a promising alternative for sustainable and standardized gelatin production. ***Bacillus subtilis*** has emerged as a promising host for gelatin production due to its ability to secrete high levels of recombinant proteins and its ease of genetic manipulation. However, the efficiency of protein secretion is dependent on the interplay between the secretion tag and protein sequence. Therefore, in this study, we investigate the production efficiency of 32 different secretion signal peptides coupled with the same gelatin sequence from a strain of ***B. subtilis***. The genes encoding the secretion tag and gelatin peptide were cloned into a vector and integrated into ***B. subtilis’*** genome. The strains were accessed for their protein production capabilities ***via*** Western blot after 72h of cultivation. Our results demonstrate that gelatin can be sufficiently detected in most samples, even without concentrating the supernatant, indicating the high titers and efficient secretion. However, further optimization of the microbial cell factory and production process will be necessary to fully realize the strain’s potential for food-related applications.

References:

Báez, J., Olsen, D. & Polarek, J.W. Recombinant microbial systems for the production of human collagen and gelatin. ***Appl Microbiol Biotechnol* 69**, 245–252 (2005).

Gómez-Guillén M.C., Giménez B., López-Caballero M.E., Montero M.P. (2011). Functional and bioactive properties of collagen and gelatin from alternative sources: A review, ***Food Hydrocolloids***, 25, pp. 1813-1827

Sorushanova, A., Delgado, L. M., Wu, Z., Shologu, N., Kshirsagar, A., Raghunath, R., Mullen, A. M., Bayon, Y., Pandit, A., Raghunath, M., & Zeugolis, D. I. (2019). The Collagen Suprafamily: From Biosynthesis to Advanced Biomaterial Development. ***Advanced materials (Deerfield Beach, Fla.)***, ***31***(1), e1801651.

Su, Y., Liu, C., Fang, H., & Zhang, D. (2020). Bacillus subtilis: a universal cell factory for industry, agriculture, biomaterials and medicine. ***Microbial cell factories***, ***19***(1), 173.

[26] FEEDING FERMENTED RAPESEED AND SEAWEED (EP199) TO SOWS ALTERS GUT MICROBIOME COMPOSITION AND PIG PRODUCTION PARAMETERS

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Introduction: Diets incorporating fermented feed supplements could be a potential tool to reduce post-weaning diarrhea and improve production parameters in pig farms. Here, we aimed at determining the effect of introducing fermented rapeseed and seaweed (EP199) in the feed of sows across 35 commercial Danish pig farms on gut microbiome (GM) composition as well as pig production parameters.

Methods: We assessed the GM of sows (n=1006) from 35 Danish farms. Fecal samples were taken before introducing the feed supplement and again approximately five months after introducing the fermented rapeseed and seaweed-based feed supplement, EP199. For GM composition determination, we applied Oxford Nanopore full length 16S rRNA gene amplicon sequencing. Alpha diversity was measured based on Observed Features and Shannon Index, while Principal Coordinates Analysis (PCoA) and distance-based redundancy analysis (db-RDA) analysis was used for GM compositional analysis. Pig production data and GM was linked by linear mixed effect models.

Results: Sow GM strongly clustered according to farm. Introduction of EP199 as feed supplement clearly influenced GM as shown by db-RDA-analysis (base-line vs. 5 months after EP199 introduction). The feed supplement significantly increased the number of observed species pr. sow for the farms that belonged to the lower quartile of observed species at start, but did not have the same effect for farms, that already at start had a high number of observed species. Based on the Canberra distance method, linking GM data with farm metadata showed that changes in production parameters, such as the number of liveborn piglets from latest farrowing and the average number of litters pr. sow/year were linked with GM composition after introduction of EP199 as feed supplement. In addition to this, we observed a significant decrease in abundance of bacteria such as ***Clostridium perfringens*** and ***Campylobacter coli*** after introducing EP199 as feed supplement.

Conclusion: Introduction of EP199 as feed supplement alters sow GM composition, which is associated with changes in production parameters. Further analysis is needed to elucidate the exact mechanisms underlying the links between EP199 supplementation, GM changes and production parameters.

[27] DEVELOPMENT OF A MODEL SYSTEM FOR MONITORING SAVORY FLAVOR FROM 2-METHYL-3-FURANTHIOL (MFT) IN YELLOW PEAS

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Introduction: Design of new plant-based products with the savory flavors known from meat is central for the change of consumer behavior towards more sustainable diets. The savory flavors consist of umami and aroma compounds that together form the recognizable taste and aroma of meat. Here, we describe the aroma of yellow peas after heat treatment and supplementation with the flavor precursors thiamine, cysteine, ribose and inosine-5'-monophosphate, for the development of the highly potent meaty heterocyclic thiol 2-methyl-3-furanthiol (MFT) and its derivatives.

Methods: A method was developed using dynamic head-space extraction a long purge time (100 minutes) and splitless GC-MS analysis to track the potential meat flavors at low concentration. Although we found a clear odor reminiscent of MFT, we were only able to trace the oxidized disulfide molecule (MFT-MFT) and 2-methyl-3(methylthio)furan in the test solutions.

Results: We have established a model system with pea flour, where we have tested the effect of precursor concentration, temperature and incubation time on the flavor development. The highest score of "meaty" and "grilled pork" odor attributes in pea flour was obtained with a thiamine to ribose ratio of 5 to 1 (150 mg thiamine / 17 mg of ribose), suggesting that the ribose stimulated MFT development through an unknown mechanism. We also observed inhibition of meat flavor development in oat flour, likely because of the higher fat content.

Conclusions: MFT has a sensory threshold of 4 ng/L in model solution, and further optimization of the flavor analysis is therefore necessary. These results are the first evidence of flavor development through these precursors in pea flour, and are promising for the bio-supplementation of thiamine in solid-state fermentation experiments.

[28] EXOPOLYSACCHARIDES FROM LACTIC ACID BACTERIA AS FUNCTIONAL MICROBIAL FOOD INGREDIENTS

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Introduction: Exopolysaccharides (EPS) are secondary metabolites that have attracted attention due to their positive effects on food and feed production. In the food industry, EPS are widely used as emulsifiers, texturizers, thickeners, stabilizers, and gelling agents. They also show antioxidant, antibacterial, antibiofilm, and cholesterol-lowering activities. Due to their different biological and technological properties, the production of these metabolites and the determination of their properties are important for their use in different fields.

Methods: In this study, EPS were produced from artisanal lactic acid bacteria (LAB) isolated from fermented foods (cheese and table olives). The antimicrobial activities of EPS were evaluated using a disc diffusion method and broth microdilution assay. Their antibiofilm activities were determined by the crystal violet method. To determine the antioxidant activity, a 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity test was conducted. Their prebiotic properties were evaluated by adding the growth medium of a recognized probiotic bacterium.

Results: The partially purified EPS were evaluated to demonstrate their biologically functional activity. EPS producer 12 of ***Lactobacillus*** strains were tested, and half of them showed antimicrobial and antibiofilm activities against ***Bacillus cereus*** ATCC 14579, however, only one strain inhibited the growth of ***Escherichia coli*** NRRL B-3008. Free radical scavenging activity of four strains was found higher than 60%. In addition to anti-activities, EPS exhibited prebiotic properties and enhanced the growth of ***Lactiplantibacillus plantarum*** NRRL B-4495 selected as a model probiotic.

Conclusions: In this study, artisanal LAB isolated from fermented foods were shown to produce functionally diversified EPS. The obtained EPS can be used as novel natural functional bioingredients and additives.

[29] FROM INVENTION TO INNOVATION: INDUSTRIAL PERSPECTIVE ON SHORTENING TIME TO MARKET IN PRECISION FERMENTATION SPACE

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Very little solid innovative ideas in biotechnology find their intended market applications. Crossing the notoriously known valley of death may represent a long and costly route accompanied by numerous technical and managerial hurdles. Proper understanding of such a challenging process and clear product development strategy are key elements of the future commercial success. Time to market is a crucial parameter for many start-up companies to successfully position the product on the market and attract further investor funding to grow and broaden the product portfolio. Efficient synergy of early-stage entrepreneurial ideas and industry operating at commercially relevant volumes represents a way forward with the aim to enable the rapid development of innovative solutions.

Here we provide an industrial perspective of the innovation process from initial proof of concept stage to successful manufacturing. We will address common scale-up pitfalls in upstream as well as downstream process development at the industrial scale. Furthermore, we will point out important technical and regulatory challenges being often omitted in the initial design stage. Key process management tasks necessary to achieve a cost-efficient and commercially attractive process will be outlined. Completion of the first commercial manufacturing of recombinant collagen for food application will be discussed.

[30] PROCESSING OF VEGETABLE PROTEINS FOR FOOD PRODUCTION

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The food sector is responsible for a considerable impact on the environment. A change in the food supply chain is required to reduce the emissions and meet the increasing demands for sufficient and qualitative food products. Meat alternatives are required to decrease food's environmental impact, and are important for the intake of critical proteins, and is thus an important step.
There is shift towards an increased use of plant-based proteins around the world. This increases the need of knowledge and methods to effectively scale up industrial processes, to produce safe user-friendly products with good taste and texture and a high nutritional value.
The Agricultural and Food unit at RISE has investigated and developed methods for research as well as for industry. The aims have varied but process technology (3D printing and extrusion), nutrient quality, microbial safety, taste, and smell have been investigated.
Fermentation provides an effective method to improve properties of plant-based materials for food consumption. Through fermentation, the nutritional quality can be improved by reducing the content of antinutritional factors as phytic acid, oligosaccharides, and tannins. Furthermore, fermentation has been reported to improve protein digestibility, amino acid composition, and to increase bioavailability of iron and zinc. Taste and texture can also improve with fermentation and the consumer experience less abdominal pain and flatulence compared due to reduction of oligosaccharides.
Further, fermentation can facilitate successful extrusion, by improving protein quality and reducing the starch content of protein concentrates. Another positive effect of fermentation may be a reduced off-flavor common for extruded legume protein.
In the Like:Meat, project the aim is to develop second-generation Swedish plant-based meat analogues, which are more nutritious and attractive than currently available alternatives. The meat analogues are developed using innovative technology, where extrusion is combined with fermentation.
RISE is Sweden's research institute and innovation partner with approximately 3000 employees. RISE is an independent, state-owned research institute, which offers unique expertise and over 100 testbeds and demonstration environments for future-proof technologies, products, and services.

[31] FERMENTED RAPESEED AND SOYBEAN IN COMBINATION WITH MACRO ALGAE INHIBITS HUMAN AND LIVESTOCK PATHOGENIC BACTERIA.

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Abstract:

Higher plants produce secondary metabolites expressing antimicrobial effects as a defense mechanism against opportunistic microorganisms living in close proximity with the plant. Fermentation leads to bioconversion of plant substrates to these bioactive compounds and their subsequent release via breakdown of plant cell walls. Fermented feed products are being widely used in the pig industry to overall reduce the disease pressure and has been found to reduce e.g. post-weaning diarrhea. In this study we investigate the antimicrobial potential of fermented soybean- and rapeseed-based pig feed additives, with and without added seaweed. The antimicrobial effect is tested in a plate well diffusion assay, against a range of known human and livestock pathogenic bacteria. Further, we investigate the metabolite profiles based on an LC-MS analysis of the fermented products in comparison to unfermented control counterparts. We observed pronounced release of potential antimicrobial secondary metabolites, such as benzoic acids, when the plant material is fermented, and a significantly increased antimicrobial effect compared to the unfermented controls against several pathogenic bacteria, especially ***Salmonella enterica*** Typhimurium and also ***Listeria monocytogenes***, ***Yersinia enterocolitica*** and a strain of the atopic dermatitis causing ***Staphylococcus aureus*** CC1.

[32] FERMENTATION-BASED PROCESS FOR THE PRODUCTION OF RED BEET COLOR WITH AN IMPROVED SUSTAINABILITY PROFILE

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Introduction: Betanin derived from red beets is the most commonly used natural red food color. While synthetic food colorants are used extensively in the food industry, consumer awareness regarding safety is rapidly driving their replacement with natural alternatives. Current betanin production relies on red beet cultivation, however the low native betanin content in red beets (~0.2% wet weight) makes this an inherently wasteful process unable to sustain the growing demand for natural food dyes.

Methods: The development of industrial biotechnological processes is investment intensive, therefore we initially estimated the economic and environmental viability of betanin production by traditional extraction-based methods against the biosynthetic production of betanin from renewable feedstocks by yeast fermentation. Hereafter, we rationally engineered the oleaginous yeast *Yarrowia lipolytica* to produce red beet betalains (betanin and isobetanin) and assessed the performance of our biotechnological process from an economic and environmental perspective.

Results: An investigative assessment of the environmental impact of replacing traditional extraction-based betanin production with a biotechnological process indicates that fermentation-based betanin production has a favorable environmental sustainability profile. We demonstrate that betanin can be produced in brief fermentation cycles at gram-scale in bioreactors, and that our process currently outperforms extraction-based betanin across a variety of environmental impact categories.

Conclusions: While microbially-produced betanin is a promising sustainable alternative to plant-extracted betanin and other synthetic / natural food dyes, it must be cost-competitive – which is conditioned on its efficient production. Through further metabolic engineering and fermentation process optimization, betanin produced by microbes will soon be able to compete in the current food colorant market.

[33] WATER KEFIR AND DERIVED PASTEURIZED BEVERAGES MODULATE GUT MICROBIATE AND PROMOTES IMMUNOMODULATION

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Traditional fermented beverages like water kefir have been used by different civilizations for centuries and is perceived as health promoting drink. In comparison to the dairy products, water kefir which uses sugary water and fruits, have received less attention to elucidate the potential health benefits and its mechanisms. We performed a study where the combination of in vitro tools mimicking colonic fermentation and the intestinal epithelium have been applied to study the effect of different pasteurized and non-pasteurized water kefir products on gut microbiota, epithelial barrier function and immunomodulation. Water kefir increased beneficial short-chain fatty acid production at the microbial level, reduced detrimental proteolytic fermentation compounds and increased Bifidobacterium genus abundance. The observed benefits are enhanced by pasteurization. Pasteurized products also had a significant effect at the host level, improving inflammation-induced intestinal epithelial barrier disruption and increasing IL-10 and IL-1β compared to the control condition. Metabolomics of the fermented product show molecular shifts detected in the different fermentation stage, these can have an impact on taste and on potential health benefits. Our data support the potential health benefits of water kefir and demonstrate that pasteurization, performed to prolong shelf life and stability of the product, also enhanced these benefits.

[34] CONSTRAINT-BASED METABOLIC MODELLING OF CYANOBACTERIA FOR BRANCHED-CHAIN AMINO ACIDS OVERPRODUCTION.

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Introduction: Branched-chain amino acids (BCAA), L-valine, L-leucine and L-isoleucine, are essential amino acids that cannot be synthesized de novo in mammals, and therefore must be supplied in the diet. Due to their vital role in biological processes, BCAA are widely used in food, medicine, and feed industries. In the face of an intensifying climate crisis, and the substantial growing societal demands for bio-based products, the development of sustainable and efficient manufacturing processes for these components is needed. Photosynthetic microbes, such as cyanobacteria, offer an attractive cell platform to produce BCAA, due to their capability to utilize solar light and CO_2 as the sole energy and carbon sources. In order to make BCAA produced from cyanobacteria commercially feasible, more efforts for metabolic engineering of the strains are required. A key challenge is the understanding of the effects of genetic or environmental changes on the cellular behavior.

Methods: Constraint-based modelling (CBM) is a mathematical tool used for determining the in-silico flux distributions through a metabolic network, under given constraints. Here, we applied CBM-based methods, namely flux balance analysis (FBA) and flux sampling, on the genome-scale metabolic model (GEM) of the cyanobacterium ***Synechocystis*** sp. PCC 6803, and analyzed the solution space of the admissible flux values. Different carbon sources and secretion rates of L-valine and L-leucine amino acids were set as constraints, and a comparison between metabolic models was conducted.

Results: Key enzymes that take effect during BCAA over-secretion and shift from autotrophic and mixotrophic growth modes were identified.

Conclusions: These findings can guide the rational strain improvement towards BCAA production in cyanobacteria, designed for specific food applications.

[35] STRUCTURAL CHARACTERIZATION OF MULTI-DOMAIN, EXTRACELLULAR PROTEASES FROM LACTIC ACID BACTERIA

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Introduction: Some lactic acid bacteria (LAB) perform extracellular hydrolysis of environmental proteins to satisfy their amino acid requirements. The remaining peptides and amino acids can be useful in different food matrixes where changes of plant protein properties are needed to facilitate the demand for plant-based food developments. The extracellular PrtP proteases are large multi-domain subtilisin homologs, which display diverse proteolytic properties. Structure analysis of PrtP homologs of dairy LAB stains are limited, leaving large domain regions uncharacterized. Their 3D structures may revealed functional differences of these proteases, but the large size and cell envelope attachment of the PrtP homologs challenge experimental structure determination. The recent accessibility of the neural network-based algorithm AlphaFold have inspired to this work where we aim to clarify structural characteristics and prevalence of PrtP homologs among LAB species from dairy, human and plant environments.

Methods: PrtP homologs were identified in LAB isolated from a culture collection, containing LAB strains isolated from diverse plant sources. These proteases were compared with well-characterized PrtP homologs from dairy and human associated LAB strains. The 3D structures of the PrtP homologs were modeled using AlphaFold 2.

Results: The PrtP homologs represent 12 phylogenetic clusters, representing the protease diversity among LAB strains from diverse environments. Proteases of each cluster have the same domain composition though the clustering are based on the catalytic domain region. The C-terminal flanking regions of the catalytic domains display different domain compositions, which were revealed from the structural details of the domain boundaries.

Conclusions: The domain compositions of the PrtP homologs are not necessarily limited to the environment from which the LAB strains are isolated. Domain delimitations show domain variants and domain compositions that result in different proteolytic activity, stability, and potentially adhesive properties for PrtP homologs.

Oral abstract

[36] FECAL VIROMES DEPLETED OF ENVELOPED VIRUSES EFFICIENTLY TREATS CLOSTRIDIOIDES DIFFICILE-ASSOCIATED DIARRHEA IN A MURINE MODEL

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Introduction: Fecal microbiota transplantation (FMT) from a healthy donor to recurrent ***Clostridioides difficile*** infection (CDI) patients have proven efficient in curing the disease, possibly through bacteriophage-mediated (bacterial viruses, in short phages) modulation of the gut microbiome landscape. Fecal virome transplantation (FVT, sterile filtrated donor feces) has also been shown to be very efficient in treating the disease. FVT has the advantage over FMT that no bacteria are transferred, but FVT does not exclude the obvious risk of transferring eukaryotic viruses.

Methods: Here we aimed to develop methodologies to obtain safer FVT by removing and/or inactivating eukaryotic viruses, while maintaining an active phage community. Three different strategies were tested: 1) Donor feces were used as inoculum for a chemostat fermentation to remove eukaryotic viruses by dilution (FVT-ChP). 2) Sterile filtrated donor feces underwent solvent-detergent treatment to inactivate enveloped viruses (FVT-SDT) and 3) pyronin Y treatment to block the replication of RNA viruses (FVT-PyT). The safety and efficacy of these treatments were assessed in a CDI mouse model and compared with untreated FVT (FVT-UnT), FMT, and saline treatment that constituted the controls.

Results: Intriguingly, 8 out of 8 mice receiving FVT-SDT survived until planned euthanization and expressed limited symptoms of CDI on the parameters of health scoring, cecum histology, ***C. difficile*** toxin levels, and cytokine levels. On the other hand, only 2 out of 7 saline treated mice survived. Compared to the saline treatment, lower ***C. difficile*** abundance ($p < 0.005$) in the FVT-SDT treated mice suggested that the intervention had hampered ***C. difficile*** colonization. The mice receiving FVT-ChP and FVT-UnT tended to express alleviated CDI symptoms compared to the saline control. FMT had surprisingly low treatment efficacy.

Conclusions: This proof-of-concept study may constitute the initial step of developing a therapeutic tool that targets a broad spectrum of gut-related diseases and thereby substituting FMT with a safer phage-mediated therapy.

Oral presentation

[37] FERMENTED SPIRUILINA AS A POTENTIAL BIOACTIVE AND NUTRITIONAL FOOD INGREDIENT

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Introduction: ***Arthrospira platensis*** (***Spirulina***) is rich in nutrients and bioactive compounds. To further enhance its nutritional value and bioactivity, lactic acid fermentation (LAF) can be used, as it has been previously shown to improve nutritional properties, functionality and extend the shelf life of foods.

Methods: We studied the LAF of ***Spirulina*** as the sole substrate using ***Lactobacillus plantarum***. Fermented (FB) and non-fermented broth (NFB) were analysed by means of pH, lactic acid bacteria (LAB) count, lactic acid concentration, microbiological safety and nutritional composition. Additionally, water and ethanol extracts were prepared on which total phenolic conten (TPC), DPPH radical scavenging activity and cellular antioxidant activity were determined.

Results: The maximum increase in LAB count, lactic acid concentration and drop of pH was observed in the first 24 h of fermentation, the DPPH radical scavinging activity of water extracts decreased with fermentation, while ethanol extracts showed higher antioxidant activity. Similar results were obtained for TPC, where after fermentation a decrease in TPC in water extracts and an increase in ethanol extracts were observed. NFB ethanol extracts significantly decreased intracellular oxidation level, but even greater decrease was observed when FB ethanol extracts were used. Further antioxidant activity of FB ethanol extract was confirmed by measuring lipid oxidative damages in yeast cells. Cells that were first exposed to FB ethanol extract and then to menadione as an oxidative stress inductor showed a lower level of oxidative lipid damage compared to the cells exposed only to menadione. The content of proteins, lipids, dietary fibres, carbohydrates and non-protein nitrogen was determined. Analysis showed that during fermentation the substrate changed significantly in the content of non-protein nitrogen, which increased and fat content, which decreased in comparison to NFB. Microbiological analysis showed no presence of pathogenic bacteria, as well as yeasts and moulds in FB after 24 h fermentation.

Conclusions ***Arthrospira platensis*** was shown as suitable substrate for lactic acid fermentation. TPC and DPPH radical scavinging activity of ethanol extracts increased after fermentation. Ethanol extracts of FB have been shown as a potential source of antioxidants, which efficiently lowered oxidation level in the yeast cells as well as oxidative damages of lipids. Additionally, the level of non-protein nitrogen increased indicating higher protein bioavailability. No presence of pathogenic bacteria and low pH indicate enhancement of FB microbiological stability**.** Therefore, inclusion of fermented ***Spirulina*** into food products could lead to added-value foods based on microalgae.

[38] THE EFFECT OF COLONIC PH ON MICROBIAL ACTIVITY AND METABOLITE PRODUCTION USING COMMON PREBIOTICS AS SUBSTRATES: AN IN VITRO STUDY

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Introduction: "The interplay between gut microbiota (GM) and host via degradation of dietary components leading to the production of metabolites such as short-chain fatty acids (SCFAs) is affected by a range of factors including colonic pH and carbohydrate source."

Methods: " Here we investigate how differences in colonic pH influence GM composition and metabolite production using different substrates including inulin, lactose, Galactooligosaccharides (GOS), and Fructooligosaccharide (FOS) in an ***in vitro*** colon setup. We investigated 3 different pH regimes (low, 5.2 increasing to 6.4; medium, 5.6 increasing to 6.8 and high, 6.0 increasing to 7.2)."

Results: "The results showed that ***Bacteroides*** spp decreased but ***Bifidobacterium*** spp. became abundant under lower pHs, suggesting complex interactions of the bacterial community in the face of pH fluctuations in the colon. The butyrate producer ***Butyricimonas*** and ***Christensenella*** were enriched at higher pH conditions, where also butyrate production was increased using inulin as substrate. The relative abundance of ***Phascolarctobacterium***, ***Bacteroides***, and ***Rikenellaceae*** was increased at higher colonic pH, which was accompanied by increased production of propionate using GOS and FOS as substrate."

Conclusions: "The gastrointestinal factors are linked in a complex network, where microbial activity leads to the production of SCFAs and other compounds that influence pH, which in turn seems to influence microbial activity. Taken together, our results show that dynamic changes in colonic pH under ***in vitro*** simulated conditions have a strong effect on gut microbial activity with SCFA production being higher at colonic pH conditions close to neutral. Further, our study sheds light on possibilities for influencing colonic microbial activity via dietary components modifying colonic pH and provides references for further ***in vivo*** validations."

[39] SUPPLEMENTATION WITH FIVE HUMAN MILK OLIGOSACCHARIDES CHANGES THE MICROBIOME OF FORMULA-FED INFANTS AND BRINGS MICROBIAL DEVELOPMENT CLOSER TO THAT OF BREASTFED INFANTS

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Abstract: Breastfeeding is the optimal source of infant nutrition and confers established health benefits to the infant. Some of these benefits are associated with the human milk oligosaccharides (HMOs), a unique group of carbohydrates that comprises the third most abundant solid component of human milk. HMOs remain undigested by the host and serve as a nutritional source for certain gut bacteria, thereby shaping the microbiome in the neonates. HMOs are also known to provide positive immunomodulatory and antimicrobial effects to the human host. Until recently, infant formula has not included HMOs, but innovation in HMO production now allows supplementing formula with more of these functional carbohydrates.

We conducted the first clinical study with five different HMOs, namely 2'FL, 3FL, LNT, 3'SL and 6'SL at 5.75 g/L in infant formula in healthy term infants. Metagenomic sequencing of the fecal microbiome from a 5 HMO mix supplemented group compared to a control formula group shows significant changes in the microbiome of the 5 HMO mix supplemented infants within the first months of life. We show structural changes in the microbiome with an increase in the abundance of ***Bifidobacterium*** species and a decrease in potentially pathogenic bacteria. Analysis of bacterial metabolic modules reveals that the function of the microbiome is changed towards HMO metabolism and important glycosyl-hydrolases are more prevalent. In conclusion, our data indicate that supplementation with 5 HMO mix shifts the metabolic function of the microbiome and the microbial development closer to that of breastfed infants.

[40] IS THERE LIFE IN PLANT-BASED YOGURT ALTERNATIVES?

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Introduction: "Consumer demand for plant-based dairy alternatives has skyrocketed and the market has been flooded with animal-free foods. In order to succeed, these products need not only to hit the mark with vegans but attract a wider audience of flexitarians and omnivores. They need to have superior taste and texture and resemble their animal-based originals in terms of usability and nutritional profile. While traditional yogurt is made by fermenting milk with lactic acid bacteria and the product properties are universally standardized, the composition, production technology, and attributes of plant-based yogurt analogs, may vary significantly. The aim of this study was to expand our understanding of the composition and characteristics of commercially available plant-based yogurt alternatives focusing specifically on the content of live bacteria and to obtain a better overview of the processing technologies involved in the production of such products."

Methods: "The bacterial composition, with a specific emphasis on live bacteria, was evaluated using metagenetic sequencing of the 16S rRNA gene amplicons in combination with the novel PMAxx treatment approach. The profiles of organic acids, sugars, and volatiles were determined using HPLC-UV/RI and GC-MS, and descriptive sensory analysis was carried out to comprehensively describe the 25 yogurt alternatives made from soy (10), oat (9), coconut (4), and lupin (2)."

Results: "As it was presumed, the main ingredients defined the general characteristics of the products, yet considerable diversity was observed in chemical and microbiological composition, and sensorial attributes even among the yoghurt alternatives made from the same main ingredient. The estimated number of bacterial cells per gram of product ranged from log 4.4 – 9.4, while the proportion of living cells among all bacterial cells varied between 0.4 – 100.0 %. ***Streptococcus thermophilus*** and ***Lactobacillus rhamnosus*** dominated the bacterial communities, but according to the measurement of only living cells, the samples were far more diverse containing significant proportions of lactic and bifidobacteria."

Conclusions: "PMAxx treatment in combination with metagenetic sequencing proved to be a powerful tool to assess the number of live bacteria in plant-based yogurt alternatives and could be used to explore the microbial composition of other fermented foods. While the aim of this study was also to see, if based on the microbiological and chemical characteristics it would be possible to obtain an insight into the production processes behind the evaluated product, it became clear that the fine nuances of the production of plant-based yogurts are not easily revealed."

[41] HYBPI-CHEESE - A POSSIBILITY FOR ANIMAL PROTEIN REDUCTION WITHOUT LOSING ORIGINAL PRODUCT PROPERTIES

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Introduction: Replacing animal with plant-based proteins in nutrition is in trend. Many plant-based food alternatives are available. However, final products raw materials are often highly processed, fortified with additives and nutrients for improving textural und nutritional properties. So-called hybrid products could be an alternative to increase sustainability while maintaining sensory and nutritional properties. In this study, we tested the effect of partial replacement of milk by lupine mass in semi-hard cheese analogue.

Methods: Using the same starter culture, we developed a hybrid cheese analogue starting from micro-scale to pilot scale. With micro-scale in deep well plates (5ml) we evaluated the solubility and coagulation properties of different concentrations of milled lupin in milk. In mini-scale (250 ml) different alternatives to chymosin and the influence of $CaCl_2$ were tested and optimized. At a pilot scale (30 L) 25% of raw milk was replaced by lupine mass. The production followed the traditional recipe of a semi-hard cheese. After ripening for two months, the samples were analyzed for chemical and biochemical properties. Hybrid cheese analogues with 5, 10 and 15% of lupine mass were tested for acceptance.

Results: At micro scale level, a maximum of 25% lupine mass can be added while maintaining the original technology. At mini-cheese scale, Fromase was confirmed as optimal enzyme for coagulation visually by syneresis value. The addition of $CaCl_2$ was necessary for improved coagulation. At pilot scale, control and hybrid cheese analogue with 25% lupine had similar lactic acids contents after 24h. Due to lower syneresis and additional water absorption during the salt bath, the hybrid products had higher water contents (53.6±1.7 g/100 g vs. 42.1±2.3 g/100g). Salt content in the samples with lupine (2.99±3.1 g NaCl/100g) was significantly (p=0.002) higher than without lupine (1.9±1.3g NaCl/100g), even though the bath time was reduced. These factors seemed to increase protein degradation and to intensify the formation of aroma active compounds. Consumer acceptance was not significantly different for products containing 5, 10 and 15% lupine mass compared to the control.

Conclusions: The substitution of raw milk with up to 25% lupine mass allowed fermentation and ripening of a cheese-like product. Addition of compounds for textural and nutritional properties were not necessary.
Nevertheless, technological adaptions are needed to optimize control of the process and to decrease salt content. Further research is needed to evaluate the impact on sustainability and food safety risk of the new raw materials.

[42] GROWTH AND METABOLIC PROFILING OF LACTIPLANTIBACILLUS PLANTARUM IN CHEAP ALTERNATIVE GROWTH MEDIA

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Fermentation of lactic acid bacteria (LAB) are used in a wide variety of products for food, preservation, cosmetics, and supplements purposes. Growth media for LAB should therefore be versatile to fulfill the required needs for the specific products, both in terms of product efficacy but also industry specific regulatory needs.

In this study several growth media were developed from cheap and accessible raw materials in which ***Lactiplantibacillus plantarum*** were fermented. Each growth medium was tested for its ability to facilitate adequate bacterial growth (10^9 CFU/ml) and largescale production ease. Liquid and gas chromatography-mass spectrometry (GC-MS, LC-MS) was subsequently conducted on several of the media after end fermentation in order to quantify lactic acid and short chain fatty acids concentrations. Relative concentrations of all metabolites after end fermentation were also measured by LC-MS. In total 29 metabolites of interest were identified, in the different growth media after fermentation. Several antimicrobial metabolites were identified in the media as interesting in relevance to food preservation, while both antimicrobial, anti-inflammatory and antioxidant metabolites were identified as interesting for cosmetic use. Based on growth and metabolic profile, one medium was selected as the most suitable for cosmetic applications.

[43] EXPLORING THE POTENTIAL ANTIHYPERTENSIVE PROPERTIES OF SELECTED LACTIC ACID BACTERIA AND THEIR INCORPARATION IN YOGURT PRODUCTION

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Introduction: In current food industry, producing foods with an additional health benefit is the one of the main aims to provide conscious consumers' demands. Lactic acid bacteria (LAB) can secrete several compounds such as gamma-aminobutyric acid (GABA), exopolysaccharides (EPS). Also, fermentation is a method to convert food product to healthier form of itself. LAB metabolism may improve the bioactivity of compounds. Due to their enzyme portfolio, specific strains of LAB, proteolysis and acidification interaction occurs during fermentation, and thus bioactive peptides are formed. The inhibition of angiotensin-converting enzyme (ACE) activity is one of these properties of fermented protein fragments, which is associated with a potential antihypertensive effect. GABA is a non-protein amino acid and can be produced by LAB. It is related with several health benefits, especially its antihypertensive potential has been well explored. Thus, fermented foods can play an important role in the reduction of blood pressure.

Methods: *Lactobacillus delbrueckii* ssp. ***bulgaricus*** strains bTY5, bTY8, bTY11, bTY14a and bty27a was used in this study. GABA production abilities of these strains were screened by thin layer chromatography method. Also, these strains were used to ferment skim milk (10% w/v) at 42°C. ACE inhibitor activity potential in these fermented milks were investigated by RP-HPLC method. Selected strains were used for the yogurt production together with ***Streptococcus thermophilus*** strains. The ACE inhibitor activities and antioxidant activities of yogurt samples were investigated by DPPH and ABTS methods. The difference in these activities in yogurt by addition of GABA producer lactic acid bacteria is also studied. Physicochemical properties and microbial growth were also evaluated in the yogurt product.

Results: None of these strains showed GABA production abilities in MRS medium with 1% monosodium glutamate. Skim milks fermented by bTY5, bTY8, bTY11, bTY14a, bty27a and bTY30 showed 76,5%, 80,8%, 50,5%, 64.0% and 77.7% ACE inhibitor activities. pH values of fermented milks were in the range of 3.54 -3.77, respectively. Physicochemical properties of yogurts were found acceptable. Total viability of microorganisms was found above 8 log CFU/ml.

Conclusions: In this research, five ***Lactobacillus delbrueckii*** ssp***. bulgaricus*** strains were evaluated for their ACE inhibitor activities and GABA production abilities and selected strains were evaluated for their potential in yogurt production. Based on our results, ***in vivo*** studies using animal and human models are needed to confirm the possible antihypertensive effects of yogurt consumption.

[44] MODULAR METABOLIC ENGINEERING AND SYNTHETIC COCULTURE STRATEGIES FOR THE PRODUCTION OF AROMATIC COMPOUNDS IN YEAST

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Abstract
Microbial-derived aromatics provide a sustainable and renewable alternative to petroleum-derived chemicals. In this study, we used the model yeast ***Saccharomyces cerevisiae*** to produce aromatic molecules by exploiting the concept of modularity in synthetic biology. Three different modular approaches were investigated for the production of the valuable fragrance raspberry ketone (RK), found in raspberry fruits and mostly produced from petrochemicals. The first strategy used was modular cloning, which enabled the generation of combinatorial libraries of promoters to optimize the expression level of the genes involved in the synthesis pathway of RK. The second strategy was modular pathway engineering and involved the creation of 4 modules, one for product formation: RK synthesis module (Mod. RK); and three for precursor synthesis: aromatic amino acids synthesis module (Mod. Aro), ***p***-coumaric acid synthesis module (Mod. ***p***-CA) and malonyl-CoA synthesis module (Mod. M-CoA). The production of RK by combinations of the expression of these modules was studied and the best engineered strain produced 17.3 mg/L RK from glucose, which is the highest production described in yeast, and 0.87 mg RK/g glucose, which is the highest yield reported in any organism without ***p***-coumaric acid supplementation. The third strategy was the use of modular cocultures to explore the effects of division of labour on RK production. Two two-member communities and one three-member community were created, and their production capacity was highly dependent on the structure of the synthetic community, the inoculation ratio and the culture media. In certain conditions, the cocultures outperformed their monoculture controls for RK production, although this was not the norm. Interestingly, the cocultures showed up to 7.5-fold increase and 308.4 mg/L of 4-hydroxy benzalacetone, the direct precursor of RK which can be used for the semi-synthesis of RK. This study illustrates the utility of modularity in synthetic biology tools and their applications to the synthesis of products of industrial interest.

Keywords
Synthetic biology, combinatorial engineering, division of labour, microbial communities, ***p***-coumaric acid, raspberry ketone

[45] GROWTH RATE AND LIMITING SUBSTRATE DEFINE THE NUTRITIONAL COMPOSITION AND CELL SIZE OF MICROBIAL BIOMASS FOR FOOD APPLICATIONS

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Introduction: The biomass of microorganisms, such as bacteria, yeasts, fungi, or microalgae, can be a more sustainable alternative to conventional food-protein sources. Current research in microbial biomass production focuses on assessing the biomass quality only at the end of a batch process and often does not consider how limiting substrate conditions shape cell composition and product quality. At the same time, studies frequently prioritize maximizing the protein content or productivity neglecting other nutritionally relevant parameters such as content of specific proteins, amino acid (AA) profile or nucleic acid (NA) content.

Methods: The aim of this work was to address these knowledge gaps by evaluating the macromolecular composition as well as the protein and AA profile of growing microbial cells in function of (i) the availability of carbon and nitrogen, (ii) the growth rate and (iii) the cell size, using well-established bulk analyses and high-resolution analytical techniques such as flow cytometry and mass spectrometry-based proteomics (**Figure 1**). The results obtained using the biotechnologically relevant ***Cupriavidus necator*** and ***Komagataella phaffii*** were analyzed in the context of microbial biomass production for food applications. The key trade-offs between production rate, substrate availability and nutritional microbial biomass quality (with a focus on protein and NA) as well as microbial cell size were identified.

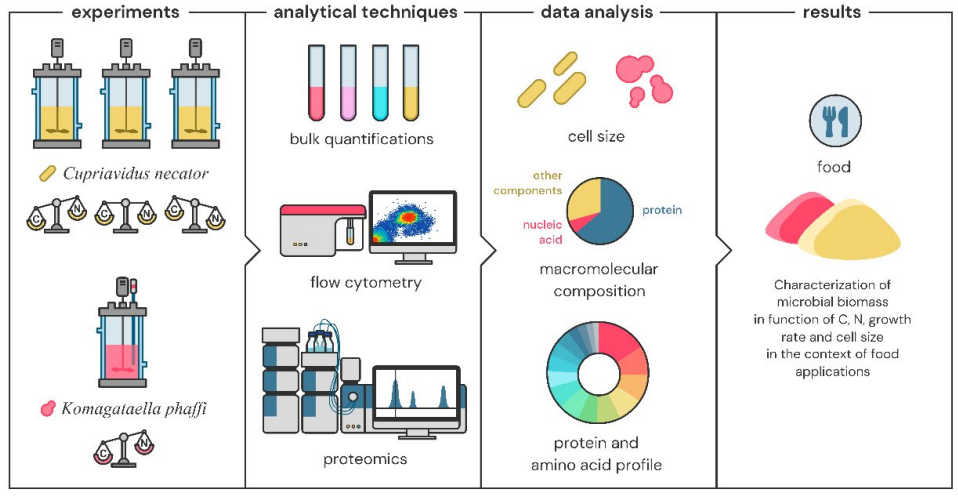

Figure 1: *Methodology followed for evaluating the characteristics of microbial biomass for food applications based on substrate availability, growth rate, and microbial cell size.*

Results: Low growth rates resulted in decreased NA and increased protein content, thereby increasing the nutritional quality of the product. Conversely, high growth rates resulted in larger cells, which could enable more efficient biomass harvesting. Limitation of carbon or nitrogen variably impacted the product quality, where nitrogen-limited cells were larger and contained more polyhydroxyalkanoates and neutral lipids. Distinct protein profiles and AA distributions were observed at different growth rates, which could substantially influence the techno-functional food properties of the produced microbial biomass. Specifically, lower growth rates yielded lower amounts of ribosomal proteins and higher glycine, phenylalanine, tryptophan and tyrosine content.

Conclusions: This work investigated the relationship between growth rate, substrate availability and cell composition and size of ***Cupriavidus necator*** and ***Komagataella phaffii***. We demonstrated that the highest nutritional quality of microbial biomass is achieved by cultivation at low rates after carefully selecting the limiting substrate.

Oral abstract

[46] GASTRONOMICS OF PLEUROTUS OSTREATUS MYCELIUM AS A NOVEL FOOD

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Introduction: In recent years, the demand for more sustainable and alternative food sources has increased, leading to a growing interest in the potential of fungal mycelium as a food ingredient. In this study, we examine the mycelium of the oyster mushroom, ***Pleurotus ostreatus,*** as a potential culinary material while also evaluating its food safety.

Results: We used whole-genome sequencing and pan-genome analysis to determine the taxonomic classification of the ***P. ostreatus*** strain M2191 and found a high degree of genetic variability within the genus. Our findings showed that the core of the genus consists of 990 protein families, which only represents 8% of the average proteome of the genus. This core includes functions related to central metabolism and housekeeping, while the accessory genome of strain M2191 includes functions involved in cell cycle regulation, transport, and catabolism. Metabolomic analysis showed that the mycelium of ***P. ostreatus*** does not contain detectable amounts of known mycotoxins. Furthermore, the expression levels of the ribosomally encoded peptide toxins Ostreatin and Ostreolysin A/Pleurotoysin B (OlyA/PlyB) were found to be lower in the mycelium compared to the fruiting bodies, which are considered safe for consumption. Additionally, using the sensory analysis of an oyster mycelium-based dish, we present the main attributes and sensory perception for this product, indicating consumer liking and openness to novel foods based on fungal mycelium.

Conclusions: Based on the relative sustainability, safety, culinary potential, and consumer acceptance, the findings of this study suggest that ***P. ostreatus*** mycelium has great potential for use as a novel food source.

[47] MICROBIAL PROTEIN FROM RECOVERED NITROGEN: NUTRITIONAL QUALITY, SAFETY AND FEASIBILITY ASSESSMENT

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Introduction: Microbial protein (MP), biomass from microorganisms rich in protein, produced with recovered N, could drastically increase the sustainability of our current food system. However, clear proof of the impact of recovered N-sources on process performance parameters and the nutritional and safety characteristics of the final product is lacking.

Methods: The suitability of seven recovered N-sources originating from different waste streams for MP production was evaluated in two sets of experiments (Figure 1). First, the impact of recovered N on microbial kinetics and yields was assessed through screening tests using 96-well plates. Second, the nutritional quality and safety of the biomass produced with recovered N via a fed-batch process was evaluated.

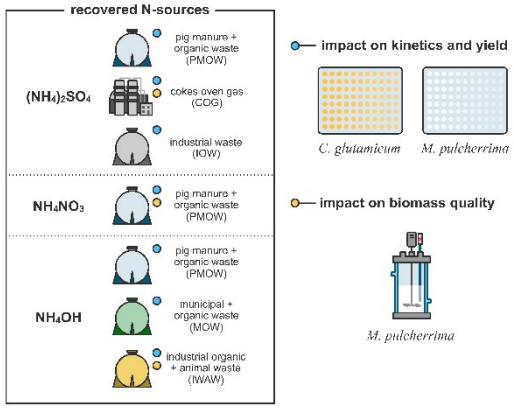

Figure 1: *Experimental methodology followed to assess the impact of selected recovered N-sources on the kinetics, yield, quality and safety of MP production*

Results: Six of the seven tested recovered N-sources presented insignificant differences in the specific growth rates of ***M. pulcherrima*** in comparison to their commercial equivalent. The biomass yield for recovered N was equal to or lower than (1.0-1.3 times) commercial N for both ***C. glutamicum*** and ***M. pulcherrima***. Also the macromolecular composition showed little variation over the different recovered N-sources tested in fed-batch. Moreover the ***M. pulcherrima*** biomass contained all essential amino acids and fatty acids for human consumption and a daily intake of 161-189g would cover the dietary reference intake of protein.
Heavy metals like As, Cd, Hg, Pb and Ni, were all detected in the ***M. pulcherrima*** biomass. However, an average person with a body weight of 62kg would need to consume more than 114-780g of biomass daily to exceed the provisional tolerable daily intake of these metals suggested by the European Food Safety Agency depending on the recovered N-source. None of the analyzed antibiotics were present in the biomass. In two of the three biomass samples, traces of two fungicides were found. Finally, ten out of eleven analyzed polyaromatic hydrocarbons (PAH) were found in the biomass. Moreover, all ***M. pulcherrima*** exceeded the maximum levels of PAH4 set by the European commission for all foodstuff including fishery products, oils and fats.

Conclusions: This work delivered a proof of concept that estimates the feasibility of using recovered N sources for MP production. We showed that ***M. pulcherrima*** biomass produced with recovered N had a similar nutritional value as soy flour and could be safe for human consumption if PAH are removed from the final product.

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