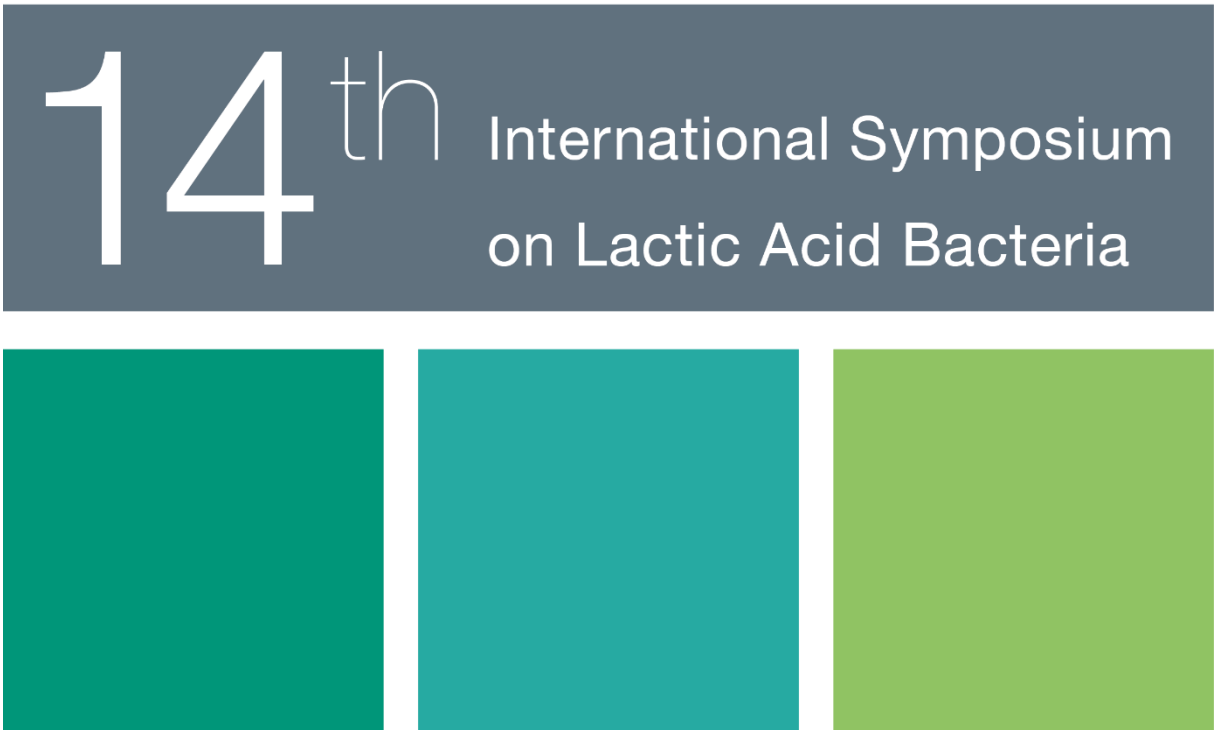
BOOK OF ABSTRACTS

14th Symposium on Lactic Acid Bacteria



27/08/23 – 31/08/23

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SHORT TALKS

Lipochaperon activity of Lo18 sHSP: from interaction forces to the residues involved.

Tiffany Bellanger, David Da Silva Barreira, Frank Wien, Patrice Delarue, Patrick Senet, Aurélie Rieu, Fabrice Neiers, Paloma Fernandez-Varela, Sophie Combet, Stéphanie Weidmann

Number
46

Themes
Fermentation and Metabolism, including protein transition

Abstract
Lactic acid bacteria (LAB), such as *Oenococcus oeni* involved in wine malolactic fermentation processes, are subjected to important environmental stresses. Most of these stresses will affect their membrane fluidity. In consequence, bacteria have developed different strategies of regulation. One of them is the production of small heat shock proteins (sHSP). Indeed, if all sHSPs are described for their role as molecular chaperones, some are also known to act as lipochaperone to maintain membrane fluidity in its optimal state following cellular stresses. These both activities have been observed on the Lo18 sHSP produced by *O. oeni*. Nevertheless, the molecular mechanisms involved in the lipochaperon activity are still poorly understood. The aim of this study was therefore to investigate these mechanisms involved in the binding between Lo18 and membranes, using *in silico, in vitro* and *in vivo* approaches such as anisotropy and Synchrotron Radiation Circular Dichroism (SRCD) measurements, as well as immunolabeling techniques. First, the membrane domains (in particular the type of phospholipids) and the amino acid residues of Lo18 involved in this interaction were identified. Secondly, the involvement of different forces such as hydrophobic and electrostatic interaction forces was highlighted for this interaction. Finally, the contribution of structural modifications of the Lo18 protein was shown to be essential to interact with the membrane.

Keywords
Lipochaperon activity, sHSP, Protein structural modification.

Lactobacillus crispatus produces a novel class IV lanthipeptide active against vaginal pathogens

Jelle Dillen, Sarah Ahannach, Thibaut Maeyens, Tom Eilers, Angus Weir, Eline Cauwenberghs, Isabel Erreygers, Stijn Wittouck, Peter Bron, Joleen Masschelein, Sarah Lebeer

Number
45

Themes
Microbial Communities
Bacteriophage and Antimicrobials

Abstract
Lactobacillus crispatus is the most dominant and prevalent species in the vaginal microbiome of healthy women. Its occurrence is clearly associated with urogenital and reproductive health, but the mechanisms that underlie its ecological dominance and beneficial mode of action are still largely unknown. In this study, we investigated the role of strain- and/or species- specific antimicrobial ribosomally-synthesized and post-translationally modified peptides (RiPPs). First, more than 100 *L. crispatus* strains were isolated from a subset of the 3,345 Flemish women that participated in our Isala citizen-science project on the vaginal microbiome (https://isala.be/en). These isolates were screened for their antimicrobial capacity against the most common vaginal pathogens *Streptococcus agalactiae, Gardnerella vaginalis* and *Candida albicans*. The genomes of a subset of 54 promising isolates were sequenced, followed by phylogenetical mapping of the occurrence of RiPP clusters via genome mining. Strain 815 displayed very high inhibitory activity against the tested pathogens, and this trait was matched at genome level with the presence of a novel class IV lanthipeptide cluster, a class with only few characterized members, unique to strain 815. The cluster was characterized *in silico*, identifying three putative propeptides, predicting their cross-links and the lanthipeptide-processing enzyme. The activity of the propeptides was experimentally validated through heterologous expression in *Escherichia coli.* Based on the promising activity of isolate 815, the isolate has potential applications as a live biotherapeutic for the treatment of vaginal infections.

Keywords
Vaginal microbiome, Genome mining, Antimicrobials

Riboflavin is a key mediator of metabolic interactions between Lactobacillaceae and the vaginal immune system.

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Number
34

Themes
Microbial Communities
Host Microbe Interactions

Abstract
The vaginal microbiome is essential for women's health, by being involved in reproduction, resistance to infections and mental wellbeing. Lactobacilli play a well-established role in determining healthy vaginal ecosystems and symbiotic interactions with the host, however the mechanisms behind their activity and functionality are not yet well-understood. Our Isala study investigated the vaginal microbiome of healthy women (n=3300) in Flanders (https://isala.be). We confirmed that *Lactobacillus crispatus* is most common in healthy women. We also observed a clear co-occurrence between *L. crispatus*, *Lactobacillus jensenii*, and low abundant but prevalent *Limosilactobacillus spp*. (detected in 47.6% of the participants at an average relative abundance of 0.4%) into a module of interacting taxa. Interestingly, the members of this module are well-known for riboflavin (vitamin B2) production, which is emerging as a key bio-energetic molecule for mediating metabolic cooperation in microbiome ecosystems. We experimentally confirmed riboflavin production by many vaginal Lactobacillaceae isolates from the Isala project, with *Limosilactobacillus reuteri* AMBV339 showing exceptionally high riboflavin production. Using untargeted metabolomics (HILIC), microbial riboflavin intermediates were detected in two thirds of vaginal swabs (n=64). To experimentally investigate interactions of riboflavin-producing Lactobacillaceae with the host, the expression of the riboflavin host-immune receptor, Major histocompatibility complex class I-related protein (MR1), was validated for vaginal epithelial cells (VK2) and overexpressing mutants (VK2.hMR1) were created. In addition, interleukin 7 and 12, commonly associated with microbial riboflavin signaling, were shown to be upregulated upon co-incubation with riboflavin-producing lactobacilli. This indicates that riboflavin has an important role in vaginal Lactobacillaceae-host interactions.

Keywords
Vaginal microbiome, riboflavin, immune response, metabolic cooperation

Molecular mechanisms underlying the structural diversity of complex rhamnose-rich cell wall polysaccharides in lactococci

Dr Hugo Guérin, Pascal Courtin, Alain Guillot, Pr Jennifer Mahony, Pr Douwe Van Sinderen, Dr Saulius Kulakauskas, Dr Christian Cambillau, Dr Thierry Touzé, **Dr Marie-Pierre Chapot-Chartier**

Number
24

Themes
Genetics and Genomics
Bacteriophage and Antimicrobials

Abstract
Rhamnose-rich cell wall polysaccharides (Rha-CWPS) are crucial cell wall components in a number of ovococci, which are functional analogs of wall teichoic acids. In lactococci, Rha-CWPS comprise two components: a conserved rhamnan embedded inside the peptidoglycan layer covalently linked to a surface-exposed polysaccharide pellicle (PSP). Notably, the structurally diverse PSP has an important function as a receptor for numerous bacteriophages infecting lactococci. We have proposed a model biosynthesis scheme in which rhamnan and PSP are synthesized independently, with PSP constituting a decoration added extracellularly onto rhamnan. Using *in vitro* enzymatic tests with lipid acceptor substrates combined with LC-MS analysis, modeling of protein 3D-structure, complementation experiments and phage assays, we examined the first two steps of PSP biosynthesis. We showed that PSP subunit is synthesized on an undecaprenyl-phosphate (C55P) lipid carrier, with a monophosphate linkage between the lipid tail and the subunit. Synthesis is initiated by WpsA/WpsB complex with C55P-GlcNAc synthase activity. PSP is then elongated by glycosyltransferases with variable specificity among strains, thus resulting in the synthesis of PSP with diverse structures. Moreover, we engineered the PSP biosynthesis pathway in lactococci to obtain a chimeric PSP structure, which allowed us to pinpoint the importance of a single residue of the PSP subunit in phage recognition. In conclusion, our results validate the biosynthesis scheme of PSP on a lipid-monophosphate intermediate as an extracellular modification of rhamnan, and shed light on the molecular mechanisms supporting the structural diversity of complex Rha-CWPS in lactococci.

Keywords
Lactococcus, cell wall, polysaccharide, bacteriophage receptor, glycosyltransferase

AI-driven design of microbial communities for dairy applications

Willi Gottstein
DSM

Number
73

Themes
Microbial Communities

Abstract
Designing microbial communities with a specific phenotype is a challenging task due to the complexity and very limited predictability of interactions among different species in the community. Microbial communities are composed of diverse populations that can interact with each other in multiple ways. Currently, we lack predictive computational approaches to select a subset of strains out of a set of available strains that leads to the desired phenotype once it is exposed to a certain environment. Therefore, an iterative approach is needed to design microbial communities.

Here, we present an AI-driven iterative approach that can be used to design cultures for dairy applications. Our case study is based on a real-world use case: Can we extend the shelf-life of a yogurt product without negatively impacting other KPIs, such as acidification rate, texture and taste? We started with a combination of a starter culture and a bioprotective strain; while the shelf-life is indeed extended, the post acidification is negatively impacted. The challenge we faced was to select the correct strains and their respective abundances to reach all specified targets simultaneously. Here, we explain how we designed several novel blends with the desired properties using AI in an iterative manner.

Keywords
Dairy, community design, AI, formulation, data-driven

The interplay between growth conditions and probiotic effector molecules; the case of *Bifidobacterium longum* NCC 2705 serine protease inhibitor

Dr Stéphane Duboux, Dr Biljana Bogicevic, Dr Annick Mercenier, Prof. Dr. Michiel Kleerebezem

Number
28

Themes
Genetics and Genomics
Fermentation and Metabolism, including protein transition

Abstract
To date, the potential impact of manufacturing conditions on bifidobacteria has not been studied at functional molecular make-up level. We used *B. longum* NCC2705 and its serine protease inhibitor (serpin) as a model to study how production processes may influence i) the amount of this important bifidobacterial effector molecule; ii) the strain metabolism and overall physiology. We demonstrated that the production of serpin in *B. longum* is regulated by the carbon substrate utilized, supporting that its production may involve catabolite repression. By implementing a fluorescent promoter reporter system coupled to flow cytometry, we confirmed the existence of glucose-mediated catabolite repression in NCC2705 and demonstrated that the level of repression elicited by the presence of glucose may be dependent on the alternative sugars substrate used. To clarify if glucose-catabolite repression influences other physiological properties of NCC2705 we used a combination of transcriptome analyses and physiological *in vitro* assays. This approach enabled us to show that industrially relevant physiological and metabolic features of the strain were significantly modulated upon growth on glucose or galactose as sole carbon source. Intriguingly, we discovered that part of those changes may be the subject of post-transcriptional regulation, arguing that sRNA may play a prominent role in the catabolite repression mechanism of *B. longum.* In conclusion, our results underline that industrial probiotic applications may benefit from a deeper understanding of fundamentals aspects of bacterial physiology and its impact on effector molecule expression, to provide a path to the development of effect-oriented, robust, and reproducible probiotic concepts.

Keywords
Bifidobacterium, probiotic, effector molecule, processing, catabolite repression, sRNA

Growth inhibition of lactic acid bacteria during starter culture production

Oscar Van Mastrigt, Sylviani Hartono, Tamara Bendig, Tjakko Abee, Eddy J. Smid

Number
13

Themes
Fermentation and Metabolism, including protein transition

Abstract
Lactic acid bacteria (LAB) are widely used as starter culture to make fermented dairy products, such as cheese and yoghurt. In these fermentations LAB produce lactic acid, thereby acidifying the food product and prolonging its shelf life. However, lactic acid production also poses a potential problem during the preparation of these starter cultures by inhibiting bacterial growth and thus decreasing biomass productivity, titer and yield. To provide an optimum pH for growth and alleviate inhibition by undissociated lactic acid, the pH is controlled at near-neutral pH values, where the dissociated lactate anion prevails. However, despite pH control, growth is inhibited as indicated by incomplete lactose utilisation in pH-controlled batch fermentations. Surprisingly, the underlying mechanism for this growth inhibition is still unclear. Inhibitory factors may include intracellular accumulation of the lactate anion, ionic and/or osmotic stress from lactate and counter-ions and/or another unidentified stress factor. In this study, we are quantitatively studying all the different potential inhibitory factors to decipher which factor mainly inhibits growth during pH-controlled batch fermentation of *Lactococcus lactis*. Applied methods include high-throughput growth rate measurements at varying lactate concentrations, pH values and osmolarities as well as pH-controlled batch fermentations and chemostat cultivations under specific compound limitations. Lactic acid concentrations and pH will be quantified intra- and extracellularly and linked to proteomes of selected samples. With this combined approach, we aim to get fundamental insights into microbial physiology during starter culture production and provide directions on how to improve biomass production and performance of starter cultures.

Keywords
Starter culture, Lactococcus, Physiology, Lactate, Osmotic, Transport

Galactose-positive adjunct cultures prevent gas formation by *Paucilactobacillus wasatchensis* WDC04 in a model gas production test

Dr. Taylor Oberg, Ireland Green, Jeff Broadbent, Craig Oberg, Donald McMahon, Randall Thunnell

Number
71

Themes
Microbial Communities
Fermentation and Metabolism, including protein transition

Abstract
Gas production by heterofermentative lactic acid bacteria such as *Paucilactobacillus wasatchensis* is a sporadic problem in Cheddar cheese and results in undesired slits and cracks in the cheese. A gas production model test was developed to investigate the effect of galactose and ribose utilization on gas production by *Pa. wasatchensis* and determine whether galactose-fermenting adjunct cultures could prevent gas formation. *Paucilactobacillus wasatchensis* WDC04 was inoculated at 101 to 106 cfu/mL into carbohydrate-restricted MRS broth containing different ribose and galactose levels and incubated for 21 days at 23°C. Gas production was detected using an inverted Durham tube. Cells were enumerated and residual galactose was measured at 4, 8, and 12 days. Gas production was sporadic except when 105 cfu/mL of WDC04 was inoculated into broth containing 0.3% ribose and 0.7% galactose. In those tubes, gas production was consistently observed after 8-day incubation, by which time galactose levels had decreased to 0.15%. Co-inoculation of WDC04 with as few as 103 cfu/mL of a lactose-negative galactose-positive adjunct culture resulted in galactose depletion by day 4 and no observable gas production by day 12. With less galactose available to the slower-growing WDC04, growth was limited to 108 cfu/mL when any of the adjunct cultures was co-inoculated, compared with 109 cfu/mL when grown independently. We concluded that galactose fermenting adjunct cultures have potential for preventing unwanted gas production in cheese by removing the 6-carbon galactose before it can be utilized for energy and gas production by heterofermentative lactobacilli such as *Pa. wasatchensis*.

Keywords
Protective cultures, Gas Defect

Knock-out and knock-in of lactococcal phage neck passage structure gene

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Number
70

Themes
Genetics and Genomics
Bacteriophage and Antimicrobials

Abstract
Members of the *Skunavirus* viral genera are the most isolated lactococcal phages in cheese factories and have been extensively studied because of their negative impact on milk fermentation. Whole genome sequencing was performed on 54 cheese-plant-isolated phages all infecting the *Lactococcus* strain SMQ-1001. Comparative genomic of their genomes revealed that the neck passage structure (*nps*) has a high sequence diversity.

While the function of most of the *Skunavirus* structural proteins is known, the *nps* still has an elusive function related to host recognition. The *nps* is present in 70% (143/213) of sequenced *Skunavirus* phages. Ranging from 134 to 888 amino acids, it shares a low percentage of identity of 41.3%. As previously reported, the first 180 N-terminal amino acids of the Nps proteins are conserved while the C-terminal is more diverse.

Using a previously developed CRISPR-Cas9 based method, we report the construction of a *nps*-knock-out mutant and a *nps*-knock-in mutant highlighting the high modularity of lactococcal phage genomes. First, a deletion of 2,486 bp encompassing the whole Nps protein and part of the flanking regions was generated in phage JeB4 (JeB4âˆ†*nps*) infecting SMQ-1001. Second, a knock-in of the gene coding for a NPS protein and its flanking regions (2,190 bpwas performed in the model lactococcal phage p2 (p2*nps+*). These specific phage genome editions were confirmed by Sanger and Illumina sequencing.

Electronic microscopy observations confirmed the absence and the presence of the NPS in phages JeB4âˆ†*nps* and p2*nps+*, respectively. Characterization of phage adsorption and infection kinetics are underway.

Keywords
genome editing, bacteriophages, neck passage structure, adsorption

Cultivation of *Limosilactobacillus reuteri* DSM 17938 on Galactooligosaccharide (GOS) reduces prophage activation, and increases stress tolerance, stability and bioactivity

Mr Ludwig Ermann Lundberg, Dr Yanhong Pang, Dr Roger Karlsson, Dr. Hans Jonsson, Prof. Stefan Roos

Number
36

Themes
Fermentation and Metabolism, including protein transition
Bacteriophage and Antimicrobials

Abstract
Today, most probiotics are produced with glucose as carbon source. We queried if this is a stressor in itself and evaluated if the prebiotics galactooligosaccharide (GOS), could be a more suitable substrate for production of stress tolerant and efficient *L. reuteri* DSM 17938. Initially, we discovered that cultivation on GOS instead of glucose improved several properties of importance for probiotics, including bile tolerance, acid tolerance, adhesion to mucus and storage stability after freeze drying. Furthermore, GOS cultivated bacteria gave a better protection against ETEC induced leakage in an epithelial cell model containing cocultures of Caco-2 and HT-29-MTX. To understand the underlying mechanisms of these effects, we performed a quantitative proteomic analysis using tandem mass tags, comparing GOS- and glucose-cultivated bacteria. The results revealed that 12 proteins were upregulated and 53 were downregulated 2 times or more. The upregulated proteins were associated to GOS metabolism. Interestingly, 29 of the 53 downregulated proteins were associated to prophages, indicating that cultivation on GOS gives a lower activation of the prophages than on glucose. Sixteen of these proteins were downregulated 10-30 times and thirteen were downregulated 1.5-10 times. We suggest that there is a link between the increase of stress tolerance and storage stability of GOS-cultivated *L. reutei* DSM 17938 and a lower activation of prophages. These findings show that carbon source optimization could improve the properties of *L. reuteri* DSM 17938 and potentially allow probiotic strains with poor tolerance profiles to better cope with the stressors of the production and encountered *in vivo*.

Keywords
Galactooligosaccharides, prophage activation, carbon source, tolerance, bioactivity

Comparative genomics and methylome analysis of *Bifidobacterium longum* subsp. *longum* reveals an extensive defense mechanism against DNA acquisition.

Francesca Bottacini, Keisuke Yoshida, Richard J. Roberts, Ortensia Catalano Gonzaga di Cirella, Brian McDonnell, Jin Zhung Xiao, Toshitaka Odamaki, Douwe Van Sinderen

Number
23

Themes
Genetics and Genomics

Abstract
Bifidobacteria are Gram-positive gut commensals, key representatives of the gut microbiome of healthy and breast-fed infants. Their beneficial effects on human health have made functional genomic applications particularly relevant for understanding the role of this genus within the intestinal gut community. It is well established that R/M systems in *Bifidobacterium* are the main obstacle to HGT and foreign DNA acquisition, however, little is known about their biological implication in gut colonisation.

In the current study, we applied comparative genome and methylome analysis to a set of 31 novel strains of *Bifidobacterium longum* subsp. *longum*, one of the most prevalent gut commensals across the lifespan and especially abundant among the Japanese population.

Our comparative and methylome analysis established that the *B. longum* genomes harbour a unique and diverse set of R/M systems, rendering genetic accessibility of this species particularly challenging. The presence of a large set of R/M systems seems to be driven by the necessity of counteracting DNA acquisition in the challenging gut environment. Particularly in the case of Type I R/M systems, we observed a wide and strain-specific range of methylated sites associated with only four systems distributed across this species. These results suggest that *B. longum* developed a diverse defense mechanism to counteract foreign DNA acquisition, mostly relying on hypervariable Type I R/M systems. Overall, our study indicates different strategies adopted by bifidobacterial species in surviving the challenging gut environment and represent an important step forward in the expansion of functional investigations to species that are genetically intractable.

Keywords
Bifidobacterium, metylome, comparative genomics, functional genomics, HGT

Adaptative evolution of the wine bacterium *Oenococcus oeni* triggered the slowdown of citrate metabolism: Transcriptomic investigation of the citrate locus

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Number
50

Themes
Genetics and Genomics

Abstract
Oenococcus oeni is a Lactic Acid Bacteria responsible for malolactic fermentation in wine and able to resist to different wine related stresses. Nevertheless, stuck or delayed fermentation can occur when the environmental conditions are too drastic for the bacterium such as in very acid wines.

A directed evolution procedure was conducted on the ATCC BAA-1163 *O. oeni* strain in order to increase its acid-tolerance (Julliat *et al*., 2023). Genome sequencing of the evolved populations obtained revealed mutations in non-coding regions of the citrate locus. Transcriptional analysis showed that these mutations impact the whole operon expression level, associated with the slowdown of citrate uptake in the evolved populations. Two transcription start sites were identified and confirmed that mutations present in the evolved populations are positioned in -10 and RBS regions. Furthermore, these changes in citrate consumption led to an optimization of the bacterial growth in acid conditions in presence of citrate.

On the other hand, screening of citrate consumption in various *O. oeni* isolates highlighted that the ATCC BAA-1163 strain consumes citrate very quickly. Genomic comparison highlighted a one base deletion in this strain leading to a premature stop-codon in *citR*, the gene encoding a putative transcriptional regulator of the operon. Complementation of ATCC BAA-1163 with a functional *citR* gene confirmed that *citR* encodes a transcriptional repressor.

To date, no study has been mentioned concerning the expression and regulation of the *O. oeni cit* locus. Thus, the present work brings new information concerning the regulation of citrate metabolism in *O. oeni*.

Keywords
Oenococcus oeni, Evolution, Citrate operon, transcriptional regulation

Living Therapeutic Materials - Gels with engineered lactobacilli for smart drug delivery

Dr. Shrikrishnan Sankaran, Sourik Dey, Marc Blanch-Asensio, Varun Sai Tadimarri

Number
6

Themes
Genetics and Genomics
Host Microbe Interactions
Bacteriophage and Antimicrobials

Abstract
Lactobacilli colonize a wide range of host microenvironments like the gut, skin, vagina, oral cavity, etc. and provide health benefits. Several probiotic strains are being developed as live biotherapeutics, some by genetically engineering them to produce and secrete specific drugs in the body. While this approach has shown promise, it faces 2 major challenges – (i) scarcity of genetic tools to control drug production and (ii) unpredictability of drug doses due to variable bacterial colonization.

We tackle these challenges by combining synthetic biology with biomaterials. To address (i), we are expanding lactobacilli's genetic toolbox by identifying new promoters and retention systems[1, 2], developing stimuli-responsive regulatory modules for gene expression and encoding the secretion of therapeutic peptides, whose bioactivities are being tested *in vitro*. To address (ii), we develop hydrogel matrices that control lactobacilli's growth and metabolism, ensuring predictable drug doses. These bacterial gels can be fabricated in different formats to suit different body sites while improving biosafety. Through these advances, our Living Therapeutic Materials concept introduces a novel approach to treat diseases with lactobacilli.

[1] S.Dey, et al. Microbial Biotechnology, 2023, https://doi.org/10.1111/1751-7915.14228

[2] M.Blanch-Asensio, et al. PLOS ONE, 2023, https://doi.org/10.1371/journal.pone.0281625

Keywords
Lactobacilli, genetic-toolbox, promoter, toxin-antitoxin, regulator, synthetic-biology, drug-delivery

The stressostat: a novel approach in adaptive laboratory evolution to improve end-product resistance

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Number
21

Themes
Microbial Communities
Fermentation and Metabolism, including protein transition

Abstract
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, but none of the existing ALE methods could select for improved end-product resistance. Therefore, 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 lactate is applied. During the fermentation, the lactate concentration increases *in situ* through LAB fermentation. In this study, we used *Lactococcus lactis* FM03P as a model organism. During 35 days of stressostat cultivations, we isolated 34 variants in which most of them could grow at higher lactate concentrations than the wild type. However, the variants grew slower than the wild type at control media without lactate 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. In conclusion, 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.

Keywords
adaptation, chemostat, lactate, robustness, Lactococcus, starter culture

EndPointFBA: a novel mechanistic modelling approach to predict cross-feeding interactions in yoghurt starter cultures

Francesco Moro, Sebastian Mendoza, Julia Lischke, Frank Bruggeman, Bas Teusink

Number
48

Themes
Microbial Communities
Fermentation and Metabolism, including protein transition

Abstract
In the last two decades, mathematical modelling has contributed to better understanding and prediction of microbial metabolism of microbes in monoculture, exploring integrative aspects that are not easily accessible by experiments or intuition. In the context of food biotechnology, this paved the way for improved starter culture selection and functionality. However, most fermented foods require microbial consortia for their productions, and current computational methods come short in modelling microbial community behaviour, especially when it involves cooperative interactions.

Here we present a new method, EndPoint Flux Balance Analysis (EndPointFBA), which predicts the most effective metabolic behaviour for a microbial consortium in a predictable dynamic environment, assuming its members are well adapted to the process. One such environments is repeated batch culture as traditionally done by back-slopping of fermentation processes, such as for kefir or yoghurt.

We therefore applied EndPointFBA to a coarse-grain model of a yoghurt starter culture, composed of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*. Back-slopping allowed *S. thermophilus* and *L. bulgaricus* to co-evolve and adapt not only to the milk matrix, but also to each other. The development of mutualistic behaviours led to faster growth and acidification rates, key features for high quality yoghurt production.

Our method effectively predicted cross-feeding interactions based the individual microbes' metabolic networks. Including exchange kinetics subsequently improved dynamic resolution. We foresee its application to genome-scale models which will allow *in silico* screening of large strain libraries and select promising combination of microbes, contributing to the design of more effective starting cultures for food fermentations.

Keywords
Microbial communities, Metabolic modelling, Yoghurt, Cross-feeding, FluxBalanceAnalysis

THEMATIC SESSION 1: MONDAY 28

Selection of starter cultures for novel food fermentations

Prof. Dr. Michael Gänzle

Number
41

Themes
Fermentation and Metabolism, including protein transition

Abstract
Fermentation as a unit operation in food processing is currently experiencing a resurgence, with increasing interest both in traditional fermented foods such as sourdough bread or fermented vegetables, and in novel, innovative fermented foods such as fermented vegetable juices, or plant/insect-based analogues for fermented meat and dairy products. The classical approach to development of starter cultures – sample traditionally produced templates, isolate fermentation microbes, select for competitiveness and functionality, and the amenability to large scale production of biomass – is not applicable for these products. The selection of fermentation / starter cultures for novel fermented foods can be partially based on traditional fermented foods that are produced with traditional knowledge systems in South America, East Asia and Africa. In addition, current knowledge linking the phylogeny of food fermenting microbes to their ecology and metabolic activities provides a sound framework to guide the selection of functional starter cultures for applications in novel food fermentations. This presentation will communicate how phylogenetic position of lactic acid bacteria relates to metabolic traits relating to metabolism of carbohydrates, organic acids, amino acids, and (poly)-phenolic compounds, and now these clade-specific traits can be exploited in selection of starter cultures for traditional and innovative fermented foods.

Keywords
Fermented foods, starter cultures, Lactobacillus, metabolism,

Biopurification – Microbial Conversions to Improve The Quality of Plant Proteins

Dr Avis Nugroho, Dr Herwig Bachmann, Saskia Van Schalkwijk, Simon Jacobs, Wilma Wesselink, Andrei Prodan, Ann Stijnman, Emma, Kerensa Broersen

Number
143

Themes
Fermentation and Metabolism, including protein transition

Abstract
Securing a sustainable food supply in the decades to come requires a shift toward a more plant-based diet. Plant-based ingredients such as protein concentrates and isolates are increasingly used, but they are limited by unpleasant sensory-active compounds. However, current processing solutions, including traditional fermentation, exchange off-flavours with (techno-)functionality of proteins or require lengthy incubation where spoilage microbes can thrive.

Here, we systematically screen more than 100 food-grade microorganisms for their potential to remove off-flavours in almond, oat, pea, and potato proteins. To produce a purified rather than fermented ingredient, strains with limited production of fermentation end products, particularly those derived from pyruvate, were selected. We demonstrate that various Lactic Acid Bacteria (LAB) and yeasts removed “green” volatiles belonging to aldehydes and ketones. Using CATA (Check-All-That-Apply) analysis for sensory evaluation, a decrease of the sensory attributes pea and green were confirmed. Process optimization allowed us to achieve such conversions in less than an hour and at a low protein hydration content. We could further confirm that under the chosen conditions protein solubility, emulsification, foaming abilities, and digestibility were unaltered. Next to volatiles, the optimization of strains through adaptive evolution, the degradation of bitter or antinutritional non-volatiles, and the use of genome-based pathway prediction will be shortly discussed. Finally, the overall application perspective, e.g., positioning of biopurification in the processing chain in combination with physicochemical processes, will be provided. The presented results will help to improve consumer acceptance of plant-based food.

Keywords
Plant proteins, LAB, yeasts, non-growing, fermentation, sensory

Dairy cultures go Vegan!

dr Ineke Van Boeijen, dr Sofia Dashko, Fleur Bollee, dr Neleke Van Nieuwenhuijzen, dr Clair Price, MSc Maarten Van de Graaf

Number
19

Themes
Fermentation and Metabolism, including protein transition

Abstract
The demand for plant-based dairy alternatives, including fermented products, is on the rise due to various factors such as health concerns, environmental impact, and ethical considerations. This has created a growing market for vegan or dairy-free alternatives. As a result, there is a need for suitable starter cultures to produce these products. However, traditionally, dairy cultures are cultivated on media containing dairy ingredients, like milk, lactose, and whey-components.

When talking about dairy alternatives, there is not one alternative to milk, but a large variety of plant bases that can be used. Therefore, the project involved screening various *Streptococcus thermophilus* and *Lactobacillus bulgaricus* strains to identify those with optimal performance on acidification speed, taste and texture in various plant-based substrates such as soy, oat, coconut and pea. In the next step we have developed dairy-free media for starter cultures to fit fermented plant-based dairy alternatives. Of the strains selected, some strains showed impaired performance upon conversion to a dairy-free format, highlighting the challenges involved in developing suitable dairy-free starter cultures.

Following successful lab scale tests, the vegan starter cultures were scaled up to operations. Subsequently, the application team developed a number of blends to meet customer needs for producing a wide variety of high-quality fermented dairy alternatives. Recently, the vegan starter cultures were launched as part of the Plant Power Toolkit.

Keywords
vegan, starter cultures, dairy-free media, fermentation

Selection of proteolytic LAB starter cultures for acidification of soy based dairy alternatives.

Blandine Genet, Hang Xiao, Lise Friis Christensen, Ida Nynne Laforce, Mohammad Amin Mohammadifar, Claus Heiner Bang-Berthelsen, Egon Bech Hansen

Number
100

Themes
Fermentation and Metabolism, including protein transition

Abstract
61 strains isolated from different environments in Denmark (plant, fermented food, dairy and animal faeces) were investigated for their capacity to produce a curd structure during fermentation of Soy Based Milk Alternative (SBMA). The acidification dynamic was determined in commercial SBMA at 30 °C and the production of metabolites was investigated by HPLC analysis. The proteolytic activity was determined and for selected strains proteomics was used to determine the hydrolysis profile for individual soy proteins. SBMA samples fermented with strains identified to be suitable as starter cultures were characterized with rheology and the genome sequence was analysed. From an initial selection of 61 strains, 29 (5 *Lactococcus lactis*, 4 *Lactiplantibacillus plantarum*, 6 *Leuconostoc mesenteroides*, 3 *Leuconostoc pseudomesenteroides*, 1 *Leuconostoc citreum*, 1 *Leuconostoc lactis*, 6 *Lactobacillus*, 2 *Pediococcus pentosaceus* and 1 *Weissella*) were found to acidify SBMA, with final pH ranging from 4.46 to 6.07. Lactic acid concentration ranged from 1.8 mg/mL to 4.3 mg/mL. Among the acidifying strains, 10 were found to grow on raffinose, and all showed growth on saccharose. Two strains of *L. lactis* and three strains of *Ln. pseudomesenteroides* were found to have proteolytic activity in soy, and the proteolysis patterns of glycinin G4 (UniProtKB: P02858) and β-conglycinin α subunit 1 (POD016) were investigated further in depth. Moreover, they were the only investigated strains presenting a PrtP sequence in their genomes. In view of the results in all the analysed parameters, these five strains are candidates of particular interest to produce a soy-based yogurt-like product.

Kewords
cell envelope proteases, dairy alternatives, Genomics, Proteomics

Cell-to-cell non-conjugative molecular transfer between *Bacillus subtilis* and Lactic Acid Bacteria: novel routes for natural strain improvement.

Luiza Morawska, Oscar Kuipers

Number
112

Themes
Microbial Communities
Genetics and Genomics

Abstract
In nature, bacterial genetic modifications commonly occur via horizontal gene transfer (HGT) mechanisms, where co-existing microorganisms can acquire new genes to gain beneficial traits from their neighbours. *Bacillus subtilis* is a soil-dwelling bacterium that interacts with a plethora of other microorganisms in its natural habitat. Due to its versatile interactions with neighbouring bacteria and the ability to form nanotubes, i.e., recently described membrane structures that trade cytoplasmic content between neighbouring cells, we investigated the potential of molecular transfer from *B. subtilis* to industrially relevant members of Lactic Acid Bacteria (LAB) in mixed bacterial co-cultures.
In this work, we developed a co-culturing protocol and provided proof of transfer of a small high copy non-conjugative plasmid from *B. subtilis* to LAB and vice versa. We demonstrated that intra- and interspecies plasmid transfer did not involve conjugation or competent state activation in *B. subtilis*, and for the first time, we showed transfer of a high copy, non-conjugative plasmid in mixed co-cultures of either *B. subtilis* and *Lactococcus lactis* or *B. subtilis* and *Streptococcus thermophilus*.
Besides the plasmid transfer, we explored the potential to transfer the competence transcription factor ComK to *comK*-deficient strains of *B. subtilis* and provided evidence for transient competence induction and plasmid transformation in co-culturing conditions with a hyper-competent donor strain.
Our study indicates that cell-to-cell transformation and molecular transfer is a ubiquitous form of HGT and can be potentially utilized as an alternative tool for natural (non-GMO) strain improvement and transient induction of the competent state.

Kewords
nanotubes, horizontal gene transfer, natural strain improvement

Stability and evolution of microbial communities in water kefir fermentation in response to strain and phage invasions

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Number
210

Themes
Microbial Communities
Genetics and Genomics
Bacteriophage and Antimicrobials

Abstract
Microbial communities in nature exhibit intricate interactions between diverse prokaryotes, eukaryotes, and phages. In contrast, fermented foods represent simplified, yet undefined microbial systems primarily composed of yeasts and lactic acid bacteria. Historically, they are produced in non-sterile environments that promote microbial exchange and interactions with the external environment. Despite continuous passaging in small batches, these microbial communities reliably and safely ferment foods. While we are aware of specialized LAB species, we are lacking an understanding of genomic diversity. Here, we study population responses at a high genomic resolution to different households, i.e. microbe and phage invasions, Within the framework of a master course, we let 35 students passage water kefir for several generations in their households using various carbon sources. By shotgun sequencing the water kefir over time, we document population responses on an ecological and evolutionary timescale. Our findings indicate a substantial replacement of accessory (i.e. rare) species, while minimal exchange or mutations were observed within strains of the dominant lactic acid bacteria. Surprisingly, these strains remained stable even in the presence of household-specific phage occurrences. These observations suggest the existence of a diverse repertoire of phage defense mechanisms withstanding and potentially shared within the microbial community. An in-depth exploration of these ecological and evolutionary dynamic defense mechanisms holds promise for understanding the stability of microbial communities. By elucidating the intricate interactions between microbial species, strains, and phages, we can gain a deeper understanding of the processes underlying the reproducibility and safety of fermented foods.

Kewords
Eco-evolutionary dynamics, phage-host dynamics, shotgun metagenomics

StrainInSight: a unique private strain collection and innovative tools to facilitate novel strain discovery in the food and feed industry.

Charlotte Peeters, Eliza Depoorter, Anneleen D. Wieme, Ilse Cleenwerck, Ann Hellemans, Peter Vandamme

Number
163

Themes
Microbial Communities
Genetics and Genomics

Abstract
The Laboratory of Microbiology at Ghent University (LM-UGent) presents a unique opportunity for researchers to access its extensive private strain collection that originates from research projects focusing on bacterial diversity and ecology in very diverse environments. The StrainInSight collection currently conists of over 45,000 bacterial strains, of which approximately 7000 strains are lactic acid bacteria. Our strains were identified using state-of-the-art methods available at the time of isolation and extensive efforts using modern methods (i.e. MALDI-TOF MS and whole-genome sequencing) are ongoing to further refine strain identifications.
To facilitate novel strain discovery, we have developed two innovative tools. Firstly, our interactive dashboard provides researchers with a visual and intuitive interface to explore the metadata (e.g. identification, isolation source and geographical origin) associated with our strain collection. This user-friendly interface streamlines the strain selection process and allows researchers to quickly identify strains of interest.
Secondly, we developed a comprehensive genome database that currently comprises over 1,000 annotated genomes of food-grade bacteria. Equipped with a user-friendly interface and customizable filters, this genome database allows users to search for specific enzymes or metabolites within the genomes of our strains. This feature empowers researchers to identify strains that possess desired genetic traits, facilitating targeted investigations and applications.
By accessing the StrainInSight private strain collection and utilizing our innovative tools, researchers can tap into a wealth of microbial diversity, facilitating the selection of novel strains with interesting functionalities for the food and feed industry.

Kewords
Novel strain discovery, User-friendly databases, Genome database

A plasmid encoded sortase-mediated pilus is involved in the formation of strong biofilms in histamine-producing *Lentilactobacillus parabuchneri*

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Number
105

Themes
Microbial Communities
Genetics and Genomics

Abstract
Lentilactobacillus parabuchneri is the main responsible for the accumulation of the toxic biogenic amine histamine in cheese. Previous studies have also shown the ability of some *L. parabuchneri* strains to form strong biofilms on various surfaces, including stainless steel. These biofilms are a source of contamination in the Dairy Industry that finally cause the accumulation of histamine, particularly in post-ripening processed cheeses such as those grated and sliced.

The objective of this study was to identify the genes of *L. parabuchneri* involved in strong biofilm formation and determine their functionality. For this purpose, the genomes of six *L. parabuchneri* strains with different biofilm production capacities -strong, moderate, and weak- were sequenced and analyzed. The presence of the histidine decarboxylase gene cluster responsible of histamine production was confirmed in all strains. Additionally, a four genes cluster (8 kb) was identified only in strains with strong biofilm-forming ability. The cluster showed similarity to genes implicated in pili formation through a sortase-mediated pilus model (SpaAâ€type), suggesting a potential role in surface adhesion. Cloning and heterologous expression in *Lactococcus cremoris* NZ9000 confirmed its implication in adhesion, and subsequently in biofilm formation. In addition, PacBio sequencing confirmed that the pilus-cluster is located in a plasmid of 33.4 kb, which would favor the horizontal transmission of the adhesion capacity and strong biofilm formation.

Kewords
Lentilactobacillus parabuchneri, histamine, biofilm, pili, plasmid

Exploring Phage-Host Interactions through CRISPR-cas Loci in Dairy Cows

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Number
166

Themes
Microbial Communities
Genetics and Genomics
Bacteriophage and Antimicrobials

Abstract
The fecal microbiome of dairy cows provides the opportunity to mine a relatively under-explored resource of biological and economic relevance. Using metagenomic analyses, we investigated the bacterial and bacteriophage populations in diverse dairy cow fecal samples, with a specific focus on CRISPR-Cas systems as a means to decipher the interplay between hosts and phages within the gut environment. We analyzed CRISPR arrays to establish genetic linkage between specific host spacers and homologous viral sequences. An analysis showcasing the relationship across cow samples spanning multiple breeds co-located within a farm revealed viral interrelatedness within a herd subset. Specifically, our results showed that the dairy cow fecal microbiome has unexpected diversity and variability in bacterial and bacteriophage composition, especially with regards to *Siphoviridae* associated with species of the *Streptococcus*, *Lactobacillus, Lactococcus* and *Bifidobacterium* genera. In particular, we discovered consistent interactions across multiple cows through the presence of Type I-E CRISPR spacers that specifically targeted the prophage regions of *Bifidobacterium pseudolongum*. To evaluate the potential functionality and biotechnological potential of select single effector Cas proteins, we utilized an *E. coli*-based cell-free transcription-translation system. Specifically, we identified and characterized novel and diverse Cas9 and Cas12 orthologs with various guide RNA and PAM-targeting features showcasing promising and competitive activity levels. This approach provides a framework to explore bacteriophage-host dynamics using hypervariable CRISPR arrays in metagenomic datasets, and the repurposing of CRISPR-Cas systems and their effectors to manipulate the composition and function of important microbiomes, such as the dairy cow microbiome.

Kewords
Metagenome, Bacteriophage, CRISPR-Cas, Microbiome, Virome, Bifidobacterium

Genetic variation in *Saccharomyces cerevisiae* impacts fitness in the presence of lactic acid bacteria

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Number
61

Themes
Microbial Communities
Genetics and Genomics

Abstract
Introduction: Yeast and bacteria often coexist in the same niche, such as in fermented foods like sourdough. Understanding the genetic and evolutionary mechanisms promoting the fitness of different species is crucial to better comprehending their ecological interactions. Here, we investigate the impact of genetic variation in *Saccharomyces cerevisiae* on its fitness in the presence of two lactic acid bacteria, *Lactobacillus plantarum* or *Fructilactobacillus sanfranciscensis*.

Methods: We measured the fitness of 1,011 genetically variable *S. cerevisiae* strains co-cultured with either *L. plantarum* or *F. sanfranciscensis* on Sourdough Media Agar, pinned on top of a lawn of lactic acid bacteria. We assessed the growth rate of *S. cerevisiae* strains and identified strains with altered fitness.

Results: We found that genetic backgrounds of *S. cerevisiae* strains displayed both inhibition and facilitation of growth in the presence of *L. plantarum* or *F. sanfranciscensis*. We identified strains of *S. cerevisiae* where the fitness was significantly affected in the presence of either LAB.

Discussion: These results suggest that genetic variation in *S. cerevisiae* can impact its fitness in the presence of LAB, indicating the potential for genetic adaptation to specific ecological niches. Further investigations using quantitative trait loci mapping will help to identify specific genetic loci contributing to the observed fitness variation.

Conclusion: Our findings provide insights into the genetic mechanisms that promote the coexistence of *S. cerevisiae* with lactic acid bacteria in sourdough, and could lead to the development of strategies for manipulating microbial communities in fermentation processes.

Keywords
sourdough, genetics, yeast, quantitative trait loci mapping

Synthetic ecology as a tool to unravel the mechanisms behind lactobacilli dominance in the vagina

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Number
62

Themes
Microbial Communities
Fermentation and Metabolism, including protein transition

Abstract
The vaginal microbiome is generally dominated by *Lactobacillus* spp. in optimal conditions. Yet, a deeper understanding on why these lactobacilli dominate the vagina and how they interact with other vaginal microorganisms is still limited. Synthetic microbial communities (SynComs) enable the study of community interactions, dynamics and disturbances in more controlled conditions. Within the Isala project, four modules of intercorrelated taxa were observed in vaginal microbiome samples; each named after the most abundant taxon. The *L. crispatus*-module, containing also *L. jensenii* and *Limosilactobacillus,* is of interest as it showed to be mostly associated with health. To better understand the architecture of this module, we subsequently aimed to examine it in a SynCom. In a top-down experiment, swabs were cultivated and diluted over several days. A more simple community was established, dominated by *L. crispatus*. Moreover, all three members of the *L. crispatus*-module stabilized in this SynCom. These data suggest that the bacteria of the *L. crispatus*-module do exert synergistic interactions, which can be mimicked in SynComs. Next, we performed a bottom-up experiment, where the module was manually assembled. Metabolomics will unravel the cross-feeding network, while qPCR is used for quantitative community composition. These experiments are the first steps towards developing SynComs with the aim to understand how lactic acid bacteria can positively interact in the vaginal ecosystem and benefit the host. These *Lactobacillus*-dominated SynComs are also a promising tool to explore external and internal influences, invading pathogens or pathobionts, and metabolic networks.

Keywords
synthetic ecology, vaginal microbiome, microbial interactions

Discovery & Characterization of Phages Targeting Carnobacteria

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Number
164

Themes
Bacteriophage and Antimicrobials

Abstract
Carnobacterium maltaromaticum and *Carnobacterium divergens,* the most abundant species of carnobacteria isolated from natural environments and food products, have a complex role within the food and aquaculture industries. One on hand, they can be used as protective cultures and/or probiotics to inhibit the growth of undesirable bacteria, yet the uncontrolled growth of carnobacteria in food products has been associated with food spoilage, and some strains are known fish pathogens. Bacteriophages offer a selective approach to identify and, if necessary, eliminate specific carnobacteria isolates. However, in contrast to phages that target other genera of lactic acid bacteria, few phages infecting carnobacteria have been reported. Recently, we have isolated multiple phages from a sample of ground beef, that infect *C. divergens* (phages cd2-cd7) and *C. maltaromaticum* (phage cm1). Electron microscopy reveals that phages cd2-cd7 are siphophages with an elongated capsid (B3 morphotype), and genomic analysis shows that they constitute a new genus of phage, named *Carnodivirus*. We have characterized phage cd2 (growth kinetics, stability, host range) and are completing similar studies for phages cd3-cd7. Unlike phages cd2-cd7, phage cm1 has an icosahedral capsid and a longer tail, and analysis of its genome suggests that it represents yet another new genus of phage. Characterization studies of phage cm1 studies are in progress. Taken together, this work sets the foundation for understanding the biology of these new phages and their potential use in the detection and biocontrol of carnobacteria isolates.

Kewords
Carnobacterium divergens, Carnobacterium maltaromaticum, bacteriophage, siphophage, subtyping

The impact of human iPS-derived intestinal epithelial cells on the metabolism of Bifidobacteria

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Number

42

Themes

Host Microbe Interactions

Abstract

Probiotics have several health benefits via their beneficial metabolites. Therefore, for better understanding of probiotic efficacy *in vivo,* the effects of the host cells, such as intestinal epithelial cells (IECs), on the metabolic behavior of probiotics need to be investigated. In this study, we performed comprehensive analysis of metabolites produced by co-cultivation of *Bifidobacterium breve* MCC1274 with human induced pluripotent stem cells (iPS)-derived intestinal epithelial cells. We observed a significant increase in several amino acid metabolites, including indole lactic acid (ILA) and phenyllactic acid (PLA) in the co-culture condition. Both PLA and ILA are known as immunomodulatory metabolites produced by *Bifidobacterium* species harbored in infant. Corresponding to the metabolic shift, the expression of genes involved in ILA synthesis, such as transaminase and tryptophan synthesis related genes were also elevated in *B. breve* MCC1274 cells. As one mechanism, we found that ILA production was enhanced in the presence purines, which were thought to be possibly produced by IECs. These findings suggest a synergistic relationship between *B. breve* MCC1274 and IECs, which enhanced beneficial metabolite production in vivo.

Keywords

Bifidobacteria, intestinal epithelial cells, metabolite, coculture, indolelactate

POSTER FLASH SESSION 2: TUESDAY 29

Unraveling habitat adaptation within species of Lactobacillaceae

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Number
101

Themes
Genetics and Genomics
Host Microbe Interactions

Abstract
Species within the *Lactobacillaceae* are found in a multiplicity of ecological niches. However, lifestyles of lactobacilli are diverse and vary in the degree of host specificity, and it is difficult to determine the exact niches and the mechanisms underlying habitat adaptations. Here we present a machine learning tool named *aurora* that can identify allochthonous strains and lineages that are adapted to a particular habitat and determine the genes responsible for this adaptation. We validated *aurora* with two species with well-established but contrasting lifestyles: *Limosilactobacillus reuteri*, a host-adapted species composed of lineages that have become highly specialized to particular hosts, and *Lactiplantibacillus plantarum*, a generalist species without specific genomic signatures marking adaptations (a “nomadic” lifestyle). *aurora* successfully classified *L. reuteri* strains as autochthonous for rodents, poultry, porcine, primate, and human, and identified genes that underlie genotype-habitat associations, such as L-Ala-D/L-Glu epimerase, cobalt/nickel transport system, and a passive transporter to maintain Mg^{2+} homeostasis in rodent strains. In contrast, *L. plantarum* strains were identified as allochthonous to the habitats they were isolated from, confirming its status as a generalist species capable of shifting between habitats. Having successfully validated *aurora*, we now plan to apply the tool to other species of *Lactobacillaceae* to comprehensively map colonization/association factors for different habitats. In conclusion, *aurora* allows the determination of autochthony and genes that underpin habitat adaptation, and therefore provides information of paramount importance to unravel the ecological success and functional diversity of this group.

Kewords
host adaptation, GWAS, reuteri, plantarum

Rewiring the respiratory pathway of *Lactococcus lactis* to enhance extracellular electron transfer

Liuyan Gu, Jianming Liu, Sang Yup Lee, Christian Solem

Number
86

Themes
Genetics and Genomics
Fermentation and Metabolism, including protein transition

Abstract
Lactococcus lactis, a lactic acid bacterium with a typical fermentative metabolism, can also use oxygen as an extracellular electron acceptor. Here we demonstrate, for the first time, that *L. lactis* blocked in NAD^+ regeneration can use the alternative electron acceptor ferricyanide to support growth. By electrochemical analysis and characterization of strains carrying mutations in the respiratory chain, we pinpoint the essential role of the NADH dehydrogenase and 2-amino-3-carboxy-1,4-naphthoquinone in extracellular electron transfer (EET) and uncover the underlying pathway systematically. Ferricyanide respiration has unexpected effects on *L. lactis*, e.g., we find that morphology is altered from the normal coccoid to a more rod-shaped appearance, and that acid resistance is increased. Using adaptive laboratory evolution (ALE), we successfully enhance the capacity for EET. Whole-genome sequencing reveals the underlying reason for the observed enhanced EET capacity to be a late-stage blocking of menaquinone biosynthesis. The perspectives of the study are numerous, especially within food fermentation and microbiome engineering, where EET can help relieve oxidative stress, promote growth of oxygen sensitive microorganisms and play critical roles in shaping microbial communities.

Kewords
Lactococcus lactis, electron transfer, adaptive laboratory evolution

Effect of fumarate reductase on the growth of Lactobacillus delbrueckii ssp. bulgaricus

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Number
115

Themes
Genetics and Genomics
Fermentation and Metabolism, including protein transition

Abstract
Yogurt is traditionally fermented by a symbiotic starter culture of *Lactobacillus delbrueckii* ssp. *bulgaricus* (*L. bulgaricus*) and *Streptococcus thermophilus*. They exchange metabolites which meet their nutritional demands during symbiosis, resulting in a shorter fermentation time. We have demonstrated that fumaric acid or malic acid addition to the monoculture of 6 *L. bulgaricus* strains promoted their growth and shortened fermentation time (Yamamoto *et al*, 2021). Since many *S. thermophilus* strains have been confirmed to produce fumaric acid during fermentation, fumaric acid can function as one of the symbiotic substances to promote the growth of *L. bulgaricus*.

L. bulgaricus metabolize fumaric acid to succinic acid by fumarate reductase (Frd). Here, to examine the importance of Frd on the growth of *L. bulgaricus*, *frd* deletion mutants (Δ*frd*) were constructed for *L. bulgaricus* 2038 and *L. bulgaricus* NCIMB701373. The fermentation time of both Δ*frd* were slowed down compared to the parental strains and the addition of fumaric acid did not restore the delay. Coculture of Δ*frd* with *S. thermophilus* 1131 also resulted in longer fermentation time and the accumulation of succinic acid was not observed. These results indicated that the metabolism of fumaric acid to succinic acid by Frd is one of the key factors determining the fermentation ability of *L. bulgaricus*, and the fumaric acid produced by *S. thermophilus* is utilized by *L. bulgaricus* as a symbiotic substance during yogurt fermentation.

Kewords
Lactobacillus bulgaricus, fumarate reductase, yogurt fermentation, protocooperation

Development of a biosensor for the detection and isolation of agmatine-producing microorganisms from dairy samples

Angel Casado, Eva Fernández, Esther Sánchez-LLana, PhD María Fernández, **PhD Victor Ladero**, PhD Miguel A. Alvarez

Number
84

Themes
Genetics and Genomics
Fermentation and Metabolism, including protein transition

Abstract
Biogenic amines (BA) are nitrogenous compounds with biological activity that can accumulate in cheese in toxic concentrations due to the metabolism of some lactic acid bacteria (LAB). Putrescine, one of the BA most frequently found in cheese, is synthesized from arginine through the agmatine deiminase (AGDI) route. Arginine is first decarboxylated to produce agmatine, which is subsequently deaminated by the agmatine deiminase pathway (AgdI) to produce putrescine.

Our group has identified *Enterococcus faecalis* and some strains of *Lactococcus lactis* as those responsible for the conversion of agmatine to putrescine in cheese. However, little is known about which microorganisms perform the decarboxylation of arginine to produce agmatine. Moreover, these microorganisms would be of additional interest since there is evidence of the usefulness of agmatine in the treatment of neuropathies and mood disorders.

The identification of BA-producing microorganisms in food samples is usually based on the pH rise due to the decarboxylation of the amino-acid substrate, revealed by a pH indicator. However, for the detection of agmatine-producing microorganisms in cheese, this would lead to a large number of false positives, since many LAB possess the arginine deiminase activity which, from arginine, produces ornithine and ammonium, which causes a pH increase even greater. To solve this problem, we have designed a new biosensor for the detection of agmatine-producing microorganisms based on the agmatine-inducible transcription system (ACE) designed in our group. Here we describe the design and optimization of a protocol that has allowed us to isolate and identify agmatine-producing microorganisms from dairy samples.

Kewords
Biogenic amines, Putrescine, Agmatine producers, Biosensor, Lactococcus

Engineering multifunctional *Lactococcus lactis* with therapeutic potential

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Number
20

Themes
Genetics and Genomics
Host Microbe Interactions

Abstract
Lactococcus lactis has been engineered as a vector for delivery of therapeutic proteins in inflammatory bowel disease, infections, allergies, diabetes and cancer, with some examples already in clinical trials. We have developed plasmids for multiple protein expression and used them to express binders of cytokines, cytokine receptors and tumor antigens for potential synergistic effect. We used the dual nisin promoter plasmid pNZDual for controlled expression, secretion or surface display of two proteins, and pNBBX plasmid for BglBrick assembly of up to three expression cassettes. Binders of cytokines IL-6, IL-8, IL-17A and IL-23, cytokine receptors IL-17R and IL-23R and tumor antigens EpCAM and HER2 were combined in dual or triple constructs. Infrared fluorescent or mCherry proteins were concomitantly expressed to facilitate detection and imaging. Expression of all proteins was confirmed with specific antibodies, and binding of the corresponding targets was assessed with ELISA and flow cytometry. The ability of engineered *L. lactis* to remove cytokines or attach to tumor antigens was validated in human colon adenocarcinoma cells Caco-2 and HT-29, monocyte-like cells THP-1 and U-937, HEK293 cells transfected to overexpress EpCAM or HER2 receptors, and HEK-Blue IL-17 cells. Apart from static conditions, targeting ability of engineered *L. lactis* was also demonstrated under constant flow in microfluidic system. In conclusion, multifunctional *L. lactis* with therapeutic potential were produced by simultaneous expression of different therapeutically relevant proteins. The activity of the recombinant bacteria has been confirmed in various cell models, and offers a novel strategy for the treatment of intestinal inflammation and cancer.

Keywords
Lactococcus lactis, genetic engineering, multiple protein expression

HybPi-Cheese: An innovative process to decrease animal protein without reducing nutritional and sensory properties

Gabriela Purtschert-Montenegro, Ueli Von Ah, Barbara Walther, Barbara Guggenbuehl, Helena Stoffers, Hans-Peter Bachmann, Florian Loosli

Number
131

Themes
Fermentation and Metabolism, including protein transition
Host Microbe Interactions
Microbial Communities

Abstract
In recent years there has been an increasing demand of plant-based alternatives for cheese and meat. Consumers are not only looking for sustainable-eco-friendly products, but also for less processed ones. However, plant-based alternatives are often highly processed and need supplements to reach similar nutritional values as their counterparts. To address these consumer's demands, we developed a hybrid cheese analogue using milk with the addition of minimally processed lupin seeds. Initially, we fermented the milled lupin (40% w/v) using a starter culture, which showed the same acidification rate as in milk. Using a micro-cheese model (1ml), we investigated the addition of different concentrations of milled lupin (10, 25, 50, 75%) to milk with regard to solubility, coagulation and fermentation. The upscaling process was followed by producing a mini-cheese model (250ml), where we only used up to 25% of lupin, as the coagulation properties were lost when using higher percentages of it. Based on this model, we produced the cheese variety "Mutschli" using 7, 15 and 25% of lupin. The addition of lupin resulted in faster ripening and a higher uptake of salt. After adapting the cheese-making process we found the best lupin/milk ratio was 15/85%. The aroma profile indicated similarities with the milk counterpart and the hybrid-cheese had an overall good acceptance in a consumer test. We have developed a hybrid product that reduces the amount of animal protein, maintains nutritional value and is sensorially appealing without the need for highly processed raw materials or the additives required for plant-based alternatives.

Kewords
fermentation, plant-based, hybrid-cheese, cheese, starter-cultures, plant-animal-proteins, hybrid-technologie.

Quantitative physiology and proteome adaptations of Bifidobacterium breve NRBB57 at near-zero growth rates

Angela Rocio Ortiz Camargo, Oscar Van Mastrigt, Roger S. Bongers, Kaouther Ben-Amor, Jan Knol, Tjakko Abee, Eddy J. Smid

Number
119

Themes
Fermentation and Metabolism, including protein transition

Abstract
In natural environments, nutrients are usually scarce causing microorganisms to grow slow while staying metabolically active. This is the case in the human gut which harbors a dense population of microorganism generating a low concentration of nutrients despite its constant inflow. These natural conditions can be simulated using retentostat cultivations. The present study describes the physiological and proteome adaptations of the probiotic *Bifidobacterium breve* NRBB57 from high (0.4 h^{-1}) to near-zero growth rates. Lactose-limited retentostat cultivations were carried out for 21 days in which the bacterial growth rate was progressively reduced to 0.00092 h^{-1}, with a 3.4 fold reduction of maintenance energy requirement. Lactose was mainly converted into acetate, formate and ethanol at high growth rates while in the retentostat lactate production increased. Interestingly, the consumption of several amino acids (serine, aspartic acid and glutamine/arginine) and glycerol increased over time in the retentostat. Morphological changes and viable but non-culturable cells were also observed in the retentostat. Proteomes were compared for all growth rates, revealing a down-regulation of ribosomal proteins at near-zero growth rate and an up-regulation of proteins involved in the catabolism of alternative energy sources matching the shift in metabolism and providing a basis for the suggested catabolism pathways. Finally, we observed induction of the stringent response and stress defence systems. Retentostat cultivations were proven useful to study the physiology of *B. breve*, mimicking the nutrient scarcity of its complex habitat, the human gut.

Kewords
retentostat, proteomics, chemostat, stringent response, metabolism, bifidobacteria

Versatile Lactic Acid Bacteria Improve Texture in Both Fermented Milk and Plant Based Matrices

PhD Vera K Poulsen, Elahe Moghadam, PhD Stjepan Kracun, Birgit Svendsen, Wioletta M Nielsen, Gunnar Oregaard, Anders Krarup

Number
87

Themes
Fermentation and Metabolism, including protein transition

Abstract
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 flavour 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.

Exo-cellular polysaccharide production is believed to contribute positively to fermented milk and plant-base texture. It appeared as if 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.

Kewords
Exo-cellular polysaccharide, Texture, Milk, Soy, L. lactis

Evaluation of the fermentation potential of plant-derived lactic acid bacteria as starter cultures in nut-based milk alternatives

Wenkang Huang, Anran Dong, Huong Thi Pham, Cailtin Zhou, Zhaotong Huo, Anders Peter Wätjen, Sangeeta Prakash, Claus Heiner Bang-Berthelsen, Mark Turner

Number
8

Themes
Fermentation and Metabolism, including protein transition

Abstract
Fermentation of plant-based milk alternatives (PBMAs), including nut-based products, has the potential to generate new foods with improved sensorial properties. In this study, we screened 593 lactic acid bacteria (LAB) isolates from herbs, fruits and vegetables for their ability to acidify an almond-based milk alternative. The majority of the strongest acidifying plant-based isolates were identified as *Lactococcus lactis*, which were found to lower the pH of almond milk faster than dairy yoghurt cultures. Whole genome sequencing (WGS) of 18 plant-based *Lc. lactis* isolates revealed the presence of sucrose utilisation genes (*sacR*, *sacA, sacB* and *sacK*) in the strongly acidifying strains (n=17), which were absent in one non-acidifying strain. To confirm the importance of *Lc. lactis* sucrose metabolism in efficient acidification of nut-based milk alternatives, we obtained spontaneous mutants defective in sucrose utilisation and confirmed their mutations by WGS. One mutant containing a sucrose-6-phosphate hydrolase gene (*sacA*) frameshift mutation was unable to efficiently acidify almond, cashew and macadamia nut milk alternatives. Plant-based *Lc. lactis* isolates were heterogeneous in their possession of the nisin gene operon near the sucrose gene cluster. The results of this work show that sucrose-utilising plant-based *Lc. lactis* have potential as starter cultures for nut-based milk alternatives.

Kewords
Nut-based, Fermentation, Lactic Acid Bacteria, Lactococcus

POSTER SESSION 1: MONDAY 28

Inactivation of Jag enhances osmotic resistance in *Lactococcus lactis*

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Number
2

Themes
Genetics and Genomics

Abstract
Abstract

The second messenger cyclic di-AMP (c-di-AMP) is a stress signal in most Gram-positive bacteria that plays a regulator of osmotic resistance through its control of potassium and compatible solute transport. Our previous work in a model strain of *Lactococcus lactis* has revealed high c-di-AMP mutants can restore their salt resistance by acquiring mutations that simply lower the c-di-AMP level or by elevating potassium uptake. To identify more mechanisms whereby cells can alter their osmoresistance independently of c-di-AMP level changes, we carried out a salt resistance suppressor screen using a phenotypically different strain of *L. lactis*. Using a spontaneous mutant of this strain (*gdpP*) which has high c-di-AMP, we obtained 25 suppressor mutants that had restored salt resistance and identified 5 independent mutations in the *jag* gene. Jag is a putative RNA-binding protein that has been reported to be involved in cell elongation and cell size via its direct interaction with the peptidoglycan lytic transglycosylase MltG. The Jag-MltG interaction in *Lactococcus* was confirmed using a bacterial two-hybrid assay. We found *jag* mutant cells were significantly smaller than parent cells. This data suggests that cell 'downsizing' may be a coping mechanism to allow growth under low cell turgor conditions. Besides, this study also provides a potential non-GM method for designing innovative cheese-making strains.

Kewords
Lactococcus lactis, c-di-AMP, Jag, MltG, cell elongation

Bifidobacterium longum BG-L47 boosts activity of *Limosilactobacillus reuteri* DSM 17938 and is proven safe in a randomized clinical safety trial

Mr. Ludwig Ermann Lundberg, Mrs. Punya Pallabi Mishra, Dr. Peidi Liu, Dr. Manuel Mata Forsberg, Prof. Eva Sverremark-Ekström, Prof. Sebastian Håkansson, Dr. Caroline Linninge, Prof. Stefan Roos

Number
10

Themes
Fermentation and Metabolism, including protein transition
Host Microbe Interactions

Abstract
The aim was to find a companion strain that could improve the performance of *Limosilactobacillus reuteri* DSM 17938, one of the worlds most studied probiotic strains. Initial tests showed that coincubation with two novel strains of *Bifidobacterium longum*, BG-L47 and BG-L48, could boost the growth of *L. reuteri* DSM 17938 during *in vivo* like conditions. Characterization of the two bifidobacteria revealed that strain BG-L47 had better bile and acid tolerance as well as mucus adhesion compared to strain BG-L48 and the comparison strain *B. longum* BB536. Next, we evaluated functions of importance for host-microbe interactions and discovered that coincubation with *B. longum* cells also increased the amount of secreted 5' nucleotidase (5' NT), an enzyme that converts AMP into the potent signaling molecule adenosine. In addition, coincubation with BG-L47 boosted the bioactivity of extracellular membrane vesicles (MV) produced by *L. reuteri*. Firstly, the MV exerted an improved antagonistic effect on the pain receptor TRPV1. Secondly, the MV increased the expression of the immune development markers IL-6 and IL-1ß in peripheral blood mononuclear cells (PBMC). Finally, *B. longum* BG-L47 was evaluated in a clinical study and proven safe and well tolerated. Analysis of the microbiota composition by 16S rRNA gene amplicon sequencing showed that 10^{10} cfu/day for 28 days did not induce significant changes of the microbiota. To conclude, *B. longum* BG-L47 has favorable physiological properties, can boost the *in vitro* activity of *L. reuteri* DSM 17938, and is safe for consumption, making it a candidate for further evaluation in probiotic studies.

Keywords
Interactions, bioactivity, membrane vesicles, synergism, B.longum, L.reuteri

Intake of *Lactobacillus paragasseri* SBT2055 improves subjective symptoms of common cold in healthy adults: RCT

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[5]*Products Development Dept., MEGMILK SNOW BRAND Co., Ltd., Saitama, Japan*

Number
12

Themes
Host Microbe Interactions

Abstract
This study aimed to investigate the effects of *Lactobacillus paragasseri* SBT2055 (LG2055) on the subjective symptoms of the physical condition in healthy adults. In this randomized, double-blind, placebo-controlled, parallel-group comparative study, Japanese individuals aged 20-64 years were recruited. A total of 200 participants were randomly divided into two groups by an independent controller (100 participants per group). Drinkable yogurts containing or lacking LG2055 were used as test samples. The participants ingested one bottle of the test sample once a day for 12 weeks. A daily questionnaire survey (about common cold symptoms) was performed as the primary outcome, and immunological and oxidative stress markers were evaluated as secondary outcomes. In total, 198 participants completed the scheduled intake of the test samples, and five participants were excluded from the final analysis. Consequently, 193 participants (LG2055 group, n = 97; placebo group, n = 96) were included in the efficacy analysis. The cumulative days of each symptom were evaluated, and the LG2055 group showed a significantly higher ratio of “without symptom” in runny nose, plugged nose, sneezing, sore throat, hoarseness, cough, headache, feeling tired, and fever than the placebo group, indicating that the incidence rates of common cold symptoms were lower in the LG2055 group. Additionally, changes in the salivary secretory IgA levels were significantly higher, and the serum derivatives of reactive oxygen metabolites levels were significantly lower in the LG2055 group. These results suggest that LG2055 contributes to the maintenance of physical conditions by improving the host immune system.
(UMIN000045901)

Kewords
probiotics, clinical study, subjective symptoms, common cold

Cheesemaking processes selected for thermostable endolysins in phages infecting *Streptococcus thermophilus*

Frank Oechslin, Xiaojun Zhu, Carlee Morency, Rong Shi, Sylvain Moineau

Number
14

Themes
Bacteriophage and Antimicrobials

Abstract
Endolysins are bacteriophage (phage) lytic enzymes that degrade the bacterial cell wall to release phage progeny at the end of the viral replication cycle. We recently observed that the diversity of endolysins in phages infecting *Lactococcus lactis* can be considerable, with more than 10 different modular types (Oechslin *et al*., 2022, PLoS Biology).

In this study, we aimed to investigate the diversity of endolysins in phages infecting *Streptococcus thermophilus,* which is arguably the second most important industrial starter culture after *L. lactis*. We found that the endolysin diversity in these phages is much lower, with 91% of them belonging to only one modular type. Using X-ray crystallography, we identified a highly conserved calcium-binding motif in the cell wall-binding domain of this type of endolysin. Inactivation of the motif in purified endolysin and in the phage genome revealed its role in stabilizing the enzyme at higher temperatures and under conditions simulating a cheesemaking process.

Several fermented dairy products such as yogurt and various cheeses are made using elevated temperatures and require thermophilic lactic acid bacteria (LAB) such as *S. thermophilus*. Our results support the hypothesis that phages have adapted to cheesemaking by producing thermostable endolysins, which may have constrained the evolution of their diversity. The domestication of *S. thermophilus* for milk fermentation purposes has left genetic hallmarks such as the loss of certain bacterial genes. It now appears that LAB viruses have also adapted to the domestication of their host.

Keywords
Streptococcus thermophilus
bacteriophage
endolysin

Characterization of exopolysaccharides from tempeh-associated lactic acid bacteria with anti-adhesion bioactivity towards enterotoxigenic *Escherichia coli*

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Number
16

Themes
Host Microbe Interactions
Bacteriophage and Antimicrobials

Abstract
Enterotoxigenic *Escherichia coli* (ETEC)-mediated diarrhea can be mitigated by inhibiting the adhesion of ETEC to the intestinal surface. Exopolysaccharides (EPS) from lactic acid bacteria (LAB) have been known to have anti-adhesion bioactivity towards ETEC. In this report, we aimed to characterize EPS from LAB isolated from tempeh-related environments for its structure and anti-adhesion bioactivity. Two strains of EPS-producing LAB were isolated from tempeh (*Pediococcus pentosaceus* strain TL and *Leuconostoc mesenteroides* strain TR) and soak water used in tempeh production (*L. mesenteroides* strains AW and NW). The EPS yields of TL and TR grown in liquid culture were 0.4% each, while AW and NW gave yields of 1.3% ± 0.4 and 1.1% ± 0.3, respectively. Carbohydrate analysis indicated that the EPS samples consisted of predominantly glucose and fructose, with the exception of TR, which consisted almost only of glucose. The EPS samples were degraded with carbohydrate-degrading enzymes for identification and released oligosaccharides were subjected to high-performance size exclusion chromatography. We found that TL, AW, and NW consisted of dextran (α-1,6 linked glucan with α-1,3 glucose branches; 1100 – 1800 kDa) and levan (β-2,6 linked fructan with β-2,1 branches; 650 – 760 kDa), while EPS from TR only consisted of dextran (828 kDa). EPS extracts from the four LAB isolates inhibited ETEC-mediated yeast agglutination and showed the capability to bind with ETEC. In conclusion, dextran and levan are major components of EPS produced by tempeh-associated LAB isolates and showed promising potential as an anti-adhesive agent towards ETEC.

Keywords
tempeh, ETEC, exopolysaccharides, anti-adhesion, dextran, levan

Using *Steptococcus thermophilus* satellite prophages as a tool to increase phage resistance

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Number
18

Themes
Genetics and Genomics
Host Microbe Interactions

Abstract
Streptococcus thermophilus (*S.t*.) is one of the most widely used lactic acid bacterium (LAB) by the dairy industry. However, this bacterium of industrial interest is also sensitive to virulent phages. If virulent phages naturally present in heat-treated milk infect these carefully selected industrial LAB strains, this "phage attack" can lead to low quality fermented products. Recent studies have shown that some satellite prophages (SP) found in the genome of *Staphylococcus aureus* strains are able to slow down the replication of certain temperate phages. We noted that structurally similar SPs are present in the genome of some *S.t.* strains. The aim of this project is to characterize *S.t*. SPs in order to increase our toolbox of anti-phage mechanisms in S.t.

By using bioinformatic tools, we found that 69% of *S.t.* strains contain a putative SP with a similar genomic structure as previously characterized SPs (also known as SaPIs) in *S. aureus.*

We showed that some SPs could be excised spontaneously from the bacterial chromosome as it could be found in an overnight culture using PCR with primers specific to both SP ends. Using CRISPR interference, we could cure the SP from selected *S.t.* cells. Using these SP-free strains, we could mobilize SPs from other *S.t.* strains, by natural competence. The resulting strains with a different SP were found to be more resistant to virulent phages.

Taken altogether, this study provides new tools to develop more robust LAB strains for various industrial applications, including the development of phage-resistant strains.

Keywords
Satellite prophages, phage resistance, natural competence

How to screen for agents that prevent late blowing defect in cheese

Sofia Dashko, Internship student Sijin Liu, Principal Scientist Paul Klaassen, Innovation Manager Pim Van Hee, Principal Scientist Noel Van Peij

Number
22

Themes
Microbial Communities

Abstract
A screening system to test inhibitory substances and cultures against gas-producing *Clostridium tyrobutyricum* was developed. Four model systems were compared: Reinforced Clostridial Medium (RCM), Enriched Milk (EM), cheese slurry and miniature cheese. Each of these systems had their specific pros and cons, no single system being able to accommodate both the testing of inhibitory substances and inhibitory cultures. For anti-clostridial substances, RCM and cheese slurry are recommended. RCM serves as a preliminary test model, with the cheese slurry serving as a good follow-up model under more application-relevant conditions. To test the protective cultures, a combination of miniature cheese and cheese slurry model is recommended, because these together offer insight in the freshly-made and late ripening period of cheese manufacture.

Kewords
cheese late-blowing, Clostridium tyrobutyricum, screening, bioprotective cultures

StrainInSight: a unique genome database to facilitate novel strain discovery for the food industry

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Number
32

Themes
Genetics and Genomics

Abstract
Commercially available starter cultures often comprise the same bacterial species or even the same strains. In this project, LM-UGent and Puratos developed a new tool to explore the LM-UGent private strain collection, containing more than 7000 strains of lactic acid bacteria (LAB), in search of novel bacteria for applications in the food industry.

5000 of these LAB strains were dereplicated and identified using MALDI-TOF MS. Based on differences in the mass spectra and available strain metadata, a diverse set of 1000 strains was selected for whole-genome sequencing. The resulting annotated genomes, along with the available metadata, were uploaded into the StrainInSight genome database. This database enables users to search for specific enzymes or metabolic pathways within the bacterial genomes and combine this with searches on strain metadata such as identification or source. This novel approach provides a more efficient and targeted means of selecting potentially interesting strains from the LM-UGent private strain collection for industrial applications.

Overall, this user-friendly database provides valuable insights into the functional capacity of these strains. It facilitates the selection of novel strains with interesting functionalities, such as production of flavour compounds and organic acids that can be used in the food industry.

Keywords
genome database, MALDI-TOF MS, novel strain discovery

In *Streptococcus thermophilus*, Ammonia from Urea Hydrolysis Paradoxically Boosts Acidification and Reveals a New Regulatory Mechanism of Glycolysis

Professor Stefania Arioli

Number
33

Themes
Fermentation and Metabolism, including protein transition

Abstract
Streptococcus thermophilus is widely used in the dairy industry for the manufacturing of fermented milk and cheeses and probiotic formulations. *S. thermophilus* evolved from closely phylogenetically related pathogenic streptococci through loss-of-function events counterbalanced by the acquisition of relevant traits, such as lactose and urea utilization for the adaptation to the milk environment. The fate of ammonia and carbon dioxide derived by urea hydrolysis in several biosynthetic pathways have been depicted, and the positive effect of urease activity on *S. thermophilus* growth fitness and lactic acid fermentation in milk has been already addressed. This study aimed to assess the effect of urease activity on the growth and energy metabolism of *S. thermophilus* in milk. In milk, 13C-urea was completely hydrolyzed in the first 150 min of the growth, and urea hydrolysis was accompanied by an increase in cell density and a reduction in the generation time. By using energetically discharged cells with gene transcription and translation blocked, we showed that in the presence of fermentable carbon sources, urease activity, specifically the production of ammonia, could dramatically boost glycolysis and homolactic fermentation. Furthermore, we showed that ammonium ions were potent effectors of phosphofructokinase, a key glycolytic enzyme. Finding that ammonia-generating enzymes, such as urease, and exogenous ammonia act on phosphofructokinase activity shed new light on the regulatory mechanisms that govern glycolysis. Phosphofructokinase is the key enzyme known to exert a regulatory role on glycolytic flux and, therefore, ammonia as an effector of phosphofructokinase acts, in cascade, modulating the glycolytic pathway.

Keywords
Streptococcus thermophilus, glycolysis, ammonia, urease, phosphofructokinase

Predicting metabolic product formation in *Lactococcus cremoris* with a dynamic model

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Number
38

Themes
Fermentation and Metabolism, including protein transition

Abstract
Lactococcus cremoris is a cheese starter bacteria that uses homolactic fermentation to produce lactate, a key metabolic product that contributes to acidification and flavour in cheese. However, when lactose is limited or substituted for sugars that support lower growth rates, such as galactose or maltose, mixed acid fermentation occurs, resulting in the production of formic and acetic acid and ethanol. This metabolic adaptation is becoming increasingly relevant in the transition from dairy to plant-based fermented products that often contain “slow” sugars. Therefore, it is important for industries to understand the critical factors that affect *L. cremoris* ability to convert different sugars into lactate or mixed acids.

Much research has been carried out to understand the mechanism of the metabolic shift from homolactic to mixed acid fermentation. Despite these efforts, specifics about how metabolic regulation works are not totally clear. Current kinetic models of central metabolism of *L. cremoris* have not focused on this phenomenon or were inconclusive. This motivated us to revisit the topic and design a detailed kinetic computer model that captures the most important characteristics of this regulatory behaviour.

Based on previous research and available experimental data, we have developed a computer model that mimics the shift present in *L. cremoris*. Through parameter sensitivity we aim to identify critical regulatory interactions and parameters that influence the metabolic shift. This should improve the prediction of end products for different bacterial strains and conditions and ultimately may lead to better process control or strain selection.

Keywords
Lactococcus cremoris, Metabolic shift, Fermentation, Kinetic model

Developing a standardized cloning toolbox to metabolically engineer *Lactiplantibacillus plantarum* as an aroma-adding microorganism in beverage fermentation

Xiangang Li, Pascal Schönberg, Tabea Wucherpfennig, Christoph Hinze, Prof. Thomas Henle, Prof. Thorsten Mascher

Number
44

Themes
Genetics and Genomics
Fermentation and Metabolism, including protein transition

Abstract
Lactiplantibacillus plantarum is an organism commonly used for sour beer and other beverage fermentations. Previous studies found that the major compounds that contribute to the flavour of beer are the two monoterpenoids linalool and geraniol present in hops. Here, we aim to genetically engineer *L. plantarum* WCFS1 as an efficient aroma producer for a hoppy sour beer. First, we developed a modular and standardized Golden Gate Assembly-based toolbox for the *de novo* assembly of shuttle vectors from *Escherichia coli* to lactic acid bacteria (LAB). A collection of the most relevant genetic parts, e.g. different origins of replication and promoters, was incorporated and characterized in our toolbox. Each genetic part can be combined freely into a plasmid, due to their standardized fusion sites. Next, we screened four plant-derived linalool and geraniol synthase genes and co-expressed the best performer with two bottleneck enzymes of the mevalonate pathway using our toolbox. Detectable amounts of linalool and geraniol were produced with the linalool titer well above the odor threshold. Overall, we have developed a highly efficient and flexible cloning toolbox for engineering LAB as promising probiotics and biofactories. Two flavourful monoterpenoids were produced in *L. plantarum* with potential applications in food processing.

Keywords
Lactiplantibacillus plantarum, synthetic biology, cloning toolbox, terpenoid

Building a novel thermogenetic circuit in probiotic Lactobacillus

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Number
52

Themes
Genetics and Genomics

Abstract
The *Lactobacillus* genus comprises many species of great importance to the food and healthcare industries, with numerous strains identified as beneficial for humans and used as probiotics. Hence, there is a growing interest in engineering these probiotic bacteria for healthcare applications in animals and humans. However, the genetic switches needed to fine-tune gene expression accurately in these bacteria remain limited compared to model bacteria.

In this study, we explored a rational design-based strategy to create a thermogenetic switch that controls gene expression in lactobacilli. We first identified and tested several heterologous genetic parts with the potential to regulate transcription in lactobacilli . Next, we combined these genetic parts to thermo-responsive protein domains and rationally optimized the design of these chimeric transcription regulators to achieve switch-like performance in physiologically relevant temperature ranges.

We believe that all these insights could positively contribute to the enhancement of the genetic programmability of *Lactobacillus* for healthcare and industrial applications.

Keywords
Thermogenetics, Genetic circuit, Lactobacillus

Inside Out: Quantification of Intracellular Lactic Acid in *Lactococcus lactis* in dynamic conditions

Tamara Bendig, Eddy J. Smid, Tjakko Abee, Oscar Van Mastrigt

Number
54

Themes
Fermentation and Metabolism, including protein transition

Abstract
Lactococcus lactis is recognized for its rapid production of lactic acid and acidification, which is an important feature in dairy fermentations. The acidification not only improves the taste but also helps to extend the shelf life of the product. Despite the vital role of lactic acid in the physiology of lactic acid bacteria, little is known about the dynamics of intracellular lactic acid concentrations in growing cells. Quantification of intracellular lactic acid pools under dynamic conditions is a challenging task because of i) high extracellular volumes that greatly exceed intracellular volumes, ii) high extracellular lactic acid concentrations, iii) fast metabolite turnover times and iv) potentially low and changing cell densities, which renders NMR unsuitable as a method. Therefore, we developed a novel method to monitor intracellular lactic acid concentrations in *L. lactis* during fermentation, without the need for high cell densities. First, cells are quenched using cold (-20°C) glycerol-saline, to halt metabolism immediately without compromising the cell membrane, and washed with glycerol-saline to remove extracellular lactic acid. Subsequently, an optimized cell lysis step allows efficient extraction of lactic acid, which is quantified using high-performance liquid chromatography. Finally, we developed an image analysis pipeline that enables the estimation of the intracellular volume, allowing to accurately calculate concentrations. Overall, our approach provides a reliable way to quantify intracellular lactic acid concentrations, which can deepen our understanding of cellular physiology and may support the design and optimization of industrial fermentation processes.

Kewords
intracellular lactic acid, metabolomics, extraction, fermentation

Orally administered *Lacticaseibacillus rhamnosus* GG can transfer to the upper respiratory tract in children with otitis media

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Number
56

Themes
Microbial Communities

Abstract
Background: The role and ecology of lactic acid bacteria (LABs) in the upper respiratory tract (URT) is not yet well-understood. Several orally ingested LABs, including the probiotic *Lacticaseibacillus rhamnosus* GG(LGG), can reduce the incidence/symptoms of URT infections in randomized placebo-controlled trials, including moderate effect on otitis media with effusion(OME). However, it is not yet known how LGG could mediate its effects via transfer to the URT and local infection modulation.

Aim: Here, we investigated the potential of two model LAB probiotics (LGG & *Bifidobacterium lactis* BB-12) to transfer to the URT and impact the URT microbiome and pathogen colonization.

Methods: Children with OME(n=20) received a commercial liquid oily formulation of LGG and BB-12 (Probactiol®Mini) orally for 4 weeks. Presence of LGG, BB-12 and endogenous OM pathogens was analyzed using qRT-PCR and 16S amplicon sequencing compared with a placebo control group(n=20).

Results: No significant change was observed in the overall microbiome composition. Up to 10^5CFU/sample of LGG was detected by qRT-PCR in the nasopharynx of 5/20 treated, but not control patients, while BB-12 was not detected.

Conclusion: Orally applied LGG transfers to URT niches such as the nasopharynx, suggesting it is more suited to thrive as a probiotic in the URT than BB-12. This is in line with LGG's

documented stress tolerance and adherence capacities via SpaCBA pili, while BB-12 is more oxygen-sensitive and lacks specific adherence factors. Future studies will explore whether LGG's URT persistence is associated with URT health benefits and how its molecular properties are involved.

Keywords
Lacticaseibacillus rhamnosus GG, upper respiratory tract, probiotics

The Impact of a Multistrain Probiotic on Fecal Microbial Ecosystem in Non-Constipated Irritable Bowel Syndrome: Results from the ESA-19 Study

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Number
64

Themes
Microbial Communities
Host Microbe Interactions

Abstract
Irritable bowel syndrome (IBS) is a chronic gastrointestinal disorder that causes abdominal pain and changes in bowel habits. Probiotics are commonly used as an adjunct treatment for IBS. The ESA-19 study was a randomized, double-blind, crossover, placebo-controlled trial involving 34 patients with non-constipated IBS. Each patient was given a daily sachet of Enterolactis® Ultra containing 5×109 CFU of six strains of bacteria, including *Lacticaseibacillus paracasei*, *Lactiplantibacillus plantarum*, *Bifidobacterium breve*, *Bifidobacterium animalis* subsp. *lactis*, and *Bifidobacterium bifidum*. The study aimed to evaluate the impact of this multistrain probiotic on the fecal microbial ecosystem of non-constipated IBS patients. Results showed a significant decrease in the Actinobacteria species and an increase in the Bacteroidetes species, which led to a significant reduction in the propionate/butyrate ratio. Unsupervised clustering based on fecal microbiomic data at baseline identified two groups: C1 (n=12) and C2 (n=22). C2 patients had lower α-diversity and a higher abundance of Proteobacteria, along with higher stool frequency, fecal type, and abdominal pain compared to C1 patients. The probiotic intervention decreased the abundance of Coriobacteriaceae and the propionate/butyrate ratio in the C2 group only. However, the study failed to find significant differences between probiotic and placebo on clinical parameters due to high placebo effect and a small sample size. Nonetheless, the improvement in the propionate/butyrate ratio, an emerging IBS marker, may have positive clinical implications for IBS patients. The probiotic under study could be more effective in individuals with more pronounced alterations in the intestinal microbial ecosystem, such as those in group C2.

Keywords
IBS-D, short-chain fatty acids, propionate/butyrate ratio

Key role for lactobacilli in the gut-vagina-axis: implications for endometriosis?

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Number
66

Themes
Microbial Communities

Abstract
Lactobacilli are widely studied members of the vaginal and gut microbial communities. Although they are a minority of the gut microbiome, their beneficial and probiotic effects have been mainly studied in the gut, and less is understood about their role in the vagina and gut-vagina axis. Recently, we have launched Isala, a citizen science project on the female microbiome and women's health with a focus on vaginal lactobacilli (https://isala.be/en). In this study, we aim to come to a better understanding of the vaginal microbiome and its lactobacilli in women with endometriosis, a disease characterized by endometrium-like tissue outside the uterus potentially causing pelvic pain and/or infertility. In a first phase, the vaginal microbiome profiles of 71 endometriosis patients were compared to the data of the 3265 healthy controls from the Isala cohort. Associations between endometriosis and self-reported health indicators were studied. Lactobacillus taxa, most notably L. crispatus and L. iners were the most dominant taxa in endometriosis patients, similar as for the overall Isala population. The sequencing data was complemented by qPCR analysis to compare the absolute abundances of several lactobacilli subspecies between women with endometriosis and the control group. Finally, higher comorbidities were reported by endometriosis patients than by the healthy controls. In particular, the occurrence of irritable bowel syndrome was found to be almost three times higher among endometriosis patients compared to the control group. These data suggest that the gut could play a role in the pathogenesis of endometriosis and that there exists a gut-vagina axis.

Keywords
Isala, vaginal lactobacilli, gut-vagina axis, endometriosis

LEGEN: a pangenome database for evolutionary genomics of Lactobacillales

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Number
68

Themes
Genetics and Genomics
Host Microbe Interactions

Abstract
The order Lactobacillales is the largest taxon of Lactic Acid Bacteria, containing species that are vital members of the human microbiome and species that are used in food fermentation and medicine. With the availability of whole genome sequences for numerous Lactobacillales strains, there is an opportunity to gain unprecedented insights into the evolution of these bacteria. However, the lack of computational tools to analyze large and diverse genome datasets has hindered progress in this field.

To address this gap, we developed SCARAP, a toolkit for comparative genomics of large and diverse datasets, and used it to build LEGEN, a pangenome database of 6,715 dereplicated genomes across all known groups of Lactobacillales. Our analysis identified 434,750 gene families within Lactobacillales, revealing novel insights into the evolution and ecology of these bacteria.

Specifically, our analysis of the Lactobacillaceae family revealed two polyphyletic groups that corresponded almost entirely to known host-adapted versus free-living lifestyles. Our findings suggest that convergent gene loss, particularly in a gene for methionine synthesis but also other genes, drives the gene content differences between these groups. This discovery confirms the hypothesized lifestyles of Lactobacillaceae and provides a model for predicting the lifestyle of poorly characterized species from their genomes.

Overall, our study demonstrates the power of the LEGEN database in revealing novel insights into the ecology and evolution of Lactic Acid Bacteria. The LEGEN pipeline and database are publicly available at github.com/swittouck/legen.

Keywords
Lactobacillales, Lactobacillaceae, comparative genomics, lifestyle adaptation, bioinformatics

Control outgrowth of non-starter lactic acid bacteria in cheddar cheese ripening by using nisin producing starter cultures

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Number
72

Themes
Microbial Communities

Abstract
A starter culture to make cheddar cheese comprises either mesophilic lactic acid bacteria (LAB) or a combination of mesophilic and thermophilic LAB, depending on the desired application of the culture (Bulk starter or cell concentrate as Direct Vat Inoculation-DVI), temperature and speed of the processes. The starter culture ensures acidification of the milk and will contribute to flavour development during ripening of the cheese. During ripening, defects can develop such as off-taste or slits and crack formation, which will result in a lower valued cheese or even economical losses. It is believed that slits and cracks are formed because of gas producing non-starter LABs (NSLAB). Gas producing NSLAB are generally from the genus *Lactobacillus* and sensitive to the bacteriocin nisin. A nisin producing starter culture was developed to inhibit the outgrowth of NSLAB and thereby control slits and cracks formation during cheddar cheese ripening. Beside nisin production, the starter culture maintained all other desired characteristics such as acidification speed at desired temperatures, salt tolerance and taste.

Keywords
nisin, cheddar, non-starter lactic acid bacteria, ripening,

Comparative Genomics of *Loigolactobacillus coryniformis* With an Emphasis on *L. coryniformis* Strain FOL-19 Isolated from Cheese

Fatih Ortakci, Ismail Gumustop

Number
74

Themes
Genetics and Genomics

Abstract
Loigolactobacillus coryniformis is a member of lactic acid bacteria that has been isolated from various ecological niches. We isolated a novel *L. coryniformis* strain FOL-19 from artisanal Tulum cheese and performed the whole-genome sequencing for FOL-19 using Illumina NextSeq. Then, genomic characterization of FOL-19 against seven available complete genome sequences of the same species isolated from kimchi, silage, fermented meat, air of cowshed, and dairy was performed. The average genome size of 2.86 ±0.06 Mbp, GC content of 42.86% ±0.06, number of CDS of 2835 ±188, number of tRNA of 54.5 ±9, and number of CRISPR elements of 6.87 ±1.53 were achieved. A single Type II-Cas cluster was found in all strains except for meat and cheese isolate, five strains harbored at least one intact prophage. The presence of CRISPR elements and Cas clusters suggests that *L. coryniformis* holds a promising potential for being a reservoir for new CRISPR-based tools. These findings put a step forward for genomic characterization of *L. coryniformis* strains for biotechnological applications via genome-guided strain selection to identify industrially relevant traits.

Kewords
Lactobacilli, Comparative genomics, CRISPR/Cas, Prophage, Fermented foods

Characterization of genes involved in the metabolism of arabinose-containing complex glycans by *Bifidobacterium longum* subsp. *longum* NCIMB 8809

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Number
76

Themes
Genetics and Genomics
Fermentation and Metabolism, including protein transition

Abstract
Members of the genus *Bifidobacterium* can be found in the human gut and are purported to elicit health benefits to their host. Strains belonging to *B. longum* subsp. *longum* (*B. longum*) can utilise plant-derived glycans, common to the adult diet, that would otherwise not be digested by the human host. In order for *B. longum* to metabolize such complex dietary glycans, it will need to encode specific carbohydrate-active enzymes. Due to their narrow substrate specificity, multiple enzymes are required to utilise and fully break down large carbohydrates, particularly if they contain various substitutions, such as is the case for arabinoxylan. In the current study, we describe seven enzymes, encoded by two genetically distinct clusters, and represented by AbfII_1, AbfII_2, AbfII_3, AbfII_4, AbnA2 as well as AbfIII_1 and AbfIII_2. Synthetic and natural substrate assays were performed, and the obtained enzymatic data together with an in-silico analysis suggest that: (i) AbfII_1 represents an extracellular exo-arabinofuranosidase; (ii) AbfII_2 is an intracellular exo-α-arabinofuranosidase; (iii) AbfII_3 acts as an intracellular endo-α-L-arabinofuranosidase; (iv) AbfII_4 represents an intracellular β-L-arabinofuranosidase; (v) AbnA2 is an intracellular exo-arabinase; (vi) AbfIII_1 operates as an extracellular α-L-arabinofuranosidase, and (vii) AbfIII_2 acts as an extracellular α-L-arabinofuranosidase. This study provides a further understanding of the metabolism of a *B. longum* subsp. *longum* strain on dietary glycans containing arabinose.

Kewords
Bifidobacterium, genomics, gut, glycans

Characterization of *Bifidobacterium kashiwanohense* that utilizes both milk- and plant-derived oligosaccharides

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Number
80

Themes
Genetics and Genomics
Microbial Communities

Abstract
Bifidobacteria are prominent members of the human gut microbiota throughout life. The ability to utilize milk- and plant-derived carbohydrates is important for bifidobacterial colonization of the infant and adult gut. The *Bifidobacterium catenulatum* subspecies *kashiwanohense* (*B. kashiwanohense*) was originally isolated from infant feces. However, only a few strains have been described, and the characteristics of this subspecies have been poorly investigated. Here, we characterized genotypes and phenotypes of 23 *B. kashiwanohense*-associated strains, including 12 newly sequenced isolates. Genome-based analysis clarified the phylogenetic relationship between these strains, revealing that only 13 strains are genuine *B. kashiwanohense*. We defined specific marker sequences and investigated the worldwide prevalence of *B. kashiwanohense* based on metagenome data. This revealed that not only infants but also adults and weaning children harbor this subspecies in the gut. Most *B. kashiwanohense* strains utilize long-chain xylans and possess genes for extracellular xylanase (GH10), arabinofuranosidase and xylosidase (GH43), and ATP Binding Cassette (ABC) transporters that contribute to the utilization of xylan-derived oligosaccharides. We also confirmed that *B. kashiwanohense* strains utilize short- and long-chain human milk oligosaccharides and possess genes for fucosidase (GH95 and GH29) and specific ABC transporter substrate-binding proteins that contribute to the utilization of a wide range of human milk oligosaccharides. Collectively, we found that *B. kashiwanohense* strains utilize both plant- and milk-derived carbohydrates and identified key genetic factors that allow them to assimilate various carbohydrates.

Kewords
Bifidobacterium_kashiwanohense, xylan, human_milk_oligosaccharide, plant-derived_carbohydrate, extracellular_xylanase, ABC_transporter_SBP

The Culturability of Five South African Vaginal Probiotic Lactobacilli in a Low-Cost, Plant-Based Soytone Growth Medium

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Number
82

Themes
Fermentation and Metabolism, including protein transition

Abstract
A comparatively low-cost, plant-based growth medium was assessed for the culturing of South African lactobacillus isolates intended for application as probiotics to treat bacterial vaginosis. This plant-based growth medium uses soytone as the primary protein source and is proposed as an alternative to the expensive and animal-derived MRS Broth. Though MRS Broth is widely used to isolate and cultivate lactic acid bacteria, it can be unsuitable for products intended for human consumption and its animal derived components lead to expensive bioprocess scale-up and industrial application.

The plant-based medium was constituted to have the same carbon concentration as standard MRS Broth. An analysis of carbon-to-nitrogen ratios revealed that the plant-based medium has a higher C/N ratio than MRS Broth, of 8.1 ± 0.04 versus 6.6 ± 0.06. The growth of five lactobacillus vaginal isolates were tested in the two media. Four of the five cultures grew better in the plant-based medium than the MRS Broth in terms of average final cell densities and growth rates (49% and 11% higher, respectively). MRS Broth only supported similar growth kinetics upon tripling the concentration of its constituents, making the medium even more expensive. The costs of the proposed plant-based soytone growth medium and MRS Broth were modelled, and the plant-based medium was shown to be 44% less expensive per volume than MRS Broth.

This work thus demonstrates the feasibility and attractiveness of using such a plant-based growth medium for lactobacilli, from an economic and sustainability standpoint, and resolving limitations posed by animal-derived products.

Kewords
Probiotics, Growth Medium, Fermentation, Soytone, Scale-Up, Lactobacillus

Lactococcus lactis mutants resistant to lactococcin A reveal missense mutations in the man-PTS sugar transport domain

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Number
88

Themes
Bacteriophage and Antimicrobials

Abstract
Lactococcin A is a bacteriocin from *Lactococcus lactis* that permeabilizes the membrane of sensitive lactococcal cells and requires the presence of the membrane-bound components IIC and IID of the mannose phosphotransferase system (man-PTS). Recently it was reported through cryo-EM analyses of man-PTS and several bacteriocins fused to a maltose binding protein, including lactococcin A, that these bacteriocins create pores by inserting themselves between the Core and Vmotif domains of man-PTS. In our study we obtained a dozen spontaneous mutants of *L. lactis* IL1403 resistant to lactococcin A. All mutants of IL1403 had mutations located in the genes encoding the IIC and/or IID proteins. These mutations also resulted in resistance to garvicin Q, a bacteriocin from *Lactococcus garvieae* with a broad inhibition spectrum and very little homology to lactococcin A. Missense mutations were found in the sugar transport domain of man-PTS of bacteriocin resistant IL1403 mutants. These missense mutations also impeded the uptake of mannose. When lactococcin A, garvicin Q or pediocin PA-1, an anti-listerial bacteriocin, were fused to a maltose binding protein, we observed reduced or no antibacterial activity. Taken together, the precise mechanism of action of bacteriocins using the man-PTS remains to be fully understood.

Kewords
bacteriocin, resistance, mannose phosphotransferase system

Carbohydrate metabolism in *Streptococcus thermophilus*: co-utilization of saccharides in mixtures and role of sugar nature and concentration in gene regulation

Dr Cécile Gasser, Dr Peggy Garault, Dr Christian Chervaux, Dr Véronique Monnet, Dr Jean-Michel Faurie, Dr Françoise Rul

Number
90

Themes
Fermentation and Metabolism, including protein transition

Abstract
Streptococcus thermophilus is a bacterium widely used in the production of yogurts and cheeses, where it efficiently ferments lactose, the saccharide naturally present in milk. It is also employed as a starter in dairy- or plant-based fermented foods that contain mono or di-saccharides other than lactose (e.g., sucrose, glucose), often in the form of mixtures. We explore here the growth and carbohydrate gene expression regulation of *S. thermophilus* LMD-9 in presence of single or mixed sugars. *S. thermophilus* preferentially used lactose and sucrose in a similar way when the two saccharides are in mixtures with 2 other saccharides. Here, we characterized promoter activity for genes involved in lactose, sucrose, glucose, galactose, and fructose metabolism. Using a transcriptional fusion approach, we discovered that lactose and glucose repressed - in a concentration-dependent manner - the activity of the *lacS* and *scrA* promoters, that encode, respectively, the lactose and glucose/sucrose transporters. When mixed with lactose, glucose also repressed the two promoter activities; when mixed with sucrose, lactose still repressed *scrA* promoter activity. We determined that catabolite control protein A (CcpA) played a key role in these dynamics. We also showed that promoter activity of *lacS* and *scrA* was linked with glycolytic flux, which varied depending on saccharide type and concentration. Overall, this study identified key underlying mechanisms in carbohydrate metabolism—autoregulation and partial hierarchical control—and demonstrated that they are partly mediated by CcpA.

Kewords
metabolism, LAB, promoter activity, regulation, saccharide consumption

Novel mild yogurt starters deliver a more sustainable and valued product.

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Number
92

Themes
Genetics and Genomics

Abstract
Mild and convenient *Streptococcus thermophilus* starter cultures were developed using classical mutagenesis combined with a highly automated screening and selection process.

Streptococcus thermophilus strains were exposed to sublethal NTG concentrations. Recovered mutants were analyzed at the phenotypic level on different criteria including acidification in milk, growth kinetics, post-acidification, and textural properties. Screening assays were conducted in microtiter plates using milk as selection medium. Selection of mild yogurt starters within the mutant pool was done in a step-wise manner. Selected mild mutants and wild type should have comparable fermentation time needed to reach pH=4.6 including similar viscosity measurements in milk. On the other hand, the mutants should generate milder yoghurts, meaning that they are not able (or to a significant lesser extent) to decrease the pH in yogurt during storage.

Further tests included producing yogurt prototypes at lab scale to validate performance of the mild strains. We observed a good correlation between expected acidification speed and fermentation time performance of the mutants at both scales. Post acidification and textural properties displayed a less strong correlation between scales.

Through the approach of classical mutagenesis and screening combined with strong support of robotics and automation, mild yogurt starters have been developed successfully. Using these starters can therefore fulfill the consumers need of taste (mild and thick), without the need for refrigeration (energy /cost saving) for preserving its taste.

Kewords
Streptococcus thermophilus, yogurt, mild, genetic screening

Dynamic Metabolic and Microbial Alterations During Industrial Kimchi Fermentation

Jekaterina Kazantseva, Emili Aus, Anne Meikas, Marina Junusova, Rain Kuldjärv

Number
94

Themes
Microbial Communities
Fermentation and Metabolism, including protein transition

Abstract
Kimchi is a traditional Korean fermented food that has recently attained popularity due to its nutritional benefits, including dietary fiber and probiotics.

Together with Estonian company Kadarbiku Köögivili OÜ specializing in vegetable farming and sauerkraut production, we performed a pilot study to adapt Asian kimchi recipes for European consumers using different local vegetables. Microbiological and metabolic parameters were monitored over a two-week period during fermentation aiming to compare the microbiota of different vegetables and understand its changes during kimchi preparation and find correlations between bacteria and metabolic profiles.

The microbial composition of kimchi identified using 16S rRNA metabarcoding on Illumina platform showed a similar trend for all four starter materials, with *Leuconostoc* dominating early in the process and *Lactiplantibacillus* and *Levilactobacillus* predominating towards the end. A notable increase in bacterial population and taxonomic switch in consortia occurred on the fourth day of fermentation, coinciding with the shift in various sugars and organic acids detection.

The final products were sensory assessed and received high organoleptic ratings from experts.

Kewords
kimchi fermentation, 16S rRNA metabarcoding, metabolites, LAB

The probiotic potential of natural Leuconostoc isolates

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Number
96

Themes
Microbial Communities
Fermentation and Metabolism, including protein transition
Host Microbe Interactions

Abstract
Various gastrointestinal but also extra-intestinal health issues are associated with gut dysbiosis and gut barrier impairment. Therefore, there is a great need to find new probiotics that can potently reinforce the gut barrier by foremostly surviving in gut conditions and adhering to the intestinal mucosa. Only a limited number of studies focus on *Leuconostoc* species, even though they naturally occur in health-promoting foods, such as fermented milk products. For this reason, this study explores the probiotic potential of *Leuconostoc* sp. strains regarding mucoadhesion, stress resistance, and other probiotic features promoting gut homeostasis. Under these fall B-group vitamin, short-chain fatty acid (SCFA), lactate and exopolysaccharide (EPS) production. Among 46 natural isolates that have been tested, only a few *Leuconostoc* strains exhibit high adhesion to mucins and medium stress resistance. However, most strains were found to potently produce EPS, which is known to enhance adhesion and resistance in the GI tract. Less than 40 strains (38) were further selected for vitamin production, where most have grown well in the absence of B1, B2 and B9 vitamins. A further selection of 20 strains was tested for antibiotic resistance showing mostly adequate sensitivity toward common antibiotics. Moreover, mass spectrometry analysis revealed high levels of lactate, acetate and B-group vitamins produced, especially B2, B3, B5 and B6. Next, *in vitro* cell culture and *in silico* validation steps will be undertaken to select the most promising probiotic strain for an *in vivo* disease mouse model.

Funded by The National Science Centre (NCN) [2021/41/B/NZ9/02236].

Kewords
Gut barrier, probiotics, mucoadhesion, exopolysaccharide, gut homeostasis

Towards a system view of the *Lactococcus lactis* cell envelope stress response

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Number
98

Themes
Bacteriophage and Antimicrobials

Abstract
Lactococcus lactis is widely used as a dairy starter and is becoming a recognized cell factory. In both applications, cells are exposed to stressful conditions that compromise the integrity of the cell envelope, a cellular structure crucial for survival. To understand how *L. lactis* responds to damage to its cell envelope, we have pursued an evolutionary experimental approach to evolve laboratory and industrial strains able to cope with Lcn972, a bacteriocin that inhibits cell wall synthesis during cell division. Lactococcal cultures were successively exposed to increasing concentrations of Lcn972 (adaptation) followed by growing in the absence of the stressor (stabilization) to fix mutations in the population. Through genome sequencing and transcriptional studies, it was found that resistance to Lcn972 is accomplished by induction of *llmg2447*, specifying a putative anti-ECF sigma factor in the laboratory strain MG1614. In industrial strains and field isolates, mutations accumulated mostly in genes specifying for a Bce-like module involved in sensing and resistance to antimicrobial peptides. In several Lcn972R clones, this Bce-like module, which has been proven to confer resistance to Lcn972, is constitutively activated. Nevertheless, variant analyses and genome-wide transcriptomics pointed to additional mechanisms and plausible metabolic adaptations whose protecting role against Lcn972 remains to be assessed. Long-read based genome sequencing also informed us of the loss of plasmids bearing important technological traits such as resistance to bacteriophages. All-in-all, a wealth of information has been gathered that will help us to understand the grounds of the cell envelope stress response in *L. lactis*.

Kewords
Bacteriocin, cell envelope, signal transduction, adaptive evolution

Complementation of the 1,2-propanediol pathway in propionic acid biosynthesis in selected lactic acid bacteria

Tamara Aleksandrzak-Piekarczyk, Lidia Stasiak-Różańska

Number
102

Themes
Fermentation and Metabolism, including protein transition

Abstract
Selected species of lactic acid bacteria (LAB) have the ability to convert lactate to 1,2-propanediol (1,2-PDO) (group I), which in turn can be converted to propionic acid (PA) by group II bacteria. Based on genome analyses of selected LAB obtained in this study, strains *Carnobacterium maltaromaticum* and *Lentilactobacillus buchneri* were assigned to group I, while *Pediococcus acidilactici*, *Levilactobacillus brevis*, and *Furfurilactobacillus rossiae* were included in group II. To create a stable consortium of individual pairs of strains, the risk of mutual inhibition of activity between them was determined and the production of organic acids by 15 coculture variants was analyzed. Each pair of microorganisms contained one species, capable of fermenting lactate to produce 1,2-PDO, and another, converting 1,2-PDO to propionate. Of all the cocultures tested, three pairs of LAB strains (*L. buchneri* 2047 and *P. acidilactici* 2065, *C. maltaromaticum* 3437 and *L. brevis* 6.14, and *C. maltaromaticum* 2857 and *L. brevis* 2.19B) induced the highest degree of acidification of the medium, equal to 4.2 pH. The decrease in pH in the medium can be due to the formation of several short-chain fatty acids such as acetic acid and propionic acid.

This work was supported by grant no. 2018/29/B/NZ9/02278 from the National Science Centre (Poland).

Kewords
propionic acid, cocultures, 1,2-propanediol, lactic acid bacteria

Dairy GABA-producing *Lactococcus lactis*: isolation, genomic and technological characterization, and optimization of GABA production

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Number
104

Themes
Genetics and Genomics
Fermentation and Metabolism, including protein transition

Abstract
GABA (γ-aminobutyric acid) is the main inhibitory neurotransmitter in the central nervous system. Therefore, it promotes relaxation and prevents sleeplessness and depression. Indeed, the disfunction of GABA homeostasis contribute to the development of major depressive and stress related disorders. The consumption of GABA can reverse this imbalance due to some of its pharmacological properties (reduction of anxiety, stress, etc). In fact, the US Food and Drug Administration has approved it as food ingredient, and even more, GABA-enriched food has been defined as "foods for specified health use" in some countries. The health benefits attributed to GABA has motivated us to screen and characterize GABA-producing LAB to use them i) as starters for the development of GABA-enriched fermented foods and, ii) as cell factories for industrial production of natural GABA.

This communication describes the isolation of GABA-producing *Lactococcus lactis* strains from camel milk, their characterization, their successful use as unique starters to elaborate GABA-enriched functional cheeses, and the optimization of GABA biosynthesis to use them as microbial cell factories.

Kewords
Lactococcus lactis, GABA, starter cultures, cell factory

Replacing animal-based ingredients by plant alternatives in hybrid food products

Wim Engels, Saskia Van Schalkwijk, Marjo Starrenburg, Simon Jacobs, Herwig Bachmann

Number
106

Themes
Microbial Communities
Fermentation and Metabolism, including protein transition

Abstract
To increase the sustainability of food supply there is a move towards the increased use of plant proteins. This shift comes with many technological challenges, as current food production processes are mostly optimized for animal, e.g. dairy, proteins. In addition, the creation of full vegan dairy-mimic products may come with challenges related to (off-)flavour or (off-)taste, antinutritional factors, and insufficient nutritional quality. The combination of plant-based ingredients with dairy in hybrid products aims to offer the best of both worlds to consumers by providing optimal taste and health benefits, through increasing the intake of plant-based foods, while limiting animal product consumption.

We here investigate the potential to reduce off-flavour and to produce desired cheese flavours in milk to which plant protein was added (hybrid “milk”) by applying fermentation with lactic acid bacteria (LAB). For this, pea, mungbean and chickpea proteins were selected as target plant proteins. Standardized hybrid “milk” formulations were prepared by adding a dispersion of plant protein to UHT dairy milk. Fifty LAB strains of the genera *Lactococcus*, *Lactobacillus*, *Leuconostoc* and *Streptococcus* were selected for the fermentations focused on cheese flavour formation, which was assessed by GC-MS analysis.

The results showed strain-specific differences in their potential to form desired cheese flavour compounds and/or to reduce undesired flavours in dairy-plant hybrid emulsions. The data suggest that combinations of strains might allow further improvement of sensorial properties and it demonstrates the potential of fermentation to facilitate the development of innovative plant-dairy hybrid products.

Kewords
lactic acid bacteria, hybrid plant-dairy, flavour, screening

Gene diversity of fructose metabolism in Fructobacillus

Florencia Mohamed, Fátima Romina Elizabeth Molina, Luc De Vuyst, Raúl Ricardo Raya, **Fernanda Mozzi**

Number
107

Themes
Genetics and Genomics

Abstract
The genus *Fructobacillus* is a group of fructophilic lactic acid bacteria reclassified from *Leuconostoc* that requires fructose or other external electron acceptors for growth. Fructose can be used as an external electron acceptor and reduced to mannitol by mannitol-2-dehydrogenase (MDH) or as carbon substrate through the phosphoketolase pathway by fructokinase (FK) and glucose-6-phosphate isomerase (GPI). This work studied the distribution of *mdh*, *fk*, and *gpi* genes and their genomic context in 13 *Fructobacillus* species and their gene expression in *Fructobacillus* sp. CRL 2054 and *F. tropaeoli* CRL 2034 when growing on fructose and/or glucose. The genomic region harboring the *mdh* gene was well-conserved in all genomes studied, with a gene for a fructose permease located downstream of the *mdh* gene. In contrast, two *fk* genes and three *gpi* genes were widely distributed within the genus. The genes *fk1* and *gpi1* were found in different parts of each genome, whereas *fk2* and *gpi2* were contiguously located in a presumptive operon (except for *F. broussonetiae*) with a variable genomic context. Additionally, *cre* repression sites were detected around some *fk* and *gpi* promoters. Differential gene expression studies showed a slight increase in *mdh* expression and a significative inhibition of *fk* and *gpi* expression when glucose was present compared to the expression values in the presence of fructose. The quantification of MDH, FK, and GPI enzymatic activities mostly supported these findings. The results obtained suggested catabolite repression of the *fk* and *gpi* genes by glucose for the *Fructobacillus* strains tested.

Kewords
Fructobacillus, mdh, fk, gpi

High-throughput antimicrobial resistance test of more than 3000 *Lactococcus lactis* strains.

Olivier Harlé, Karin Schlichter, Anna Koza, Rikke Dollerup Bech, Camilla Maciel Rabelo Pereira, Tuan Tung Tran Duc, Peter Nielsen, Gunnar Oeregaard

Number
108

Themes
Bacteriophage and Antimicrobials

Abstract
Lactococcus lactis have qualified presumption of safety (QPS) status. Despite the QPS status, there is additional focus to limit the spread of antimicrobial resistance (AMR), which is attributed to be responsible of million deaths ("Antimicrobial Resistance | EFSA" 2023). ISO 10932 | IDF 223 method is used to determine whether lactic acid bacteria are antibiotic resistant whereas, CLSI standards and genome sequencing are common practices to evaluate if putative antibiotic resistance genes are likely to spread (*e.g.*: horizontal gene transfer due to location on mobile genetic elements). Although these techniques are robust, they are generally low throughput and expensive. In this study, we have developed and implemented a high-throughput antimicrobial resistance screening assay miniaturized in SBS microplates for monitoring growth of bacteria at various concentrations of selected antibiotic compounds, using biomass determination by optical density measurements. In this study, high throughput antimicrobial test and antimicrobial gene presence of 3025 *L. lactis* strains was screened and compared. The data shows that 3010 out of 3025 *L. lactis* strains (99.5%) do not present any described antimicrobial resistance genes towards the following antibiotics: ampicillin, chloramphenicol, clindamycin, erythromycin, tetracycline, or vancomycin. 2811 strains (89.0%) were confirmed phenotypically to be antimicrobial susceptible by high throughput growth determination. The developed high-throughput antimicrobial resistance screening assay described in this study allows to increase the screening capacity, save time and costs to get insights on potential antimicrobial resistance.

Kewords
Lactococcus, screening, antibiotic resistance

Functional properties of a fermented pomegranate (*Punica granatum L.*) juice enriched with pomegranate seed oil

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Number
110

Themes
Fermentation and Metabolism, including protein transition

Abstract
Pomegranate juice (PJ) contains a wide variety of bioactive polyphenols, with hypolipidemic, anti-inflammatory, and antioxidant properties. Its seed oil (PO) is rich in conjugated linolenic acid isomers, mainly punicic acid, that have several biological effects. Nowadays, fruit juices fermented by lactic acid bacteria are increasingly becoming of interest within the functional beverage market. A PJ (60 %, v/v, pH 4.5) enriched with edible PO (0.5 %, v/v) was fermented by *Lactiplantibacillus paraplantarum* CRL2051 at 30 °C for 48 h. The functional properties of the fermented beverage (FPJO-CRL2051) were evaluated in C57BL/6 female mice fed with a high-fat diet (30 %, m/m, fatty content, HFD). Mice (5-week-old) were divided into 5 groups (n = 6) and fed *ad libitum* with (i) Biotherium control (CB): standard diet + water; (ii) HFD control: HFD + water; (iii) UPJO: HFD + unfermented PJ+PO; and (iv) FPJO-CRL2051: HFD + FPJO-CRL2051. After 8 weeks, animals were anesthetized and sacrificed; blood was collected, and tissues were removed and weighed. The results showed that FPJO-CRL2051 consumption significantly diminished mesenteric and inguinal fat deposition. Also, serum levels of triglycerides, LDL-cholesterol, and pro-inflammatory cytokines (TNF-α, INF-γ, and IL-12) were significantly reduced in treated animals compared with the HFD control group. In addition, glutathione peroxidase activity and TBARS concentration in FPJO-CRL2051 group presented similar values as CB. The fat accumulation in the hepatocytes was significantly lower in mice that consumed FPJO-CRL2051 than in HFD control animals. This study demonstrated the promising biological effects of FPJO-CRL2051 as a functional beverage for consumers.

Kewords
Pomegranate, seed, oil, fermentation, functionality, mice

Exploring the potential of lactic acid fermentation for modification of plant protein ingredients

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Number
114

Themes
Fermentation and Metabolism, including protein transition

Abstract
Consumption of plant protein sources needs to be increased to support the transition to a healthier diet and a more sustainable food system. Plant protein concentrates are common ingredients in plant-based foods such as meat and dairy products. They often suffer from poor sensory quality and may have nutritional and techno-functional limitations. Fermentation is a potential tool to improve the protein ingredients´ properties. However, there is limited understanding of lactic acid fermentation of plant proteins. This study compared 82 lactic acid bacteria (LAB) for their acidification and aroma modification ability in two plant protein concentrates without and with added nutrients. In the legume substrate, most strains needed added nutrients for proper fermentation. The grain-based substrate was a more complete growth medium. LAB differed in their innate ability to acidify the concentrates, and some trends at the genus and species level were seen. Fermentation created a range of perceived aromas, both pleasant and unpleasant, depending on the substrate, supplement, and organism. Selected strains producing a pleasant aroma were further studied. Differences in perceived aroma were reflected in volatile aroma compound profiles, which showed a decrease in off-flavour compounds and the formation of pleasant aroma compounds. The results help develop fermented plant protein ingredients with enhanced aroma and select potential cultures for different applications.

Kewords
plant protein, fermentation, aroma, acidification, VOC

Specific protease- and aminopeptidase activity of potential bioactive peptide-producing lactobacilli in media with plant protein hydrolysates

Teodora Panayotova, Zoltan Urshev

Number
116

Themes
Fermentation and Metabolism, including protein transition

Abstract
In this study cells of selected lactobacilli, potential producers of bioactive peptides, were evaluated for their specific protease activity and aminopeptidase activity after growth in milk, mMRS (MRS without peptone and meat extract) and mMRS with added pea protein hydrolysate, soy protein hydrolysate or whey protein 80 (WP 80). Five *Lactobacillus helveticus* strains (AB, h25, h48, h70, b244) and *Lacticaseibacillus casei* c1 (LB Bulgaricum PLC, Sofia, Bulgaria) were assayed. With minor exceptions the protease activity varied between strains to a much larger extent (> 10 fold) than the composition of each medium contributed to a changed value of the specific activity (up to 3 fold). This makes the selection of a highly proteolytic strains of primary importance. Interestingly, the protease activity was high even in cells grown in mMRS. Leucine- and lysine-aminopeptidase activities were highest in milk and mMRS, while addition of plant hydrolysates or WP 80 resulting in lower values. As a whole the activities of these two aminopeptidases followed a constitutive pattern. On the contrary the arginine- and proline-aminopeptidase activities showed inducible character with measurable values obtained only in milk and mMRS with added pea protein hydrolysate. In the end of the study we selected two *L. helveticus* strains (b244 and h70) with high protease and aminopeptidase activity for further experiments.

Kewords
aminopeptidase activity, lactobacilli, plant protein hydrolysates, protease

Vaginal Lactobacillus spp. dominance in Cameroonian pregnant women

Marie Josiane Kenfack zanguim, Condori Sandra, Sebastien Kenmoe, Romeo Djoundja, Modeste Ngamaleu, Micheal Bessong, Sarah Ahannach, Rose LEKE, Forgu Esemu Livo, Jude Bigoga, Rosette Megnekou, Sarah Lebeer

Number
118

Themes
Microbial Communities

Abstract
Lactobacillus spp. are common dominant members of the vaginal microbial community. Key species identified so far are *Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus iners*, *Lactobacillus jensenii.* When *Lactobacillus* is not dominant, it co-exists with strict anaerobic bacteria commonly associated with adverse health outcomes. This condition where lactobacilli are scarce or depleted is known as bacterial vaginosis (BV). BV is prevalent in Cameroon, affecting almost 20% of pregnant women. BV increases the risk of contracting more serious infections such as HIV, *Chlamydia*, *Trichomonas*, and *Neisseria*. BV can also lead to complications such as premature rupture of membranes and neonatal sepsis.

Little is known about the dominance of *Lactobacillus* spp. in African populations despite their crucial role in women's health and pregnancy. The Rose Leke project, a sister project of the Belgian Isala project (https://isala.be/en/), aims at mapping the vaginal microbiome in healthy women (also during pregnancy) living in Cameroon. In this study, pregnant women (+18 years old) donated vaginal swabs for microscopy (Nugent score), microbiome analysis (eNAT) and bacterial isolation (eSwab). Microscopy results showed that lactobacilli are abundant, low, and even rare/absent in 55%, 12%, 33% of participants respectively. In contrast, lactobacilli showed to be abundant, low and rare/absent in 37%, 23% and 41% in Pregnant women living with HIV. Sequencing technology will be used for in-depth analyses of samples. Further analysis of metadata on socio-demographics, lifestyle, dietary patterns among others is ongoing to identify the factors shaping the vaginal microbiota composition and contribute to increasing representation in vaginal microbiome research.

Kewords
Lactobacillus spp, pregnant women, vaginal microbiome

Simple & Better – Accelerated cheese ripening using a mesophilic starter based on a single strain with superior autolytic properties

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Number
120

Themes
Fermentation and Metabolism, including protein transition
Bacteriophage and Antimicrobials

Abstract
In the manufacture of rennet-coagulated cheese, autolysis is a rate-limiting step for ripening. Previously we have generated a highly autolytic and thermotolerant strain, RD07, which in preliminary laboratory cheese trials demonstrated great potential as a cheese ripening accelerant. RD07 is proteinase positive (Prt^+) and is capable of metabolizing citrate (Cit^+). In this study, we isolated a derivative of RD07 lacking the citrate plasmid, EC8, and one that in also lacks the proteinase plasmid, EC2. We found that EC2 and EC8 retained the autolytic properties of RD07, and autolyzed 20 times faster than Flora Danica (FD) and SD96, the parent of RD07. We prepared a simple starter, ERC, comprised of EC2, EC8, and RD07 (90:8:2). ERC was less sensitive to cooking when cultured in milk and autolyzed well after entering stationary phase facing sugar starvation. The ERC starter was benchmarked against FD and SD96 in laboratory cheese trials, and the content of free amino acids in cheese prepared using the ERC-culture was 31% and 34% higher than in cheese prepared using FD and SD96, respectively. Overall, the ERC culture resulted in a more rapid release of free amino acids. A large-scale (5000 L) Gouda cheese trial at a Danish dairy demonstrated that the single strain ERC starter, was comparable in performance to FD + an adjunct *Lactobacillus helveticus* culture. Furthermore, a large-scale Danbo cheese trial demonstrated that ERC could reduce the ripening period by 50% for long-term ripened (25 weeks) cheese and resulted in better cheese.

Kewords
autolysis, intracellular peptidase, thermotolerance, cheese starter

NOVEL SOURCES FOR THE ISOLATION OF FUNCTIONAL LACTIC ACID BACTERIA

Medana Zamfir, Iulia-Roxana Angelescu, Emanuela-Catalina Ionetic, Teodora-Ecaterina Chirea, Silvia-Simona Grosu-Tudor

Number
122

Themes
Microbial Communities

Abstract
BorÅŸ is an acidic liquid commonly used in Romanian cuisine to impart a sour taste to a variety of traditional soups known as *borÅŸ* or *ciorba.* It is also consumed as a refreshing drink and it is believed to have many health benefits, especially due to its high vitamin and mineral contents. Cheese rennet is traditionally prepared from animal stomach (usually lamb or young goat), which is soaked in water or cheese whey before use. The aims of this study were to evaluate the microbial content of *borÈ™* and rennet, to isolate and identify lactic acid bacteria (LAB) from these products, and to screen the newly isolated strains for potential functional properties.

From the 24 samples of *borÈ™* and 17 samples of rennet that were available, 324 presumtive LAB were isolated and purified. After dereplication by rep-PCR with $(GTG)_5$ primers, selected isolates were identified by 16S-rDNA sequencing. The most common species found in rennet were *Lactococcus lactis* and *Lactiplantibacillus plantarum*, while in *borÈ™*, a wide diversity of lactobacilli was found. In the later samples, acetic acid bacteria, belonging to *Acetobacter* genus were also found. Regarding their functionality, among the newly isolated strains, we identfied fast growing and acidifing strains, exopolysaccharide-producing ones, but also strains with antibacterial activity against other LAB and against some (potential) pathogens.

This research has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101060247 (HealthFerm project) and from the Institute of Biology Bucharest of Romanian Academy, project number RO1567-IBB05/2023.

Kewords
bors, cheese rennet, antimicrobial activity, identification

Effects of Addition of Jogi on the Production of Volatile Compounds and Correlation of Microbial Community in Baechu-Kimchi

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Number
126

Themes
Fermentation and Metabolism, including protein transition

Abstract
Kimchi is a traditional vegetable fermented food in Korea, and a generic name that mainly uses kimchi cabbage (*Brassica rapa*) as a main ingredient. *Kimchi* is a slow food that uses various ingredients by region, and fish are sometimes added as a supplementary material in coastal areas. Although a lot of research has been conducted on *kimchi*, research on *baechu-kimchi* with regional diversity is limited. Therefore, we wanted to confirm the effect of *jogi* (*Micropogonias undulatus*) addition on the volatile component of *baechu-kimchi*. *Baechu-kimchi* without *jogi* was used as the control group. Changes in volatile compounds were observed during fermentation, and sulfur-containing compounds were detected the most. Furthermore, the correlation with the previous study was conducted. In particular, correlation with volatile compounds and microbial community in *Kimchi* was compared, and it was confirmed that the volatile compound was affected according to the dominant species.

Kewords
Baechu-kimchi, Jogi-baechu-kimchi, volatile_compounds, Bacterial_community

Interactions between dairy-fermentation conditions and acidification performance of lactic acid bacteria

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Number
128

Themes
Fermentation and Metabolism, including protein transition

Abstract
In the production of fermented dairy products, acidification temperature, milk heat-treatment and composition, food additives, and metabolic by-products, are common factors that influence the quality (texture, aroma, etc.) and safety of these products. However, knowledge on how combination of these factors can influence (in simulated production conditions) the acidification performance of lactic acid bacteria (LAB) remains scarce.

Here, we investigated the effect of acidification temperatures (meso- and thermophilic ranges), milk type (5% protein and high-heat treatment milk), and additives (NaCl, sucrose, urea) on the acidification kinetics under combinations of factors. We performed a mapping study on a subset of >50,000 strains in the Chr. Hansen Culture Collection. We examined 2,436 acidifications of *Streptococcus thermophilus* (58 strains), *Lactococcus lactis* (22 strains), and *Lactococcus cremoris* (17 strains) subjected to fermentation. Within *Lactococcus*, addition of 4% NaCl was a major factor for slower acidifications, followed by sub-optimal temperatures (25°C and 40°C), and high heat-treated milk (>85°C). For *St. thermophilus*, lower acidification speeds were primarily associated with 5% protein milk (high protein), followed by 1% salt, 0.02% urea, and temperatures of 43°C and 37°C.

Our results outline the importance of characterizing LAB under a wide range of industrially relevant conditions. This will facilitate the selection of species/strains appropriate to the production conditions, and with the desired impact on product characteristics. In the future, gaining deeper understanding of the molecular interactions between bacteria and culture conditions will define a framework for fueling the development of digital tailoring of starter cultures.

Kewords
fermentation, acidification, dairy, conditions, lactococcus, streptococcus

Stimulation of probiotic Bifidobacterium strains by Human Milk Oligosaccharides (HMOs) for the development of synbiotic formulas

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Number
130

Themes
Fermentation and Metabolism, including protein transition

Abstract
Aim: This work aims at assessing the ability of four probiotic strains, *Bifidobacterium bifidum* BB10 (DSM 33678), *Bifidobacterium breve* BR03 (DSM 16604), *Bifidobacterium longum* subsp. *infantis* BI02 (DSM 24687) and *Bifidobacterium longum* subsp. *longum* BL03 (DSM 16603) to metabolize and grow on synthetic Human Milk Oligosaccharides (HMOs) (GlyCare™, DSM) to develop new synbiotic finished formula.

Methods: Strains were inoculated on de Man Rogosa Sharp supplemented with 2% (w/v) glucose or 2% (w/v) of each of the following HMOs: 2'-Fucosyllactose (2'-FL), 3'-O-Sialyllactose (3'-SL), 6'-O-Sialyllactose (6'-SL), Lacto-N-tetraose (LNT), Lacto-N-neotetraose (LnNT), 2'-Fucosyllactose/Difucosyllactose (2'-FL/DFL mix). The response of *Bifidobacterium* strains was studied in terms of a) metabolic activity by microcalorimetry (calScreener™, Symcel), b) growth by cytofluorimetric enumeration (Cytoflex, Beckman Coulter) and c) acidification kinetics (iCinac, AMS Alliance).

Results: The strains metabolized HMOs with a species (strain) specific pattern. In particular, LNT and LNnT were efficiently assimilated by all the strains and specifically for BL03 and BB10 the growth was boosted over glucose. Furthermore, all the *Bifidobacterium* strains with exception of BB10 showed limited capability to grow on sialylated HMOs, while fucosylated HMOs were selectively utilized by BB10 and BI02. Noteworthy, results showed that not in all the conditions a higher metabolic activity was correlated with enhanced acidification and growth performances.

Conclusions: This study discovers specific associations between HMOs and *Bifidobacterium* strains. This represents the first advancement in the development of innovative synbiotics

and set the bases for further studies aimed at investigating beneficial effects derived from the administration of *Bifidobacterium* strains and HMOs.

Kewords
Human Milk Oligosaccharides; Bifidobacteria; Synbiotics.

Oral health and teeth decay: a potential protective role of the synbiotic ammonia-producer lactobacilli and arginine

Teresa Graziano, Annalisa Visciglia, Angela Amoruso, Marco Pane

Number
132

Themes
Fermentation and Metabolism, including protein transition

Abstract
Introduction : This study is aimed at selecting probiotic strains capable of producing ammonia starting from arginine, preventing or modulating the progression of some diseases of the oral cavity such as dental caries.

Materials and methods : Cultures of selected probiotics, *L.brevis* LBR01 (DSM 23034), *L.reuteri* LRE11 (DSM 33827), *L.casei* LC03 (DSM 27537) and *L.rhamnosus* LR04 (DSM 16605) were inoculated (108/mL) in medium at pH 7 and 5.5 containing 0.3% I-arginine, with and without enzymes typically found in the oral cavity.

Results: *L.brevis* LBR01 and *L.reuteri* LRE11 produce ammonia in the presence of arginine causing an increase in pH as well as showing better growth capacity.

Discussion: These results suggest that these probiotics could be a useful preventive strategy in oral health management, maintaining a constant pH above 5.5 critical value for the formation of dental caries.

Kewords
Oral care,arginine,Lactobacillus,dental caries,pH homeostasis

Cellular changes occurring in LABs under the influence of additives commonly used in the production of probiotics

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Number
134

Themes
Microbial Communities

Abstract
The probiotic formulations are prepared by freeze-drying and spray drying processes. However, these methods lead to a significant decrease in viability of microorganisms and reduce the therapeutic efficacy of the probiotic preparations. The survival rate of the bacterial cells during drying can also be improved by additives of a protective nature, such as proteins, sugars, and carbohydrates. Unfortunately, some of these substances, that are considered as the protective agents (PAs), can negatively affect not only the viability of bacteria, but also their other functional properties. The latest studies clearly indicate the need for further studies about the impact of additives used in probiotic production processes.

The aim of this research was to analyze the response of lactic acid bacteria to the presence of potential PAs used in probiotic preparations. Among others, trehalose, vitamin C, arabic gum and monosodium glutamate were chosen for testing. Modifications of cell membrane permeability, surface hydrophobicity and surface roughness of bacteria were analyzed. The potential occurrence of oxidative stress in bacterial cells was also determined. The results obtained have shown that the addition of selected PAs changed significantly functional properties of the analyzed strains, compared to control cells. Moreover, the direction of changes was strongly dependent on the probiotic bacterial strains used in the research.

The research was financed by the Ministry of Education and Science as part of a grant for the statutory activity of the Poznan University of Technology (0912/SBAD/2311).

Kewords
LAB, bacterial cells properties, protective agents

Influence of fermentation temperature on the chemical, microbial and sensory profile of sauerkraut and kimchi

Rain Kuldjärv, Emili Aus, Helen Vaikma, Marina Junusova, Anne Meikas, Jekaterina Kazantseva

Number
136

Themes
Microbial Communities
Fermentation and Metabolism, including protein transition

Abstract
Sauerkraut and kimchi are well-known fermented vegetables that are gaining more popularity nowadays due to their nutritional benefits, including probiotics. In our study, we aimed to see how chemical, microbial and sensorial profiles of the products change if we ferment sauerkraut and kimchi at temperatures of 15, 20, and 25 °C. The sauerkraut and kimchi were produced by the company Kadarbiku Köögivili OÜ. Fermentation was conducted in climate chambers; each batch 4 kg. Time points for analysis: 0-, 4th, 7th and 14th day. HPLC was used to measure chemical profiles. Microbiological analysis - modified 16S rRNA sequencing technology that can discriminate between total and viable bacteria and provide the estimated number of cells. Descriptive sensory analysis was carried out in the sensory lab with trained assessors.The results showed that the most different temperature in terms of sugar consumption and organic acid production was 15 °C. Sugars were depleted for the 14th day at temperatures 20 and 25 °C, but not at 15 °C. Sensory analysis showed also clear differences between temperatures 15 °C, and 20/25 °C. Metagenomic analysis showed that the bacterial composition of kimchi and sauerkraut which were produced mainly from similar vegetables and in the same production facility is rather similar. Some differences were monitored in the proportions of lactic acid bacteria: sauerkraut had a higher proportion of *Leuconostoc*, especially at lower temperatures. *Lactiplantibacillus* and *Levilactobacillus* were dominant in kimchi. We were also able to conclude that the warmer the environment, the less *Leuconostoc* and *Latilactobacillus* occur.

Kewords
kimchi, sauerkraut, fermentation temperature

Tryptamine accumulates in cheese via lactic acid bacteria-driven tryptophan decarboxylation

Mr. David Arranz, Ms. Eva Fernandez, Dr. Beatriz del Río, **Dr. Begoña Redruello**, Dr. Miguel A. Alvarez

Number
138

Themes
Fermentation and Metabolism, including protein transition

Abstract
Tryptamine is a neuroactive compound that derives from the decarboxylation of L-tryptophan. Tryptamine may act as a neurotransmitter or neuromodulator of the human central nervous system, where it is found in low concentrations. Tryptamine is considered to be a SRA (serotonin releasing agent), a type of drug that induces the release of serotonin into the neuronal synaptic cleft. SRA have been used clinically as appetite suppressants, and they have also been proposed as novel antidepressants and anxiolytics. Therefore, we considered the screening of tryptamine-producing lactic acid bacteria (LAB).

Tryptamine has been detected in fermented food such as cheese, although data about the type of cheese where it accumulates and the concentrations that it can reach are very scarce. Supposedly, microbial tryptophan decarboxylases are the responsible enzymes for tryptamine biosynthesis in cheese. However, neither the microbial species nor the enzyme have been yet identified. Our hypothesis is that cheese accumulating tryptamine could be used as source of potential LAB able to decarboxylate L-tryptophan and produce tryptamine.

In this work, we have quantified the tryptamine content of hundreds of different cheeses. Moreover, following a knowledge discovery in databases approach we identified those technological/environmental/metabolic profiles of cheeses accumulating the highest amounts of tryptamine. Furthermore, from those cheeses we have isolated LAB with the ability to convert tryptophan into tryptamine. These results open the possibility of the future use of such LAB as therapeutic psychobiotics for conferring a mental health benefit to the host.

Kewords
Cheese, neuroactive compounds, tryptamine, psychobiotics

Optimizing microbial viability in freeze-dried powders containing probiotics and LBPs through lyoprotectant screening

Anneloes Groenenboom, Lu Wang, Jolanda Lambert, Herwig Bachmann, Janneke Ouwerkerk

Number
140

Themes
Fermentation and Metabolism, including protein transition

Abstract
Developing probiotic strains and live biotherapeutic products (LBPs) from laboratory to market can be a challenging trajectory. Several steps need to be taken on the development of a product for clinical study or commercial production. Robust process development can include food or pharma-grade media, optimizing fermentation and harvest conditions, and stabilizing cells during downstream processing (DSP). A widely used DSP step is lyophilization or freeze drying of the microorganisms. Lyophilization is a critical step for successful production as viable cell counts can easily decrease >90%, especially strict anaerobe next generation probiotics and LBPs.

Here we describe a pipeline that was optimized for the screening of variables that determine the overall survival of cells. On the example of *L. plantarum*, and *F. prausnitzii* we performed combinatorial screening with variations in growth conditions, lyoprotectants and freeze drying conditions with the aim to maximize cell survival during freezing, drying, and storage. All variants were eventually subjected to a shelf-life study. After analyses of viable cells using plate counting and other viability measures like flow cytometry, a quick overview of the most successful combinations can be prepared.

The results show a big impact of the 30 used lyoprotectants on the strains survival. While some lyoprotectants performed overall much better than others there were also significant strain to strain variations. While for the application such screening campaigns allow for short time to market the detailed analysis of diverse datasets should eventually also aid to generate more generic understanding of cell survival in industrial applications.

Kewords
Probiotics, Lyophilization, Survival, Anaerobe, Fermentation, Stability, Yield

Exploring Lactococcus strain diversity with the help of genome-scale metabolic modeling

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Number
142

Themes
Genetics and Genomics
Fermentation and Metabolism, including protein transition

Abstract
Understanding strain diversity is essential when developing new starter cultures for the dairy industry, as it allows for compounding using the right building blocks. For understanding this diversity, strain-specific genome-scale metabolic models (GEMs) stand as a promising solution. Current methods for generating this type of models are either designed for handling large sets of strains, or for accurately reflecting the metabolism of a few strains. However, both quantity and quality are important for analyzing the metabolic differences among many strains of the same or closely related species.

In this study, we present a workflow for the high-throughput generation of high-quality GEMs of closely related strains, which we apply for the generation of 439 GEMs of *Lactococcus lactis* and *Lactococcus cremoris* strains.

Comparison of the resulting GEMs under different growth conditions revealed metabolic differences between the strains in carbon-source utilization, fermentation products and nutrient requirements. Notably, over 90% of *L. cremoris* strains were unable to utilize xylose and/or showed limited ribose utilization due to the lack of key enzymes in the pentose phosphate pathway. Additionally, cysteine was able to be synthesized by only 11% of the strain-specific GEMs, where it was shown to be advantageous for growth on milk.

The workflow presented in this study enables the generation of GEMs that can be used for comparing multiple closely related strains. Furthermore, the 439 generated *Lactococcus* GEMs are a valuable resource for future studies of metabolic diversity in different applications within the dairy industry.

Kewords
Lactococcus, mechanistic modeling, high-throughput, dairy industry

Distribution and diversity of plasmid sequences in Streptococcus thermophilus genomes

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Number
144

Themes
Genetics and Genomics

Abstract
Plasmids are small, circular DNA molecules that can replicate autonomously in bacterial cells and be horizontally transferred by natural mechanisms such as conjugation and transformation. Lactic acid bacteria are well known for their rich and diverse plasmid complements, notably the *Lactococcus* and (former) *Lactobacillus* genera. In contrast, the *Streptococcus thermophilus* species is well known for its scarcity in plasmids. However, since the early eighties, several *S. thermophilus* plasmids have been described, showing an ever-increasing sequence diversity which has undermined the initial dogma of plasmid scarcity in this food-grade streptococcal species. The objective of this work was to review the existing knowledge about plasmid biology in *S. thermophilus*, and to leverage it to investigate the plasmid distribution and diversity within IFF's proprietary collection of *S. thermophilus* genomes. At least one plasmid could be identified in almost 17% of the available genomes, including public as well as proprietary sequences from commercial strains. Within draft genomes, many plasmids could be "circularized" *in silico*, leading to putative, complete plasmids of 2.8 to 6.3 kb in size. Most often, strains contained a single plasmid, rarely two; one public genome appeared as an exception with three plasmids. Plasmids identified in proprietary sequences were compared to published ones, and all were classified into a dozen distinct replication groups, highlighting the existence of a completely novel plasmid group. Seven of these plasmid groups corresponded to rolling-circle replication, while at least two groups replicate through a *theta* mechanism. Many plasmids were cryptic, just coding for their own replication system.

Kewords
plasmid, genome, lactic acid bacterium, Streptococcus thermophilus

The microbial community of a traditional fermented Zambian dairy product generates different aroma profiles upon variation of production methods.

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Number
146

Themes
Microbial Communities
Fermentation and Metabolism, including protein transition

Abstract
Aroma is a key sensory attribute that gives the first flavour impression of fermented foods, thus important in determining consumer preference and acceptability. The aroma in fermented dairy comprises the volatile compounds produced by the activity of fermenting microbes and those originally present in unfermented milk. The diverse volatile compounds coupled with certain compounds detectable by human olfactory senses characterize the odor-active profile of fermented products. We studied the influence of production method variation on the microbial community and aroma of a traditional Zambian fermented dairy product, mabisi. The microbial structure and the volatile composition of four differently produced mabisi were investigated using 16S rDNA amplicon sequence, and HS-SPME/GC-MS and PTR-QiTOF-MS techniques, respectively. Odor-active compounds were identified using GC-O-MS and the findings were validated through the Odor Activity Values (OAV) calculations.

16S rDNA amplicon sequencing of the products revealed a diverse microbial community dominated by *Lactococcus* and *Lactobacillus*. We found minor variations in the microbial composition of different mabisi products, while the functionality of the communities varied significantly between products. The PTR-QiTOF-MS run, measured 390 m/z peaks, 55 of which were tentatively identified. The GC-MS analysis detected 26 volatile compounds, consisting of ketones, aldehydes, alcohols, esters, and carboxylic acids. Twelve volatile compounds were detected by human olfaction during GC-O-MS analysis with the most prominent being the ketones and esters giving a buttery and fruity aroma respectively. Only 6 were identified as odor-active compounds by OAV. Our findings provide fundamental insights for microbial ecology and product optimization for further mabisi upscaling.

Kewords
Fermentation, mabisi, GC-O-MS, dairy, volatile-compounds, odor-active-compounds, OAV

The Impact of Different Fermentation Protocols on the Antifungal Activity of Sourdough Bread

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Number
148

Themes
Fermentation and Metabolism, including protein transition

Abstract
Sourdough is often used in bread production to improve flavour, texture, and to contribute to leavening. A recent surge in popularity has re-invigorated study into its functional and nutritional capabilities, including the antifungal effects of the primary acids produced by lactobacilli. This study aimed to determine the effectiveness of different fermentation conditions and sourdough additions in conferring these benefits.

In the first experiment heterofermentative and homofermentative lactobacilli were used to produce 10% sourdough bread of varying water activities. In the second, lactobacilli selected from the previous study were incorporated into sourdoughs, this time fermenting under 2 different conditions and at a lower temperature. Artificially acidified and straight bread doughs served as the controls. All breads were inoculated with strains of *Penicillium roqueforti* and *Aspergillus clavatus* and fungal growth was monitored over 9 days. Organic acid concentrations at various stages of bread making were quantified by HPLC.

Results show that a 10% addition of sourdough is not enough to increase the mold-free shelf life of bread regardless of the fermentation style. However, the shelf life of sourdough loaves matched the controls, and a combination of sourdough and 0.1% Calcium propionate (CaPro) are equivalent in shelf life to an artificially acidified or 0.3% CaPro control.

Tracking metabolites over the course of fermentation and baking has also provided new information about the effect of different fermentation styles, temperatures, and baking on organic acid content. The findings of this study indicate that sourdough is a suitable alternative to chemically-preserved or acidified breads.

Kewords
Sourdough, mould, shelf life, clean label, bread

Importance of surface layer protein B on lactobacilli in the uptake into THP-1 dendritic cells and following cytokine productions

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Number
150

Themes
Microbial Communities
Fermentation and Metabolism, including protein transition

Abstract
Lactic acid bacteria (LAB) strains are known to be potent inducers of proinflammatory cytokines in the gut. The uptakes of lactobacilli into DCs are closely linked with surface layer proteins (SLPs) on bacterial cells and following cytokine productions from DCs. However, the major SLPs that affect the interaction between lactobacilli and DCs remain unknown.

In the present study, we evaluated the importance of SLPs in various LAB strains for the uptakes into THP-1 DCs and following cytokine productions. Among tested 12 LAB species, *Lactobacillus helveticus* JCM 1120, *Lactobacillus acidophilus* JCM 1132, *Levilactobacillus brevis* JCM 1059, and *Lentilactobacillus kefiri* JCM 5818 showed significantly higher uptake into THP-1 DCs. Surface layer protein B (SlpB) isolated from *L. brevis* JCM 1059 essential to interact with THP-1 DCs and the receptor was identified as adenylyl cyclase-associated protein 1 (CAP-1). The SlpB coated LAB strains improved the uptakes in THP-1 DCs and cytokine productions.

These results strongly suggest that SlpB on *L. brevis* JCM 1059 via preferentially binds to CAP-1 on THP-1 DCs plays a crucial role in bacterial uptake by THP-1 cells as well as in subsequent interleukin-12 (IL-12) production.

Kewords
lactobacilli; THP-1; dendritic cells; SlpB; CAP-1; IL-12

Development of Spoilage Psychrotrophic Lactic Acid Bacteria in Ready-to-Eat Salad Under Cold Storage

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Number
152

Themes
Microbial Communities

Abstract
The consumption of ready-to-eat (RTE) foods as a convenience and fresh meal has been increasing in recent years. However, the quality and safety of RTE foods can be affected by spoilage bacteria, since they are not subjected to heat treatment. The objective of this study was to assess the effects of storage conditions on the quality of commercially produced mayonnaise-based salad in modified atmosphere packaging (MAP). The salads were kept under different temperatures (0, 2, 4, 6, and 8°C) for 7, 10, and 12 storage days to follow changes in the physicochemical and microbiological, and sensory profiles. The microbiota composition was determined by plating on the selective media (PCA, VRBG, MRS, and DRBC) followed by the MALDI-TOF technique. The results revealed that lactic acid bacteria (LAB) belonging to the species *Lactococcus piscium*, *Carnobacterium maltaromaticum*, *Leuconostoc carnosum*, and *Leuconostoc gelidum* became dominant during storage. Their numbers increased from 10^6 to 10^9 cfu/g at the end of shelf-life, depending on storage temperature. The growth of LAB was accompanied by a decrease in pH and an increase in TTA from 6.31 to 4.44 and 1 to 2.9 respectively. The sensory quality deteriorated faster at high temperatures resulting in a sour and acidic odor on the 7th day, while the storage at 0 and 2°C preserved the salad quality for up to 10 days. In conclusion, psychrotrophic lactic bacteria are dominant spoilage microorganisms in RTE mayonnaise-based salad and can be effectively controlled by appropriate storage temperature.

Kewords
Ready-to-eat salad, MAP, spoilage bacteria, storage temperature

Citizen-science mapping analysis and biostatistical analysis of the vaginal microbiome in the Isala project

Dr. Thies Gehrmann, Sarah Ahannach, Sarah Lebeer, Stijn Wittouck

Number
154

Themes
Microbial Communities
Host Microbe Interactions

Abstract
The lack of knowledge of the role that the vaginal microbiome in health and how it can be modulated by lifecourse, lifestyle and environmental factors limits our understanding of the microbiome's role in disease and treatment. Therefore, we used a citizen-science approach with 2,345 participants to map the vaginal microbiome in healthy women.

The *Lactobacillus crispatus* or *Lactobacillus iners* subgenus was dominant in 71% of our generally healthy cohort, while they were also co-dominant in 14.2%. In a taxa-taxa correlation network, we identified four modules of correlated taxa, one comprising of *L. crispatus*, *L. jensenii* and *Limosilactobacilli*, and three others representing Aerobic vaginitis, Anaerobic vaginitis, and gut-associated bacteria. These modules were validated in two other large publicly available datasets of not only healthy participants (VALENCIA & VaHMP cohorts). Within our Isala data, besides age, having children showed the largest effect size on different parameters of the vaginal microbiome analysed. Estimated estrogen levels, menstrual hygiene, contraceptive use, sexual lifestyle, and diet also showed significant associations with alpha- and beta-diversity indices, community state types, module eigentaxa, and 45 individual taxa tested by six different differential abundance testing methods. Together, these associations could explain 10.4% of the vaginal microbiome variation. This high-resolution mapping of the healthy vaginal microbiome and its metadata, together with an analysis of bacterial interactions and host characteristics provides a unique reference for follow-up case-control and intervention studies of the vaginal microbiome as well as understanding the opportunities of lifestyle interventions for women's health.

Kewords
Microbiome, vaginal niche, lifestyle, statistics

Exploring metabolic strategies to reduce excess acidification by lactobacilli: A genome-scale metabolic modeling approach

Martin H. Rau, Solvej Siedler, Susanne Bidstrup, Ahmad A. Zeidan

Number
156

Themes
Fermentation and Metabolism, including protein transition

Abstract
Excess acidification by lactobacilli during storage in fermented milk products, also known as post-acidification, 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 to effectively counteract acid formation. Notably, through the consumption of citrate and synthesis of other metabolites, the process of acidification could in effect be reversed.

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.

Kewords
Lacticaseibacillus rhamnosus, genome-scale metabolic modeling, acidification

The importance of risk assessment for the use of microorganisms in the food and feed chains

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Number
158

Themes
Genetics and Genomics
Bacteriophage and Antimicrobials

Abstract
A wide variety of bacterial cultures are intentionally used at different stages in food and feed production. These microorganisms can be used “as such” or as production organisms of substances of interest, for food ingredients and feed additives, novel foods or plant protection products. In case these organisms are subject to an application for market organization in Europe, a risk assessment by the European Food Safety Authority (EFSA) is required. This risk assessment is based on application dossiers, for which EFSA guidance documents apply.
Whole genome sequence (WGS) data play an important role in the risk assessment and should be used for the identification of the microorganisms, early in the process. Strains belonging to taxa having qualified presumption of safety (QPS) status may benefit a fast-track evaluation. WGS data should also be screened for the presence of antimicrobial resistance (AMR) genes, with focus on resistance to critically or highly important antimicrobials used in human medicine. In many cases, phenotypic antimicrobial susceptibility testing (AST - MIC determination) is also required.
At BCCM/LMG, several lactic acid bacteria (LAB) have been analysed in the frame of application dossiers. Up to 914 LAB were subjected to either AST or genome analysis in the frame of identification or the search for AMR genes. A subset of these LAB were selected by the clients for safe or patent deposit at BCCM/LMG. Offering this BCCM/LMG scientific expertise to industry can significantly speed up the pre-market authorization process.

Kewords
EFSA risk assessment, genome sequencing, MIC determination

Investigating phage-host interactions in factories employing mesophilic undefined starter cultures

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Number
160

Themes
Microbial Communities
Host Microbe Interactions
Bacteriophage and Antimicrobials

Abstract
The dairy fermentation industry relies on the activity of lactic acid bacteria, such as *Lactococcus lactis/cremoris* and *Streptococcus thermophilus* strains, in starter cultures to facilitate milk acidification. Maintenance of the composition and integrity of these starter cultures, whether defined or undefined, is essential to ensure consistent and high-quality fermentation end products. Traditionally, culture-dependent methods have been used as the primary approach to characterise complex undefined cultures; however, in recent years culture-independent methods have also been utilised. In the present study, a combination of culture-dependent and independent methods was employed to define the microbial diversity of an undefined mesophilic starter culture.

Bacteriophage infection of starter bacteria remains one of the most significant threats to the dairy fermentation process with associated costly disruptions. Therefore, in addition to defining the composition of starter cultures, it is also important to understand the diversity and impact of phages associated with certain dairy fermentation facilities and cultures. Phage infection typically commences with reversible binding of a phage receptor binding protein to a saccharidic receptor on lactococcal cell surfaces, i.e. **c**ell **w**all **p**oly**s**accharide (CWPS) structures. Therefore, improved predictions of the risk of phage infection can be garnered by defining both the CWPS type/subtype of strains within a starter culture and phage/host relationships within a fermentation system. In the present study, phage diversity in whey samples originating from fermentation factories employing this starter culture was investigated to establish the range of phage-host relationships within this complex microbial community.

Kewords
bacteriophages, undefined starter cultures, cell wall polysaccharides

Widening the class II bacteriocin landscape with circuitry and secretion elements in bacteria

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Number
162

Themes
Bacteriophage and Antimicrobials

Abstract
Facing the surge of antibiotic resistance, the medical field has a critical need for alternatives to treat bacterial infections. Among these, the use of ribosomally-synthesized antimicrobial peptides produced by bacteria (*i.e.* bacteriocins) is considered as a promising route.

In the human commensal *Streptococcus salivarius*, the production of unmodified class II bacteriocins is directly controlled at the transcriptional level by the quorum-sensing ComRS system. Here, we used an integrated approach combining bioinformatics and synthetic biology to identify novel bacteriocins from salivarius streptococci active against pathogens. We developed a bioinformatics pipeline that combines conservation of DNA motifs for genetic regulation and features of bacteriocin sequences to uncover cryptic class II bacteriocins. Notably, we discovered more than 50 novel bacteriocin candidates clustered into 21 groups from 100 genomes of *S. salivarius*. As proof of concept, we applied a similar *in silico* strategy for other streptococci species that use a different quorum sensing-based regulation such as *Streptococcus thermophilus*, *Streptococcus pneumoniae* and *Streptococcus oralis*.

The *in vitro* and *in vivo* production by synthetic biology tools of a representative set of 21 potential bacteriocins showed that most of them are active against a panel of bacteria, including clinically-relevant pathogens. To conclude, this combined approach offers a generic pipeline for the discovery of novel bacteriocins in Gram-positive bacteria that could be used in cocktail for broad applications in food industry or medicine.

Kewords
quorum sensing, predation, bacteriocins, BlpRH, ComRS, bioinformatics

Genetic Engineering of Lactic Acid Bacteria *Lactococcus lactis* for Growth on Cellulose

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Number
168

Themes
Genetics and Genomics
Fermentation and Metabolism, including protein transition

Abstract
Industries and households generate large amounts of lignocellulosic waste that could be used as a cheaper and more sustainable alternative for microbial fermentation of high-value chemicals such as lactic acid. The main component of lignocellulosic biomass is cellulose. To date, no naturally occurring lactic acid bacterium has been found that would be able to grow on cellulosic substrate on its own. The major limitation is the absence of extracellular cellulolytic enzymes that could degrade cellulose. To overcome this limitation, we expressed three cellulases from three different cellulose-degrading microorganisms in the lactic acid bacterium *Lactococcus lactis*. We designed expression vectors for the secretion and surface presentation of all three cellulases by two different surface anchors in *L. lactis*. We confirmed secretion of the cellulases into the growth medium by SDS-PAGE and western blot analysis. Detection of anchored cellulases on the cell surface of *L. lactis* bacteria was performed by dot-blot analysis of the cells. In addition, we confirmed the activity of both, catalytic and cellulose-binding domains of the heterologously expressed cellulases. The ability of cellulases to cleave β-1,4-glycosidic bonds was determined on carboxymethylcellulose (CMC) by the appearance of discoloration zones after staining with Congo red dye. The ability of cellulases to bind to cellulosic substrate was shown on crystalline cellulose. Our results confirmed the successful expression, secretion, and surface display of all three heterologous cellulases in *L. lactis*, as well as their activity on CMC-cellulose and their ability to bind to crystalline cellulose.

Kewords
Genetic engineering, Lactococcus lactis, lignocellulosic waste, cellulases

High-Throughput Screening: an efficient tool to redesign blends suitable for plant-based foods.

PhD Federica Biolcati, PhD Patrizia Buldo, PhD Fabio Dal Bello, **PhD Federica Volontè**

Number
170

Themes
Microbial Communities
Fermentation and Metabolism, including protein transition

Abstract
The consumption of plant-based foods is exponentially growing worldwide. 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.

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-flavour were identified. Finally, fast fermenting strains were selected for their ability to drive the fermentation process and to inhibit contaminants growth.

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.

Kewords
Lactic acid bacteria, high-throughput screening, plant-based matrices.

Salt-Stressed Transcriptome Analysis of *Staphylococcus equorum* KM1031 Isolated from High-Salt Fermented Salted Seafood

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Number
172

Themes
Genetics and Genomics
Fermentation and Metabolism, including protein transition

Abstract
Staphylococcus equorum is a potential starter for Korean high-salt fermented foods because of its salt-tolerance and enzymatic activities that contribute to enhanced sensory properties of the food products. However, the mechanisms of salt tolerance of *S. equorum* are not fully understood. Here, RNA sequencing was performed on *S. equorum* strain KM1031 exposed to 7% NaCl (w/v) for 2 and 4 h to determine global gene expression changes. Salt pressure for 2 and 4 h resulted in significant differential expression of 4.8% (106/2,209) and 6.1% (134/2,209) of *S. equorum* KM1031 genes, respectively. Twenty-five core genes were differentially expressed on salt-treatment for both 2 and 4 h, seven of which were related to osmoprotectant uptake and synthesis. We analyzed the genome of strain KM1031 and identified osmoprotectant uptake (Opu) systems, potassium importers, so-dium exporters, and the glycine betaine synthesis system. The RNA sequencing results indicated that the OpuD system and glycine betaine synthesis might play the main roles in the salt-tolerance of strain KM1031. Finally, the results of RNA sequencing were validated by quantitative real-time PCR of likely salt stress-related genes. This transcriptomic analysis provides evidence regarding the osmotic stress responses of *S. equorum* strain KM1031.

Kewords
Staphylococcus_equorum, salt_pressure, transcriptome, jeotgal, OpuD, glycine_betaine

Investigation of the genetic diversity of *Streptococcus thermophilus* strains isolated from artisanal cheese production systems

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Number
174

Themes
Genetics and Genomics
Host Microbe Interactions
Bacteriophage and Antimicrobials

Abstract
The cell wall polysaccharides of *Streptococcus thermophilus* strains, including Rgp (rhamnose glucose polysaccharide) and EPS (exopolysaccharide), act as the receptor for many of their infecting bacteriophages. To date, seven distinct *rgp* genotypes are described among dairy streptococcal strains. The *rgp* gene clusters that encode the RGP biosynthetic functions comprise two regions: i.e. the rhamnan backbone-encoding region and the variable side chain-encoding region. Three distinct backbone genotypes and five distinct side chain genotypes have been identified and a range of combinations of these two *rgp* regions have been observed among *S. thermophilus* strains. To explore the extent of genetic diversity of *S. thermophilus rgp* clusters beyond industrially derived strains, ovine milk, whey and curd from five Pecorino Siciliano PDO-producing farms were screened for the presence of *S. thermophilus* strains. The retrieved isolates were characterised to define their *rgp* genotype, phage sensitivity and bacteriocin production capability. Furthermore, the genomes of seven isolates were sequenced and analysed to evaluate their overall genetic diversity and to establish farm-specific populations. Through this approach, the strains were established to harbour a range of diverse anti-phage systems and a novel combination of backbone and side-cain encoding regions of the *rgp* gene cluster was identified highlighting that additional diversity exists within this species. The strain-level differentiation of isolates in this study provides significant insights into the diversity of streptococcal strains that may be derived from such foods to enhance global starter culture repertoires for the enhancement of defined and mixed starter culture systems.

Kewords
cell wall polysaccharide, genetic diversity, strain characterization

Accelerating the understanding of nutritional requirements and optimal yeast extract selection for effective culturing of lactic acid bacteria

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Number
176

Themes
Fermentation and Metabolism, including protein transition

Abstract
Lactic acid bacteria (LAB) have widespread industrial applications, such as dairy production and as probiotics. Routinely employed LAB species can exhibit distinct auxotrophies, lacking complete *de novo* synthetic pathways for specific amino acids and/or nucleotides.

Yeast extracts often act as a crucial complex source of nitrogen and growth factors essential for efficient and fast growth as required for industrial production of LAB.

The aim of this study was to elucidate the nutritional needs of various LAB and whether one specific yeast extract or preferably a combination of products would lead to improved fermentation performance.

A high throughput screening of various yeast extracts and the combinations thereof was performed in a microfermentation system (Biolector II®, Beckman Coulter) with strains belonging to different industrially relevant LAB species (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus helveticus*).

Data will be presented to show that growth in different species is highly dependent on the yeast extract being used. In addition, the benefits of using nucleotide-rich yeast peptones to improve growth kinetics and cellular biomass will be shown.

Overall, the use of a high throughput screening approach can accelerate the understanding of nutritional requirements and optimal yeast extract selection for effective culturing of LAB.

Kewords
LAB, yeast-based nutrients, nucleotides, peptides, amino acids

Wild-type Lactococcus lactis producing bacteriocin-like prophage lysins

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Number
178

Themes
Bacteriophage and Antimicrobials

Abstract
In this study, we present a wild-type *Lactococcus lactis* strain secreting prophage lysins, which behave like bacteriocins. Lactococci are known to produce more than 40 different bacteriocins, of which all belong to classes I and II, heat-resistant peptides. No class III bacteriocins, bigger heat-sensitive proteins, including phage tail-like bacteriocins (PTLB), have been found from *Lactococcus*. Unlike PTLBs, prophage lysins in wild-type bacteria have not been regarded as bacteriocins, apparently because phage lysins contribute to autolysis degrading the host's own cell wall. In this work, *L. lactis* strain LAC460, isolated from fermented idli batter, was found to kill other *Lactococcus* strains with protease- and heat-sensitive lytic activity. Three phage lysins were identified in the culture supernatant. The genes encoding the three lysins were localized in different prophage regions in the chromosome. By knock-out mutants, two of the lysins, namely LysL and LysP from defective prophages, were demonstrated to be responsible for the antimicrobial activity. The strain LAC460 was shown to be resistant to the lytic action of its own culture supernatant, and as a consequence, LysL and LysP could function like bacteriocins targeting and killing other closely related bacteria. Hence, similar to PTLBs, phage lysin-like bacteriocins could be regarded as a novel type of class III bacteriocins. As LAC460 lyses other *Lactococcus* strains, it could be useful as an adjunct starter in cheese making to accelerate ripening by releasing enzymes from starter lactococci.

Kewords
Lactococcus, prophage, endolysin, virion-associated lysin, bacteriocin

Antimicrobial resistance and safety assessment of lactic acid bacteria: *Companilactobacillus crustorum* and *Liquorilactobacillus nagelii* as case studies

Dr. Elisa Salvetti, Ms Ilaria Larini, Ms Sarah Tintori, Ms Tania Venzin, Dr. Veronica Gatto, Prof. Giovanna E. Felis, Prof. Sandra Torriani

Number
180

Themes
Genetics and Genomics
Bacteriophage and Antimicrobials

Abstract
Companilactobacillus crustorum and *Liquorilactobacillus nagelii* could be defined as non-conventional species of lactic acid bacteria (LAB), as they do not hold a QPS (Qualified Presumption of Safety) status according to the EU framework.

A key point for safety assessment is antimicrobial resistance (AMR) evaluation, as depicted in 2018 EFSA Guidance; there, AMR cut-offs for LAB are related to specific and/or metabolic groups. However, the 2020 reclassification has discontinued the reference to facultatively heterofermenters, thus creating inconsistence between scientific and regulatory terms.

The two strains analysed here, *C. crustorum* 6A1 and *L. nagelii* 4R7, isolated from goat cheese and kombucha, respectively, showed interesting pro-technological characteristics and are homofermentative species.

To assess their safety, the Minimum Inhibitory Concentration (MIC) was determined towards the most common antibiotics; then their genome sequences were obtained and analysed with the Comprehensive Antibiotic Resistance Database and Resfinder tools to exclude the presence of known antibiotic resistance genes (ARGs).

Although results suggest the safety of the two strains as MICs were below the cut-offs and no known ARG was retrieved, interpretation could not be straightforward considering vancomycin resistance for *C. crustorum*, and ampicillin, kanamycin, streptomycin and tetracycline resistance for *L. nagelii*, given EFSA Guidance.

This could be a limiting step for possible marketing authorization; therefore an update of the Guidance is hoped, with a proposal of taxonomy-based cut-off values.

Considering that non-QPS species are 88% of total *Lactobacillaceae*, such an update could contribute to unlock the biotechnology potential of LAB in food systems.

Kewords
lactobacilli, reclassification, antibiotic resistance, safety, Liquorilactobacillus, Companilactobacillus

Influence of *Latilactobacillus curvatus* on cheese quality

Stefan Irmler, Daniel Wechsler, Berthoud Hélène, **Ueli Von Ah**

Number
182

Themes
Fermentation and Metabolism, including protein transition

Abstract
In the present study, the cause of semi-hard cheese in bloated package was studied. It was assumed that the gas was due to the formation of biogenic amines, as carbon dioxide is produced in this process. Indeed, tyramine (641 mg/kg) and β-phenylethylamine (207 mg/kg) were found in the cheese. Tyramine-forming *Latilactobacillus curvatus* were isolated using tyrosine- and phenylalanine-decarboxylase-agar and the bacterium was assumed to be responsible for the formation of the biogenic amines. Quantitative PCR analyses revealed that the population density of *L. curvatus* was higher on the cut surfaces than inside. To study whether *L. curvatus* grows in cheese and forms biogenic amines, semi-hard cheeses were made with a thermophilic starter and a tyramine-producing isolate as adjunct. A tyramine-negative strain, isolated from another cheese, was used as control. After 90 days of ripening, no tyramine was found in the cheeses with the tyramine-negative strain. In contrast, 566 mg/kg of tyramine and 252 mg/kg of β-phenylethylamine were determined in the cheese with the tyramine-forming strain. The study showed that *L. curvatus* can produce considerable amounts of biogenic amines and, therefore, is an undesirable species in cheese. Since the population density was higher on the cut surfaces of the packaged cheese, the product was likely contaminated during cutting and packaging. Further studies will show whether food processing equipment represents a reservoir for decarboxylase-positive *L. curvatus.*

Kewords
Latilactobacillus curvatus, semi-hard cheese, tyramine, β-phenylethylamine, gas-formation

Iron-mediated interaction of the cheese ripening bacteria *Brevibacterium aurantiacum* and *Hafnia alvei*

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Number
184

Themes
Microbial Communities
Fermentation and Metabolism, including protein transition

Abstract
The ripened cheese ecosystem is a complex environment where lactic acid bacteria (LAB), yeasts, moulds and ripening bacteria grow sequentially or in parallel. After the fermentation carried out by LAB, the yeasts deacidify the curd, allowing the proliferation of ripening bacteria on the cheese surface. Iron plays an important role in the microbial interactions taking place on the surface, facilitating the production and exchange of iron-chelating siderophores among the microorganisms with compatible transporters. *Brevibacterium* is a genus found in a variety of cheese rinds and *Hafnia* is associated with *Brevibacterium* in French washed rind cheeses. Previous studies suggest that *B. aurantiacum* benefits from the siderophores produced by *H. alvei* and *H. alvei* benefits from the activity of the proteases produced by *B. aurantiacum,* indicating a mutualistic relationship. These strains were selected for further studies. First experiments showed differential growth responses and production of siderophores when cultured in high/low iron and casein/amino acid mediums. Genome Scale Metabolic Models (GSMMs) were built for both strains. They will be used to investigate the role of iron in the formation of volatile sulphur compounds, the main contributors to organoleptic characteristics of cheese. Omics data will be gathered from monocultures in batch bioreactors and used to constrain the GSMMs for each strain. A co-culture will be simulated to gain understanding on the mechanisms behind their interaction. The findings could assist in solving a problem that cheese producers often encounter: poor colonization of the pigment-producing *B. aurantiacum* on the cheese surface.

Kewords
Cheese ripening, iron, siderophores, genome-scale metabolic modelling

Bacteriophage monitoring of non-starter LAB cultures with protective effect

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Number
185

Themes
Bacteriophage and Antimicrobials

Abstract
Bacteriophages are under specific hygienic controls in cheese factories since potentially causing severe food and money losses.

The dairy fermentation relies on the activity of starter LAB (SLABs) because the end molecules of their metabolism are indispensable to standardise the texture and flavour of the fresh dairy products. In this regard, the killing activity of bacteriophages as a consequence of an inadequate phage monitoring results in poor quality foods or in the worst scenario even the impossibility to transform the milk.

Moreover, non-starter lactic acid bacteria (NSLABs) are crucial in dairy industries: even if not involved the main acidification of the milk, they result extremely important to ensure the quality and safety of the final products. Among NSLABs, adjunct cultures are employed to control the ripening of hard cheeses and others, showing protective effect against spoilage organisms or pathogens, to satisfy the consumer's demands for healthier and clean-label foods.

Sacco System routinary provides checks on samples received worldwide from dairy factories with the aim of investigating the presence of bacteriophages against both SLABs and NSLABs; in case of phage detection, Sacco System would suggest the most appropriate actions such as extra cleaning procedures and/or alternative blends, to overcome the problem. New phages are routinely isolated from the positive samples and used for several R&D projects such as strains selection for new phage-robust culture rotations.

Here we report how the phage screening on dairy samples has led to creation of a new rotation of cultures with antimicrobial effect.

Kewords
Bacteriophages, starter, non-starter, culture with protective effect

A study of the lactic acid bacteria community of the Nemea PDO wine (cv. “Agiorgitiko”)

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Number
188

Themes
Microbial Communities

Abstract
Wine is one of the most consumed alcoholic beverages around the globe and it is the product of fermentation by yeasts which produce ethanol during the consumption of sugars that are present in the must. The Nemea PDO wine produced from cv. “Agiorgitiko” is one of the most important Greek red wine varieties. In this study, we conducted a 4x3 (samples X producers) sampling process from different winemakers of Nemea region in order to isolate strains of lactic acid bacteria (LAB). We isolated 107 bacteria, cultured in anaerobic conditions on MRS agar containing 100 mg/L cycloheximide. Then, we proceeded with the gDNA extraction, rep-PCR and electrophoresis. After collecting their genetic fingerprints, an image analysis process took place, to distinguish the unique genetic profiles. Images were analyzed with BIONUMERICS ver.6.1, using UPGMA method and DICE coefficient. Our experiment resulted in 19 different genetic fingerprints, which were identified using an autoflex® maX MALDI-TOF/TOF. Among the identified species of bacteria were *Liquorilactobacillus mali* (42.1%), *Lactobacillus plantarum* (31.6%), *Enterococcus faecalis* (15.8%), and *Leuconostoc mesenteroides* (10.5%) . It is important to mention that only one of the three winemaker samples included all four species while the other two included only *Liquorilactobacillus mali* and *Lactobacillus plantarum* species.

Kewords
lactic acid bacteria, wine, Agiorgitiko, Nemea

Metabolic adaptation of Lactic Acid Bacteria

Dr Sybille Tachon, Aude Béranger, Dr Milica Denic, Laura Lizen, Dr Axel Magalon

Number
190

Themes
Fermentation and Metabolism, including protein transition

Abstract
Lactic acid bacteria are fermentative anaerobic aerotolerant bacteria. However, some of them are able to switch their metabolism towards respiration when the required oxygen, heme and quinones are available in the environment, leading to higher biomass and robustness of the cells. Since this switch is not molecularly regulated, and likely requires a rapid rallying of the respiratory complexes, we are considering a role of phenotypic heterogeneity in the expression and / or organization of respiration-essential proteins. Phenotypic heterogeneity in an isogenic population is an important and underestimated biological process, yet it guides cell development and evolution, and is widely used as an adaptation mechanism in pathogenic and non-pathogenic microorganisms. Characterizing the phenotypic heterogeneity regarding membrane complexes within clonal bacterial populations involves cutting-edge single-cell analyses, including fluorescence-guided flow cytometry and time-lapse, high resolution and microfluidics-associated fluorescence microscopy. Preliminary results show that the cultivation conditions that generate the most heterogeneous populations in *Lactococcus lactis* among anaerobiosis, microaerobiosis, aeration and aeration + hemin is microaerobiosis. Understanding the molecular mechanisms driving this heterogeneity would allow to modulate its level towards the most appropriate metabolism depending on the considered application, in the objective to increase performances of LAB.

Kewords
Phenotypic heterogeneity, adaptation, respiration, single-cell

Probiotics and vaginal health: a potential strategy against vaginal infections

Carlotta Morazzoni, Annalisa Visciglia, Francesca Deidda, Angela Amoruso, Marco Pane

Number
192

Themes
Microbial Communities

Abstract
Objective: Vaginal microbiome harbors various communities of microorganisms that have an important impact on women's health. During vaginal dysbiosis, when the overgrowth of pathogens causes vaginal infections such as bacterial vaginosis and vulvovaginal candidiasis, probiotics administration can serve as an alternative strategy to antibiotic and antimycotic therapy. This work aims to assess the ability of *Lactobacillus acidophilus* LA02 (DSM 21717), *Lactobacillus crispatus* LCR04 (DSM 33487), and *Limosilactobacillus fermentum* LF5 (DSM 32277), both as viable and heat-treated cells, to inhibit the growth of *Candida albicans* ATCC 10231, *Candida albicans* ATCC 60139, *Gardnerella vaginalis* ATCC 14018 and *Gardnerella vaginalis* ATCC 49145.

Methods: Probiotic strains were co-cultured with *C. albicans* in MRS broth and pathogen plate counts were executed after 24-48 hours. For *G. vaginalis*, an agar spot antimicrobial assay was performed.

Results: All viable strains demonstrated antipathogenic activity against *C. albicans*, especially LF5 inhibiting 99.44% of *C. albicans* ATCC 10231 and LCR04 inhibiting 99.99% of *C. albicans* ATCC 60193. Among the heat-treated cells, LA02 successfully inhibited 33.54% of *C. albicans* ATCC 10231 and LCR04 inhibited 14.88% of *C. albicans* ATCC 60193. Furthermore, viable LCR04 and LF5 showed the greatest reduction in the growth of *G. vaginalis* ATCC 14018 and *G. vaginalis* ATCC 49145, respectively, both with an inhibition zone of 7.25 mm.

Conclusion: This study enlightens the antipathogenic activity of selected probiotic lactobacilli, both in viable and heat-treated forms. These findings open up the possibility of an excellent, non-invasive adjuvant therapy, and a potential prevention strategy for the treatment of vaginal infections.

Kewords
Vaginal Microbiome, Vaginal dysbiosis, Heat-treated Probiotics

Studying the microbiome and the volatile profile of the Greek PDO cheese Sfela

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Number
194

Themes
Microbial Communities

Abstract
Sfela is a Greek PDO cheese produced in Messenia and Laconia regional units, which undergoes brine ripening and is made from ovine or caprine milk. In this study we examined the microbial composition of twelve Sfela cheese samples from three different producers. MALDI-TOF mass spectrometry and shotgun metagenomic analysis were used to identify the microflora of Sfela. Bacteria and yeasts isolated from the cheeses underwent DNA extraction for rep-PCR and gel electrophoresis. Clustering of strain fingerprints was performed using Dice similarity coefficient and UPGMA method in the BIONUMERICS software. Strains from each distinct cluster were selected for further analysis with MALDI-TOF MS. The most frequent bacterial species identified were *Lactobacillus plantarum*, *Lacticaseibacillus rhamnosus*, *Streptococcus thermophilus*, *Leuconostoc mesenteroides*, *Enterococcus faecium*, *Lactococcus lactis*, *Leuconostoc lactis*, and *Pediococcus pentosaceus*. Yeasts were less present with*Debaryomyces hansenii*,*Pichia fermentans*and*Candida zeylanoides*. Shotgun metagenomic analysis revealed that the dominant lactic acid bacteria (LAB) species were*Streptococcus thermophilus*, *Lactiplantibacillus plantarum*, *Lactococcus lactis*, *Latilactobacilluscurvatus*, *Lactobacillus delbrueckii*, *Lacticaseibacillus paracasei*, *Tetragenococcushalophilus*, and *Leuconostoc mesenteroides*. Partial metagenome-assembled genomes (MAGs) of *Bacillus cereus*, *Acinetobacter baumannii*, *Klebsiella oxytoca*, and *Pseudomonas putida* were detected in specific samples, highlighting the need of quality control during cheese production and storage. Furthermore, we conducted an examination of the volatilome profiles of the cheese which revealed some variations among the examined Sfela cheese samples. This study provides valuable insights into the microbial composition and volatile profile of Sfela, which are significant for both the safety and quality control associated with the cheese production.

Kewords
16 rDNA, Shotgun metagenomics, Sfela cheese, microbiome

Culture-dependent and independent approaches for evaluating the impact of stilbenoids on the gut microbiota

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Number
196

Themes
Host Microbe Interactions

Abstract
Stilbenoids, a group of plant phenolic compounds, may have multiple biological benefits, such as anti-inflammation, anticancer and antimicrobial activity. Also, they are dietary phenolics that occur in a wide range of edible fruits and seeds. It follows that bacteria of the gut microbiota are naturally exposed to different amount of these compounds. The aim of this study was the investigation of the possible effect of pterostilbene and viniferinfuran, resveratrol derivates, in affecting the overall viability of fecal samples and the culturability of specific groups of bacteria (i.e., Bifidobacteria and mucin-degrading bacteria) belonging to the gut microbiota.

Fecal samples from 5 healthy donors were collected, and exposed to selected polyphenols for 3h at 37°C. Then, the overall viability was assessed by flow cytometry by staining fecal cell suspensions with SYTO 24 and propidium iodide in order to distinguish Active Fluorescent Unites (AFUs, considered as live cells) and non-Active Fluorescent Units (non-AFUs, considered as dead cells). Also, selective media were used for investigating some relevant classes of microorganisms for GUT microbiota such as the genus of *Bifidobacterium* spp and mucin-degrading bacteria (i.e., *Akkermansia muciniphila*).

By flow cytometry we measured a decreasing AFU in AFU population by increasing Pterostilbene or Viniferinfuran (28µg/ml) concentrations in all the tested samples. Pre-treatments of the fecal samples with NaOH-SDS for the removal of Gram-negative bacteria confirmed that the most sensitive bacteria towards stilbenoids belong to the Gram-positive group. These results were confirmed also by a culture-dependent method by using selective media for Bifidobacteria and mucin-degrading bacteria.

Kewords
Stilbenoids, gut microbiota, flow cytometry, Bifidobacteria,

Harnessing the autolytic potential of *Lactobacillus helveticus*

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Number
198

Themes
Microbial Communities
Fermentation and Metabolism, including protein transition

Abstract
Lactobacillus helveticus is a Gram-positive bacterium that plays a vital role in cheese either as a starter culture - contributing to the acidification process, or as an adjunct - boosting flavour during ripening. A key feature of this organism is its ability to autolyse, a process by which the cells lyse and release their contents to the matrix therefore, accelerating the ripening process and providing new substrates to the surrounding microbial communities.

However, this phenomenon is not well understood. Why would an organism find an evolutionary advantage in sacrificing itself? What are the factors influencing autolysis? And even more relevant for the industry, how will culture conditions affect the performance of the strains in cheese?

In this abstract, we will investigate how different carbon sources and growth phases affect the robustness of *Lactobacillus helveticus*. Moreover, our results suggest that growth in glucose, lactose or galactose change the hydrophobicity of the cell capsule and therefore, the way cells aggregate.

Furthermore, microscopy images taken while cells are lysing show how bacteria are segregated into clusters where most of the population is lysing while a small part remains intact. These findings support the hypothesis that the evolutionary purpose of autolysis lies in the sacrifice of the few for the survival of the many.

In conclusion, understanding the triggers of autolysis and its context within the surrounding microbial communities will help us in the rational design of cultures for cheese ripening.

Kewords
Autolysis, cheese, lactobacillus, metabolism

Growth control of slime-producing *Leuconostoc mesenteroides* strains in Frankfurters using a preservative system consisting of vinegar and a plant extract

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Number
200

Themes
Bacteriophage and Antimicrobials

Abstract
Objective

The objective of this study was to assess a preservative system consisting of vinegar and a plant extract for reduction of slime formation caused by growth of *Leuconostoc mesenteroides* strains, known for its extreme slime formation and high resistance against organic acid based preservatives.

Materials and methods

Leuconostoc mesenteroides strains were isolated from spoiled frankfurters showing sliminess issues and spoilage levels larger than 6 log (CFU/g). Meat blocks for frankfurters were blended with 0.75% and 1.5% of dry vinegar and Nourishield D4010. Samples were inoculated with a slime-producing *L. mesenteroides* strain at 2-3 log (CFU/g). Samples were divided into 15 g portions, vacuum sealed and incubated for 44 days at 4°C. Samples were plated at regular time points during storage on MRS agar. Plates were incubated anaerobically at 30°C and counted after 48 hours. Significance of results was determined by one-way ANOVA ($P<0.05$).

Results

6 log (CFU/g) outgrowth was observed in the control treatment after 9 days at 4°C. Addition of 0.75% of vinegar and Nourishield D4010 resulted in a shelf life increase of 3 and 6 days respectively. At a use level of 1.5%, Nourishield D4010 almost doubles the shelf life with 26 days compared to 14 days for vinegar. In addition, visually less slime formation was seen in treatments containing Nourishield D4010 compared to vinegar.

Conclusion

Although slime producing *Leuconostoc mesenteroides* strains are highly organic acid resistant, addition of Nourishield D4010 has proven to be effective in reducing growth and slime formation, resulting in significant shelf-life extension.

Kewords
Preservation, sliminess, vinegar, plant extract, antioxidant, spoilage

Bacteriocins As Potential Biopreservatives: Screening of their Inhibitory Activity on a Bank Of Food-Borne Microbes.

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Number
202

Themes
Bacteriophage and Antimicrobials

Abstract
Food spoilage and safety are critical concerns for both the food industry and public health. Addressing these issues is essential to ensure the provision of safe, nutritious, and high-quality food products to consumers. Bacteriocins are ribosomally synthesized antimicrobial peptides produced by bacteria, exhibiting selective toxicity against other bacteria, enabling the opportunity to potentially utilize them as food preservatives.

Multiple microbial isolates were obtained from food products such as fish, meat and plant-based meat and investigated on the sensitivity to bacteriocins Pediocin PA-1 (Ped+), Lacticin-3147 (Ltn+) and Nisin A (NisA+) produced in GM17 by the bacteriocin producing isogenic strains of *L. lactis* MG136. The sensitivity to these bacteriocins of 218 identified strains was tested and compared to a negative control, Ped-, which represents 100% growth as there was no inhibition.

It was shown that Pediocin had the narrowest spectrum under both aerobic as anaerobic conditions. Under aerobic conditions, 59% of the strains showed low to no growth inhibition and 45% of the strains when anaerobically grown. However, Pediocin showed a medium level of inhibition on the usually pathogenic species *Salmonella enterica*.

In addition, Ltn+ and NisA+ both showed a broader spectrum of strains that were inhibited, showing high inhibition levels under aerobic as well as anaerobic conditions.

Bacteriocins offer great potential as a natural food preservative, effectively ensuring microbial safety, maintaining food quality, and extending shelf life. Their inhibitory action against a wide range of spoilage and pathogenic bacteria makes them a promising tool in food preservation.

Kewords
Bacteriocin, Antimicrobials, Preservatives, Nisin A, Pediocin, Lacticin

A selective media for the enumeration of Paucilactobacillus wasatchensis in cheese

Chase Wahlstrom, Taylor Oberg, Michele Culumber, Donald McMahon, **Craig Oberg**

Number
204

Themes
Microbial Communities

Abstract
Paucilactobacillus wasatchensis causes gas defects in aged cheese resulting in splits and puffy packages. Our goal was to develop a plating media to enumerate *Pa. wasatchensis* at 10^3 CFU/g within 72 h while inhibiting competing SLAB and NSLAB. During media development the growth of *Pa. wasatchensis* WDC04 was monitored along with 5 SLAB and NSLAB strains in a Tecan infinite 2000 plate reader using 24 well-plates. For media optimization, wells were filled with carbohydrate restricted MRS broth containing 1% ribose (CR-MRS+R), 2% Oxyrase, and a range of .01-1.0% 2-deoxyglucose (a glucose analog and glycolysis inhibitor) or 5-20 mg/mL of vancomycin and each test organism. Based on screening results, CR-MRS+R media containing 0.1% 2-deoxyglucose and 5 mg/mL vancomycin was selected. In broth culture trials, *Pa. wasatchensis* WDC04 was not inhibited by 2-deoxyglucose or vancomycin, while the other 5 SLAB and NSLAB showed inhibition. *Lacticaseibacillus casei* and *Lacticaseibacillus paracasei*, common NSLAB, showed significant inhibition between MRS broth (OD_{600} 1.28) and CR-MRS+R with 2-deoxyglucose (OD_{600} 0.60 and 0.54, respectively). *Lactococcus lactis,* a prevalent SLAB, showed nearly complete inhibition at 5 mg/mL vancomycin. On CR-MRS+R agar plates, all 5 test organisms were inhibited but not *Pa. wasatchensis* WDC04. Interestingly, *L. casei* and *L. paracasei* colony morphology was significantly altered, being reduced to almost undetectable pinpoint colonies. Addition of 0.1% 2-deoxyglucose and 5 mg/mL vancomycin into CR-MRS+R agar allows for the selective detection of *Pa. wasatchensis* in cheeses when concentrations are as low as 10^3 CFU/g cheese.

Kewords
Selective Enumeration, NSLAB, Cheese, Gas Defects

Biopreservative Potential of Indigenous Lactic Acid Bacteria against Foodborne Pathogens in Moroccan Merguez Sausage and Goat's Jben Cheese

Prof. Fouad Achemchem, Dr. Youssef Ezzaky, PhD student Kaoutar Boussif, Dr. Mariem Zanzan, Dr. Abdelkhaleq Elmoslih

Number
206

Themes
Bacteriophage and Antimicrobials

Abstract
Traditional Moroccan foods, such as goat's Jben cheese and Merguez sausage, are commonly linked to the presence of pathogenic microorganisms presenting substantial public health risks. The aim of this study was to isolate and characterize antagonistic lactic acid bacteria (LAB) from the indigenous microflora of raw materials, as well as meat and dairy goat products manufactured without starter cultures. Out of the 2400 LAB isolates obtained from goat's milk and dairy products, a total of 292 isolates showed inhibitory activity when tested using the agar-spots test. These isolates were further evaluated using the agar well-diffusion method, which revealed that 23 strains exhibited antimicrobial activity, particularly against *Listeria monocytogenes* CECT 4032. For the Merguez sausage, a screening was performed on 1440 isolates to evaluate their antagonistic activity against *L. monocytogenes*, *Escherichia coli*, and *Staphylococcus aureus*. Among the total isolates tested, only 93 isolates displayed clear inhibition zones. However, only 5.34% demonstrated measurable inhibition in liquid media. The phenotypic identification tests, coupled with API galleries and 16S rDNA sequence analysis, identified three *Latilactobacillus sakei*, one *Enterococcus faecalis*, and one *Enterococcus durans* strain from Merguez. Out of a total of 18 active strains originating from dairy products, 80% belonged to *Enterococcus durans* and *E. faecium*. Fast acid-producing strains Y17 (*E. durans*) and Y252 (*L. sakei*) were applied as starter cultures for fermented sausage, reducing L. monocytogenes loads in simulated fresh sausage by 3.6 to nearly 6 Log CFU/g. These strains may serve as adjunct cultures to enhance finished product safety.

Supporting Funds: PRIMA project ArtiSaneFood 0001/2018.

Kewords
Merguez sausage, goat cheese, antagonistic LAB, biopreservation

From women for women: citizen scientists isolating and identifying own *Lactobacillus crispatus* for vaginal probiotics development

dr. Rosanne Hertzberger, Shardelice Illidge, Eva Van Rossum, dr Wilbert Sybesma, prof dr Remco Kort

Number
208

Themes
Host Microbe Interactions

Abstract
An overwhelming number of studies have shown that a vaginal microbiome rich in *Lactobacillus crispatus* is associated with good reproductive and sexual health outcomes, such as a reduced risk of preterm birth and urogenital infections. However, the current trend of start-ups developing live biotherapeutic products based on bacteria such as *L. crispatus*, supported by intellectual property, venture capital, and prescription-dependent medical distribution, raises concerns regarding accessibility and affordability for women seeking effective prevention of serious health problems.

We question the appropriateness of such an approach for a product that utilizes a naturally occurring bacterium abundantly present in women's bodies and is likely to have a preventive rather than curative effect. To address this issue, we initiated a citizen science project, enabling female participants from the general public to isolate *L. crispatus* strains from self-sampled vaginal swabs. To facilitate identification of *L. crispatus* colonies in low-resource settings, we developed a molecular colorimetric LAMP test. Once isolated, citizen scientists license the rights to their bacteria to a non-profit foundation for ongoing research and development with industrial partners. Donors retain oversight over the applications of their isolates and also receive a share of the revenue from future license agreements.

Our project represents an innovative scientific, social, and organizational framework for the development of probiotics as medical ‘commons’, promoting active participation and empowerment, while safeguarding the collective nature of these bacteria, that are so intimately linked to the female body.

Kewords
vaginal microbiome, Lactobacillus crispatus citizen scientist

Predicting metabolic product formation in Lactococcus Cremoris with a dynamic model

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Number
212

Themes
Fermentation and Metabolism, including protein transition

Abstract
Lactococcus Cremoris is a cheese starter bacteria that uses homolactic fermentation to produce lactate, a key metabolic product that contributes to acidification and flavor in cheese. However, when lactose is limited or substituted for sugars that support lower growth rates, such as galactose or maltose, mixed acid fermentation occurs, resulting in the production of formic and acetic acid and ethanol. This metabolic adaptation is becoming increasingly relevant in the transition from dairy to plant-based fermented products that often contain “slow” sugars. Therefore, it is important for industries to understand the critical factors that affect *L. Cremoris* ability to convert different sugars into lactate or mixed acids.

Much research has been carried out to understand the mechanism of the metabolic shift from homolactic to mixed acid fermentation. Despite these efforts, specifics about how metabolic regulation works are not totally clear. Current kinetic models of central metabolism of *L. Cremoris* have not focused on this phenomenon or were inconclusive. This motivated us to revisit the topic and design a detailed kinetic computer model that captures the most important characteristics of this regulatory behaviour.

Based on previous research and available experimental data, we have developed a computer model that mimics the shift present in *L. Cremoris*. Through parameter sensitivity we aim to identify critical regulatory interactions and parameters that influence the metabolic shift. This should improve the prediction of end products for different bacterial strains and conditions and ultimately may lead to better process control or strain selection.

Keywords
Lactococcus Cremoris, Metabolic shift, Fermentation, Kinetic model

Lactococcus lactis produces GABA in vitro and alleviates visceral pain in vivo: towards a therapeutic solution in IBS

Pedro Gomes, Valérie Laroute, Catherine Beaufrand, Nathalie Ballet, Sophie Legrain-Raspaud, Muriel Mercier-Bonin, Hélène Eutamene, **Muriel Cocaign-Bousquet**

Number
216

Themes
Fermentation and Metabolism, including protein transition
Host Microbe Interactions

Abstract
IBS is a functional gastrointestinal disorder, exacerbated by stressful life events and for which visceral pain is the cardinal symptom. γ-Amino butyric acid (GABA), an inhibitory neurotransmitter of the central nervous system, plays a key role in the perception and modulation of pain and in stress. We hypothesized that GABA-producing *Lactococcus lactis*, was an adjuvant therapeutic solution to patients suffering from visceral pain. We screened a large panel of 88 *L. lactis* strains, presenting the glutamate decarboxylase (GAD) enzyme (encoded by the *gadB* gene), in order to characterize the diversity of GABA production within this species. Under the same fermentation conditions, we identified three strains as representative of GABA production diversity in *L. lactis*: NCDO2727 (low-GABA producer), NCDO2118 (median-producer) CNCM I-5388 (hyper-producer). Our results showed that ten-day oral treatment either with NCDO2118 or CNCM I-5388, but not NCDO2727, reduced visceral hypersensitivity induced by acute stress in rats, but only the GABA-hyperproducer had antinociceptive properties after five days. At the host level, these effects were demonstrated to be GAD-dependent and GABAB receptor-dependent, but independent of changes in the composition of the gut microbiota. Concerning *L. lactis* , we identified several physiological properties involved in the efficacy of the strains *in vivo*.

Keywords
Lactococcus lactis, GABA, GAD, visceral pain

POSTER SESSION 2: TUESDAY 29

Development & application of a pipeline for strain level classification of the Infant Microbiome using metagenome data

Dr Eline Klaassens, Dr Radhika Bongoni, Dr Monika Schaubeck

Number
1

Themes
Microbial Communities
Host Microbe Interactions

Abstract
At birth, the exposure to specific microbial strains shapes the infants´ microbiome and the metabolic function as we age. Human milk supports this early microbial ecosystem through probiotic strains and prebiotic compounds. When breastfeeding is not possible, infant formulae try to support the infants´ microbiome. Strain specificity is key in probiotic effects in the early 'window of opportunity' - an important time frame for the immune training in infants.

The effects of an infant formula, containing probiotic strains, was studied using an *ex-vivo* gut model (SHIME®) inoculated with infant donor-microbiomes. Each of the setups was supplemented with infant formula including 1 or both probiotics as well as hydrolyzed or non-hydrolyzed protein to study the effects on the donor microbiome, probiotic colonization and metabolic activity. To expand the microbiome analysis beyond the genus level, typical for 16S data, shotgun metagenomic data was used to perform strain-level analyses using a custom bioinformatics pipeline. We present an computational shotgun metagenomics pipeline for quantifying abundance and strain-level classification specifically within the infant microbiome by using a curated infant database.

Infant milk formula based on hydrolyzed protein indicated effects on the overall abundance of the Bifidobacterium species as well as the probiotic strains *in vitro*, highlighting the importance of the formula matrix. The abundance of the probiotic strains was increased on a strain specific level. Database optimization and bioinformatic tools comparison led to the increase of accurate and meaningful strain identification in the infant metagenome datasets.

Keywords
bioinformatics, infant, infant-formulae, microbiome, probiotic, prebiotic, database

Behavioral dynamics of Lactic acid bacteria in engineered living materials (ELMs)

Varun Tadimarri, Dr. Shrikrishnan Sankaran

Number
3

Themes
Microbial Communities
Genetics and Genomics

Abstract
Lactobacilli form one of the largest family of probiotics and are found as commensals at many sites in human body such as in the oral cavity, gastrointestinal tract, and reproductive organs. There is a growing interest to engineer these lactic acid bacteria as live biotherapeutics that produce and deliver drugs at these sites in the body. To improve their biosafety and survival within the body, they are being encapsulated within porous materials like hydrogels. This gives rise to Engineered Living Materials (ELMs) in the form of bacterial hydrogels capable of drug delivery in the body. Recent studies with *Escherichia coli* have highlighted that spatial confinement and mechanical properties of the external matrix can directly impact the metabolism, growth, and inducible gene expression rates of the encapsulated bacteria. However, such effects have not been explored in detail with lactobacilli.

In this work, we encapsulate engineered *Lactobacillus plantarum* in mechanically tunable hydrogels and study their growth, metabolism, and gene expression. Different polymer matrices encapsulating *L. plantarum* engineered to express fluorescent proteins and therapeutic peptides is being studied. Notably, we show that the viscoelastic properties of the gels have a significant effect on the growth and metabolism of lactobacilli and can be used to tune their functionality. These results and the fundamental insights derived from them will greatly help to understand the effect of mechanical forces on the behavior of lactobacilli and their protein expression capabilities within polymeric matrices, apart from providing a guideline to improving their performance when developed as ELMs.

Keywords
Lactobacillus, Polymeric matrix, therapeutic peptides

Engineered *Lactiplantibacillus plantarum* act as effective blockers for IL-6 cytokine-activated inflammation cascade

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[1]*Leibniz Institute for New Materials, Campus D2 2, 66123, Saarbrucken, Germany*

Number
5

Themes
Genetics and Genomics

Abstract
Commensal lactobacilli species play a vital role in the healthy functioning of the human body. The natural propensity of these bacteria to survive in the host microenvironment makes them an attractive choice for drug production platforms. Genetically modifying these bacteria to be used as living medicines is therefore highly desirable in the healthcare sector. However, most strains are challenging to genetically alter, lack a standardized genetic toolbox, and show insignificant responses to established genetic circuits. Our focus is to characterize efficient genetic parts[1] in *Lactiplantibacillus plantarum* to allow the sustained delivery of therapeutic proteins.

Interleukin-6 (IL-6) plays a prominent role in developing chronic inflammatory disorders and autoimmune diseases. Strategies to block the IL-6 activation cascade have shown a high success rate in treating immune-mediated disease. I would present our research on employing genetically modified *L. plantarum* to secrete an inhibitor protein for blocking the IL-6 receptor at physiological temperature to evade the pro-inflammatory cytokine activation cascade. Additional biomolecular modifications to therapeutic protein and bacterial encapsulation in biocompatible hydrogel scaffolds can also prevent host-immune rejection. This multi-faceted approach demonstrates how microbes can be used for the personalized treatment of patients suffering from long-term diseases and provides a model for Living Therapeutic Materials (LTMs).

Reference

1. Dey, S., Blanch-Asensio, M., Balaji Kuttae, S. & Sankaran, S. (2023) Novel genetic modules encoding high-level antibiotic-free protein expression in probiotic lactobacilli. Microbial Biotechnology, 00, 1–13.

Kewords
Lactiplantibacillus plantarum, cytokine, inflammation, receptor blocker

The role of a long-term co-existence and environment in the ecosystem stability

Dr Anna Alekseeva[1], Nikolai Matviiets[1], Dr Anne Kupczok, Dr Hilje Doekes[1], Dr Sijmen Schoustra[1, 2]
[1]*WUR, the Netherlands*
[2]*University of Zambia, Zambia*

Number
7

Themes
Microbial Communities
Fermentation and Metabolism, including protein transition

Abstract
Natural ecosystems vary greatly in the diversity of species and their interactions, which are thought to define ecosystem stability and functionality. Ecological theory predicts that the stability of an ecosystem relies on balanced interspecies cross-talk and how these species interact with the environment. We study a popular Zambian fermented milk product, **mabisi**, as a model system to understand the mechanisms driving community stability. Using systemic approach and experimental evolution, we aim to clarify the role of initial microbe composition, environment and a long-term co-existence in the ecosystem stability and resilience. We challenged mabisi microbial community with different milk types and long-term propagation. Although the functional output (i.e. aroma profile) of mabisi converged upon propagation, there was variation in transcriptional profiles which could have led to aroma convergence. There was little difference in mabisi aroma between cow milk types, while composition was different according to plating results. However, goat milk significantly affected the functional profile of mabisi. These findings suggest that various members of mabisi community may perform similar functions. This can stabilize the functional output of the community until the environmental change becomes more severe. We endorse mabisi as a tractable model to test evolutionary predictions and ecological stability/resilience of bacterial communities.

Keywords
LAB, traditional fermentation, meta-omics, community propagation

Biodiversity and Biofunction of Lactic Acid Bacteria (LAB): Finding Isolates that Enhance & Enable Fermented Vegetable Products

Ilenys Perez-Diaz, PhD, Clinton Page, PhD, Lesley Mendez-Sandoval, Christian Pagan-Medina, Suzanne Johanningsmeier, PhD, Meichen Pan, Rodolphe Barrangou, PhD

Number
9

Themes
Genetics and Genomics
Fermentation and Metabolism, including protein transition

Abstract
Utilization of surplus vegetables in healthy snacks, veggie-centric menus, and beneficial fermented foods are desirable to feed a growing consumer population. Because lactic acid bacteria can facilitate the development of new products from surplus vegetables, we collected 1,400 isolates autochthonous to vegetable fermentations representing 23 bacterial genera. These were evaluated by cucumber fermentation ability screening and Rep-PCR-(GTG)5 to identify unique isolates among the 243 *Lactiplantibacillus pentosus* and 131 *Levilactobacillus brevis* in the collection. Comparative genomic and phenotypic analyses of 24 *L. pentosus* and 14 *Lev. brevis* revealed that: (1) two *L. pentosus* clades, A and B, and three subclades, A.I, A.II, and A.III exist in cucumber fermentations at varied time points, and (2) *Lev. brevis* autochthonous to cucumber lack citrate lyase genes and the associated catabolic function. The CRISPR loci found in *L. pentosus* isolates confirmed recent evolutionary history and the subspeciation described above, suggesting a functional intraspecies diversity in cucumber fermentations. All the *Lev. brevis* genomes encode for 1,2-propanediol metabolosomes like those used by pathogens to colonize the human gut. Autochthonous and allochthonous *Lev. brevis* were found to remove plant sugars and spoilage implicated metabolites, features useful in enhancing the microbial stability of fermented vegetables. The incidence of *L. plantarum* and *Lactococcus lactis* in the bacterial collection was minimal (n=9 and n=4, respectively), but these species have specific properties valuable to produce relishes and bloater free pickles. We are optimizing processes for the manufacture of novel fermented vegetable products employing unique plant-derived lactic acid bacteria.

Keywords
Lactiplantibacillus pentosus, Levilactobacillus brevis, vegetable fermentation, waste

Multi-targeting bacterial gene therapy for chronic wounds and cancer

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Number
11

Themes
Genetics and Genomics
Host Microbe Interactions

Abstract
While complex diseases like chronic wounds and cancers are multi-factorial, most available treatments are unable to address multiple biologic targets. Therefore, the development and clinical testing of recombinant multi-target live biotherapeutic products constitutes an important step to solving these challenges.

Lactococci are important candidate host strains. They are non-pathogenic and have direct immunomodulatory effects. They are transient delivery vehicles and can be engineered to deliver multiple therapeutic factors (e.g. cytokines, chemokines, growth factors, antibody fragments) directly at the site of the pathology - topically for chronic wounds and intratumorally for cancer - avoiding side effects of systemic treatments. Furthermore, manufacturing is substantially easier and cheaper because the therapeutic factors are produced at the site of the disease, removing the need for purification and subsequent mixing of multiple therapeutic proteins.

In our presentation we show two examples of such a multi-target bacterial gene therapy approach. (i) Diabetic foot ulcer using a drug product that produces 3 factors (AUP-16) that are important in the healing of chronic wounds, namely hCSF-1, hIL-4 and hFGF-2. After *in vitro* and *in vivo* testing, a Phase 1 clinical study has shown safety of the product and clear indications of efficacy. A clinical Phase 2 study to confirm efficacy is currently in preparation. (ii) For cancer currently another set of therapeutic factors (AUP-55) is developed to target ovarian cancer and peritoneal carcinomatosis. The factors are hIL18, hGM-CSF and hscIL12. These studies are in the preclinical phase and the presentation will show *in vitro* and *in vivo* data.

Keywords
Lactococcus, recombinant LBP, DFU, cancer, clinical trial

READY-TO-EAT SALAD: A Potential Source of Beneficial Bacteria for the Human Gut Microbiota

Giacomo Mantegazza, Robin Duncan, Nicolò Telesca, Giorgio Gargari, Fabio Consalez, Valentina Taverniti, Patrizia Riso, Simone Guglielmetti

Number
15

Themes
Microbial Communities
Host Microbe Interactions

Abstract
While sanitation methods are widely used to prevent the proliferation of harmful microbes in food, they may also eliminate beneficial food-associated microbes. This consequence of hygiene practices might be relevant to the "*microbial depletion hypothesis"*, which suggests that reduced exposure to microorganisms from the environment and food contributes to an increased incidence of immune diseases and allergic disorders. A study was conducted to determine whether ready-to-eat rocket salad (RS) can harbour lactic acid bacteria (LAB), whether farming practices can affect RS-associated bacteria, and whether RS-associated bacteria can survive the human gastrointestinal transit. The results showed that ready-to-eat RS is a rich source of bacteria, particularly LAB, which can reach up to 10^5 CFUs/g. Wide variability was observed depending on the production lot and the cultivation method. LAB mostly belonged to the genera *Weissella* and *Leuconostoc* and were found to survive simulated gastrointestinal transit (INFOGEST) better than the other RS-associated bacteria. An intervention trial with a commercial ready-to-eat RS harboring about 10^4 CFUs/g of *Weissella* spp. determined a significant increase in live cells of this bacterial genus in feces after salad administration. Conversely, the overall fecal taxonomic community structure was not significantly affected by RS consumption. These findings suggest that ready-to-eat RS could potentially deliver live LAB to the intestinal microbiota, which could be extended to any ready-to-eat vegetable consumed raw if the abundance of harbored LAB is sufficiently high. Further studies will determine the potential benefits that LAB naturally associated with vegetables consumed raw can provide to the gut health.

Keywords
Weissella, Leuconostoc, rocket salad, gastrointestinal transit

Impact of bulk starter culture preparation on cheese quality: A multi-omics approach

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Number
17

Themes
Microbial Communities
Fermentation and Metabolism, including protein transition

Keywords
Starter culture, cheese quality, metaproteomics, metabolomics

Abstract
The control of bulk starter culture preparation is fundamental to ensure high quality and low batch-to-batch variation during cheese production. In this study, we investigated how the temperature of incubation and pH at the onset of cooling during the bulk starter preparation impacted the culture metabolism and the cheese quality by a multi-omics approach, using metaproteomics, metabolomics and metataxonomics. Our results show that both temperature and pH significantly impacted the metaproteome of the starter culture during preparation. Several pathways, important for cheese-making and cheese-ripening, were differentially expressed by the treatments. We observed changes in the abundance and diversity of proteins involved in amino acid metabolism, carbohydrate metabolism, and stress response. Interestingly, the level of free amino acids, an indicator of proteolysis and potential precursors of cheese flavour, changed significantly during ripening according to the different treatments of the bulk starter. Our findings suggest that the incubation temperature of the starter and pH at the onset of cooling of the bulk starter can significantly impact the starter culture's metabolic activity and stress response and impact cheese production and ripening. This has important implications for developing the final cheese properties and quality.

Companilactobacillus crustorum LMG 23699 is a competitive starter culture strain for Type 2 and Type 3 sourdough productions

Msc Ines Pradal, Msc Víctor González-Alonso, Msc Yohanes Raditya Wardhana, Msc Arnold Snijders, Prof. Dr. ir. Luc De Vuyst

Number
25

Themes
Microbial Communities
Fermentation and Metabolism, including protein transition

Abstract
Companilactobacillus crustorum has been indicated as an interesting candidate starter culture for sourdough production, given its metabolism. Therefore, its competitiveness and robustness has to be studied in detail. In the present study, *Coml. crustorum* LMG 23699 was used to produce Type 3 sourdoughs, prepared from wheat and wholemeal wheat flours, by an initial fermentation at 30 °C for 48 h followed by refreshments (50 %, v/v), a fermentation step of 16 h, and storage at 4 °C every three weeks during one year. A culture-independent approach revealed that *Coml. crustorum* LMG 23699 prevailed in all sourdoughs and grew together with the yeast *Wickerhamomyces anomalus* from week 12 onward. Even a perturbation of the temperature that caused an increase in the relative abundance of the background *Levilactobacillus parabrevis* could not eliminate this strain. To further examine its robustness, Type 2 sourdough productions (30 °C for 48 h) were started with different ratios of *Coml. crustorum* LMG 23699 and *Levl. parabrevis* IMDO 033007, with and without *W. anomalus* IMDO 010110, and *Coml. crustorum* LMG 23699 always prevailed these sourdoughs. Only when the yeast strain was inoculated, the four main flour carbohydrates and mannose were depleted, ethanol accumulated during the first 8 h of fermentation and was later on depleted, arabitol and xylitol accumulated, and more D-lactic acid was produced. To conclude, this study showed the potential of *Coml. crustorum* LMG 23699 as starter culture strain for Type 2 and 3 sourdough productions and indicated a trophic relationship with the yeast *W. anomalus*.

Keywords
sourdough, lactic acid bacteria, yeasts, starter cultures

Assessment of the safety of “probiotics” in food supplements

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[3]*Teagasc Food Research Centre, Ashtown, Dublin, Ireland*
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[5]*Food Safety Authority of Ireland (FSAI), The Exchange, George’s Dock, IFSC, Dublin, Ireland*

Number
27

Themes
Microbial Communities

Abstract
European Union (EU) food law defines food supplements in Directive 2002/46/EC as “foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities”. The EU food supplements Directive details the maximum safe levels of vitamins and minerals in food supplements. Other substances, including “probiotics” can be included in food supplements, provided they are safe in accordance with Regulation (EC) No 178/2002.
The most widely used “probiotics” in food supplements are strains of LAB, bifidobacteria, *Bacillus sporogenes* and the yeast *Saccharomyces boulardii*. The potential risks from “probiotics” include infection, ill effects from microbial toxins produced by the microbial strains or contaminants and immunological effects. Reported adverse events due to “probiotics” are few. Where opportunistic infections associated with “probiotics” are reported, they are usually in people at increased risk of infection where various underlying factors may enable infection by organisms that are rarely or never associated with infection in otherwise healthy people.
Here we present the recommendations and guidance made by the Scientific Committee of the Food Safety Authority of Ireland on assessing the safety of “probiotics” in food supplements in Ireland.

Keywords
Probiotics, food safety, food supplements,

Carotenoid production as scattered trait within the Lactobacillaceae family and its association with the flower habitat

Dr. Marie Legein, Tom Eilers, Jari Temmermans, Prof. Sarah Lebeer

Number
29

Themes
Genetics and Genomics
Host Microbe Interactions

Abstract
Carotenoids are pigmented terpenes known for their antioxidant and anti-inflammatory properties. Until now, carotenoid production has been described only in *Lactiplantibacillus plantarum* within the family of Lactobacillaceae. The presence of this trait in other members of this family and why these pigments are produced have not been well studied. Here, we used a pangenome approach to investigate the evolution of the key genes involved in carotenoid production (crtMN) within in the Lactobacillaceae family. 24 different species from 13 genera were shown to contain these genes and carotenoid production was experimentally confirmed in 27 in-house isolates from 11 species. Based on the phylogeny of the carotenoid genes, a clear example of horizontal gene transfer from *Lactiplantibacillus plantarum* to *Levilactobacillus spp., Lactiplantibacillus pentosus* and *Pediococcus pentosaceus* was detected, while carotenoid production was a core property in other taxa such as *Leuconostoc citreum* and *Fructilactobacillus lindneri*. In-vitro assays showed that Lactiplantibacillus strains expressing carotenoids were more resistant to UV- and oxidative stress. Based on literature and de novo isolation of Lactobacillaceae from various environments, a remarkable prevalence of carotenoid producers was found in the flower habitat, an environment characterized by UV- and oxidative stress. Taken together, this study identified new carotenoid production in Lactobacillaceae, with carotenoids playing an important role in their adaptation to stressful environments, such as the flower habitat. In addition to these fundamental insights of the eco-evolutionary role of carotenoids, our findings are also relevant for the food, feed, cosmetic, and pharmaceutical industries where carotenoids are widely applied.

Keywords
Carotenoids, pangenome, flower habitat, UV&oxidative stress

Search for novel bacteria to valorize in the food industry - A culturomics approach

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Number
35

Themes
Microbial Communities

Abstract
Culturomics is a valuable tool to discover novel bacterial strains with potential applications in the food industry. By diversifying cultivation conditions combined with high-throughput MALDI-TOF MS dereplication and genome sequence-based identification analyses, it is possible to capture a wider range of bacterial diversity and to obtain a collection of novel strains to be introduced in food applications.

The identification of LAB and other plant-associated bacteria from floral samples is particularly interesting, as these bacteria have the potential to be used as probiotics, biocontrol agents, and starter cultures in food industry. Over 1700 isolates were picked from a total of 51 floral samples, with over 1300 being identified as AAB and LAB. These isolates were classified within 21 genera, including *Leuconostoc*, *Fructobacillus*, *Apilactobacillus*, *Weissella* and *Lactiplantibacillus*. The development of natural aroma and flavour compounds using these bacteria could lead to the creation of unique and desirable food products.

Overall, the use of culturomics on environmental samples has the potential to contribute to the development of sustainable and innovative solutions that meet the evolving needs of the food industry. By exploring the microbial diversity of different sources, novel bacterial strains with unique properties can be discovered.

Keywords
Culturomics, MALDI-TOF MS, Novel strain discovery, Diversity

Diet impacts lactobacilli-dominated vaginal microbial communities

Isabel Erreygers, Sandra Condori, Sarah Ahannach, Camille Nina Allonsius, Thies Gehrmann, Stijn Wittouck, Tom Eilers, Veronique Verhoeven, Denise Medeiros Selegato, Michael Zimmermann, Sarah Lebeer

Number
37

Themes
Microbial Communities
Host Microbe Interactions

Abstract
A 'healthy vagina' has been well documented as a low diversity ecosystem dominated by *Lactobacillus* species. More diverse communities dominated by facultative or strict anaerobes such as *Gardnerella* and *Prevotella* are often associated with adverse vaginal conditions and treated with antibiotics. Due to antibiotic resistance and microbiome disruption, a clear need exists for evidence-based lifestyle and dietary advice to promote vaginal lactobacilli dominance. In our citizen-science project Isala (https://isala.be/en/), the vaginal microbiome of 3345 healthy women was mapped through 16S rRNA amplicon sequencing, showing *Lactobacillus crispatus* as most dominant and prevalent. Specific dietary habits were significantly associated with the vaginal microbiome. For example, consumption of sugary beverages was negatively associated with *L. crispatus* and co-occurring taxa *Limosilactobacillus* and *Lactobacillus jensenii*. Contrary, positive associations with this *L. crispatus*-module were found for frequent consumption of vegetables, associated fiber and a pescetarian diet. Lower levels of the *L. crispatus*-module and higher levels of the less beneficial *Prevotella*-module were linked to meat consumption, whereas lower levels of the *Prevotella*-module were observed after consuming probiotic capsules within the previous 24h. To investigate the presence of food components in the vagina, we performed metabolic profiling of 64 vaginal supernatant samples using Liquid Chromatography-Mass Spectrometry. Surprisingly, various food metabolites were detected, suggesting a food-vagina axis. However, it remains to be explored whether food components reach the vagina via the perineum and thus impact the microbiome or indirectly via intestinal absorption. Nevertheless, our findings create a new perspective to improve vaginal health through (personalized) dietary advice.

Keywords
lactic acid bacteria, lactobacilli, vaginal microbiome, diet

Bioprotective lactobacilli in different cheese models to inhibit fungal spoilage

Ms. Zheng Zhao, Mr. David Simpson, Mr. Michael Gänzle

Number
39

Themes
Microbial Communities
Bacteriophage and Antimicrobials

Abstract
Bioprotective cultures are applied control the fungal spoilage of fermented dairy products but only few studies evaluated their efficacy in cheese. To evaluate the overall performance and antifungal activity of bioprotective lactobacilli, cultures were used as single and combined adjunct cultures for laboratory-scale Crescenza cheese and for pilot-scale Gouda cheese, respectively. Growth of the bioprotective cultures was characterized by surface plating, strain-specific qPCR and nanopore sequencing of full-length 16S rRNA genes. Analysis of Crescenza cheese by plate counts documented growth by 1 - 1.5 log over 14 d of storage. In Gouda cheese, strain-specific qPCR demonstrated the growth of the lactobacilli during the first 45 d of ripening. Metagenomic sequencing demonstrated that the microbial community of Gouda cheeses was dominated by the starter and adjunct cultures with the relative abundance of other bacteria accounting for less than 0.4%. Lactobacilli inhibited *Penicillium caseifulvum* and *P. roqueforti* in Crescenza cheese; *Lacticaseibacillus rhamnosus* FUA3185 and *Lc. paracasei* FUA3413 extended the mould-free days almost 1.5-fold. Growth of yeasts was not inhibited. *Lactiplantibacillus. plantarum* FUA3183 and FUA3247 reduced the growth of *Debaryomyces hansenii* FUA4064 by 1.5 log and extended the mold-free shelf life for 3 d in 45 d-ripened Gouda cheese. Longer ripening times decreased the antifungal activity and the culture was no longer active towards yeasts. This study validates the ability of the bioprotective lactobacilli in cheese to inhibit fungal growth in cheese.

Keywords
Bioprotective, Fungal spoilage, Cheese, Microbial community

Unlocking the estrogenic potential of soy: safe and functional fermentation with kefir-derived lactic acid bacteria

Giacomo Mantegazza[1], **Robin Duncan**[1], Alessandro Dalla Via[1], Armando Licata[1], Claudio Gardana[1], Giorgio Gargari[1], Cristina Alamprese[1], Stefania Arioli[1], Valentina Taverniti[1], Matti Karp, Simone Guglielmetti[1]
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Number
43

Themes
Fermentation and Metabolism, including protein transition

Abstract
Plant based fermented foods are gaining attention for the health promoting properties they possess and as a vegan alternative to dairy products. In this context and especially for milk, soy-based products have been commonly used as an alternative for the last couple of decades. Soybeans are a nutrient-rich food that contains phytoestrogen isoflavones, compounds that have been associated with numerous health benefits. With this in mind, the aim of the study was to identify and select safe food microorganisms capable of fermenting soymilk to produce a final product with improved functional properties and an increased estrogenic activity. Milk kefir grains contain microorganisms with proven health-promoting properties. Non-commercial milk kefir grains were used as a starting inoculum for soymilk. After 14 consecutive daily passages, four lactic acid bacterial strains were isolated from the freshly inoculated soymilk, namely *Lactococcus lactis* subsp. l*actis* K03, *Leuconostoc pseudomesenteroides* K05, *Leuconostoc mesenteroides* K09, and *Lentilactobacillus kefiri* K10. These strains were found to be safe for human consumption, based on their antibiotic resistance profile and comparative genomics analysis. Furthermore, the isolated bacterial strains demonstrated their ability to ferment the naturally occurring sugars in soybeans and produce a creamy texture. The two strains belonging to the genus *Leuconostoc* were found to increase the estrogenic activity of the soybean drink, as demonstrated by a yeast-based bioluminescence reporter system. In conclusion, the results of this study suggest that a soymilk beverage fermented with the identified bacterial strains can help meet the growing demand for health-promoting alternatives to dairy products.

Keywords
isoflavones, phytoestrogen, Leuconostoc, Lactococcus lactis, kefir

Genetic engineering of lactobacilli as mucosal vaccine delivery vehicle: selection of strains and promoter evaluation

PhD student Ilke Van Tente, PhD student Tom Eilers, PhD student Jelle Dillen, PhD student Eline Cauwenberghs, postdoctoral researc Sarah Ahannach, postdoctoral researc Dieter Vandenheuvel, PhD Peter A. Bron, Professor Irina Spacova, Professor Sarah Lebeer

Number
47

Themes
Genetics and Genomics
Host Microbe Interactions

Abstract
Mucosal vaccination is an effective strategy to generate potent mucosal immunity, as it is particularly effective against respiratory infections and sexually transmitted diseases. Lactobacilli have a great potential to serve as mucosal vaccine delivery vehicles as they are generally recognized as safe, can adapt and thrive at human mucosal surfaces and have strain-specific immunostimulatory properties. In this research, we aim to develop a modular genetic system in lactobacilli for delivery of vaccine antigens to the respiratory and vaginal mucosae. Here, we aimed to select appropriate lactobacilli isolates and study activity of specific promoters in lactobacilli isolates.

First, 50 promising *Lactobacillaceae* isolates from healthy human donors were screened *in silico* and *in vitro* based on safety, genetic accessibility via electroporation, and immunostimulatory properties. Respiratory isolates *Lacticaseibacillus casei* AMBR2 and *Lactiplantibacillus plantarum* WCFS1, and vaginal isolates, *Lacticaseibacillus rhamnosus* GR-1 and *Limosilactobacillus reuteri* AMBV339, were selected as promising vaccine delivery vehicles.

Second, promoters needed for tunable/tailored expression of vaccine antigens were selected and evaluated *in vitro* for their activity in the nasal and vaginal environments. Activity of promoters (including constitutive P11, P*tlpA*, P*32(S),* P*msp2*, and P*dlt*) is evaluated via mCherry reporter fluorescence in *Escherichia coli* and in the selected lactobacilli isolates. To identify endogenous habitat-specific promoters, the lactobacilli isolates are incubated in real nasal and vaginal fluid, collected from healthy volunteers, followed by RNA sequencing.

Our results contribute to the broadening of the genetic toolboxes in these promising strains and open up new approaches for the delivery of beneficial heterologous proteins via lactobacilli.

Keywords
mucosal vaccination, heterologous expression, RNA-sequencing, Lactobacillaceae

Screening of lactic acid bacteria for the development of fermented plant based preservative ingredient solutions

PhD Pierre Guichebard, PhD Gilles Kergourlay

Number
49

Themes
Fermentation and Metabolism, including protein transition
Bacteriophage and Antimicrobials

Abstract
Leveraging on natural raw materials to provide innovative ingredients for food and beverages, pet food and aquafeed is key to fulfilling the increasing needs of the Food industry, while ensuring food quality.

This research project aims to develop fruit or vegetable antimicrobial fermentates, using lactic acid bacteria in combination with plant substrates. The objective is to create synergies between active molecules initially present in plant matrices and those produced *de novo* by fermentation, to obtain *in fine* more efficient products against a wider spectrum of microbial targets related to food spoilage and foodborne illnesses.

Thirty different bacterial strains were cultured in nine different plant substrates. Fermentation yields and fermentates antimicrobial activity were measured (MIC determined by antimicrobial susceptibility tests in Petri dishes). The data obtained show that the antimicrobial effects generated by the strains differ depending on the substrate used for growth. This demonstrates the relevancy of this type of broad screening to identify specific substrate/strain combinations of interest. A particular example illustrate that it is possible to complement the native antifungal properties of a plant substrate by fermentation, with an antibacterial activity, to ultimately obtain a product that is effective against a broader spectrum of microorganisms (MIC <1.2% against 5 bacterial targets and 5% against 3 yeast targets)

This promising work will serve as a basis to develop fermented natural preservatives solutions, for food, petfood and aquaculture applications. Further studies will focus on the identification of active substances from the crude fermentates and the optimization of their biosynthesis.

Keywords
Fermentation, plant-based, lactic acid bacteria, antimicrobials, preservatives

Bacteriocin genes identified in the genome of a promising respiratory *Lacticaseibacillus casei* strain

Eline Cauwenberghs, Ilke De Boeck, Thibaut Maeyens, Joke Bastiaenssen, Irina Spacova, Jelle Dillen, Peter Bron, Sarah Lebeer

Number
51

Themes
Genetics and Genomics
Bacteriophage and Antimicrobials

Abstract
Respiratory tract diseases form a major cause for antibiotic prescription and consumption, but antibiotics have a lot of side effects, such as increasing antibiotic resistance and disruption of the microbiome. Live biotherapeutic products (LBPs) or specifically selected micro-organisms that can target specific pathogens via multifactorial mechanisms could form an interesting alternative approach for pure antibiotics, because they can better preserve the microbiome structure and function and are less prone to resistance development due to their multiple targets.

In this study, we isolated a promising lactic acid bacterium from the respiratory tract (RT) of a healthy volunteer, named *Lacticaseibacillus casei* AMBR2 with strong inhibitory activity against the growth, biofilm formation and adhesion to human cells of important RT pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*. Ribosomally-synthesized and post-translationally modified peptides RiPPs gene clusters were identified in the genome. Further characterization of these peptides, could support the use of this strain as live biotherapeutic product for several respiratory tract diseases.

Keywords
bacteriocins, respiratory tract, antimicrobial, live biotherapeutic product

Strain identification and stability of lactic acid bacteria in back-slopped sourdough sampled over time

Vi Pham, David Simpson, Michael Gänzle

Number
53

Themes
Microbial Communities
Genetics and Genomics

Abstract
Identifying bacteria at the strain level enables source-tracking of fermentation microbes and determines the stability of microbial communities in back-slopped fermented foods. Investigations of outbreaks of foodborne-disease use whole genome sequencing of isolates with a SNP cutoff of 20 as the most accurate method for strain level identification but this method has not yet been applied to food fermenting lactic acid bacteria (LAB). This study aimed to use whole genome sequencing to determine the strain-level stability of three sourdoughs that were back-slopped over a period of 3 – 17 years between sampling dates. LAB were isolated and tentatively identified by sequencing of 16S rRNA genes. Genomes were sequenced on the Nanopore MinION platform with super high accuracy basecalling. Three additional strains for which Illumina-sequenced genomes are available were included as controls. SNPs calling was performed using Snippy. If the coverage of Nanopore sequencing was 80-fold or higher, genome sequences were identical (0 SNPs) when sequenced either on the Nanopore or the Illumina platforms. One strain each of *Pediococcus parvulus* and *Fructilactobacillus* spp. that were isolated from the same sourdough over 3 years showed a 26 SNPs difference or fewer between two isolates; 7 additional pairs of isolates remain to be analysed. The study provides an affordable and reliable method to determine the strain-level stability of microbial communities in back-slopped sourdough.

Keywords
Nanopore, sequencing, strain, identification, microbial, stability, sourdough

Comparative genomics of *Bifidobacterium dentium* reveals host adaptation and 2'/3-FL utilisation cluster in this species.

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Number
55

Themes
Genetics and Genomics

Abstract
Bifidobacteria are beneficial commensals of the human gastrointestinal tract and their presence in the gut has been associated with positive health effects on the host. They account for a vast proportion of the infant gut microbiota, when the infant is fed on a milk-based diet, with their number progressively decreasing in adult and elderly. In contrast to other bifidobacteria, *B. dentium* was initially considered an opportunistic pathogen as its presence in the oral cavity was associated with the development of dental caries. While *B. dentium* has been frequently isolated from the oral cavity of children with caries, recent microbiome investigations and preliminary genomic analyses have suggested that this species is also adapted to colonise the gastrointestinal tract, where could exert potential beneficial effects for the host. To gain a better understanding of *B. dentium* genomic diversity and metabolic potential, the current study presents analysis and chracterisation of the genome sequence of 10 novel *B. dentium* isolates from human fecal samples, obtained by next-generation sequencing (NGS). Through an extensive comparative and pan-genome analysis we investigated the genomic diversity of genetic loci involved in host interaction and gut colonsation in this species. Through a combined genotypic and phenotypic characterisation in terms of its carbohydrate metabolism we were also able to identify a 2'/3FL utilization cluster in two representative strains, thus suggesting the adaptation of member of this species to survive in the gastrointestinal tract of infants.

Kewords
Bifidobacterium, HMOs, genomics, gut, infant

Modelling Lactic Acid Bacteria Fermentation Using a Multiexperiment Calibration Scheme

Geoffrey Roudaut, Francesco Moro, David Henriques, Bas Teusink, Eva Balsa-Canto

Number
57

Themes
Microbial Communities
Fermentation and Metabolism, including protein transition

Abstract
Lactobacillus delbrueckii subsp. *bulgaricus* and *Streptococcus thermophilus* are the most commonly used Lactic acid bacteria (LAB) for the yoghurt making process in the dairy industry. The study of LAB fermentation with the help of kinetic models has the potential to provide information on the industrial process, helping to achieve a safe product that meets quality expectations. Previous studies have proposed dynamic models that accurately describe bacterial growth in single strain cultures taking into account pH, temperature, and lactic acid inhibition. However, most of the proposed models describe only one particular experimental condition. Therefore, they are of limited use for simulation or process design. Here, we propose a dynamic model to describe single-strain cultures. The model accounts for the uptake of nutrients (carbon and nitrogen sources),the inhibitory role of lactid acid in transport and growth, and the leakage of products. The model was built in two steps. In the first step, a series of experiments at different pH values were integrated into a multiexperiment calibration scheme to characterise the dependence of model parameters, such as maximum growth or uptake rates, on pH. In the second step, we further refined our model using data obtained under fermentation conditions with amino acid-supplied and casein-supplied media. Finally, we performed an identifiability analysis of the model to address the uncertainty of its parameters and predictions. The final model adequately explains all the experiments and is well suited to build a dynamic genome scale model of *L. bulgaricus* and *S. thermophilus* in yoghurt fermentation.

Keywords
Lactobacillus, Streptococcus, mechanistic modelling, parameter estimation, Yoghurt

Isala citizen-science mapping of vaginal lactobacilli

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Number
59

Themes
Host Microbe Interactions
Microbial Communities

Abstract
Vaginal lactobacilli are key for our reproduction and health, but a deeper understanding of the composition and function of the vaginal microbiome is needed in healthy women to design better diagnostics and therapeutics. Here, we used a citizen-science approach and actively motivated women to self-sample and co-create dynamic research on how health, life course and lifestyle are associated with the dominance of lactobacilli in this important habitat. *Lactobacillus* taxa were found to be dominant in 78% of the 3,345 healthy women who donated a vaginal swab, most notably *Lactobacillus crispatus* and *Lactobacillus iners*. In 15% of the women, these species co-occurred in similar amounts arguing against previously described discrete community state types. We therefore studied the microbial interactions in more detail in these communities: most vaginal taxa show small to moderate positive or negative abundance correlations with each other. Positively interacting vaginal taxa were summarized by grouping as modules of min. 3 taxa interacting (*L. crispatus-, Gardnerella-, Prevotella-,* and *Bacteroides*-modules). Interestingly, we found that the *Limosilactobacillus* genus was prevalent in almost 50% of the vaginal samples and positively correlated with *L. crispatus* and *L. jensenii*. Besides age, having children and stage of the menstrual cycle were most strongly associated with different parameters of the vaginal microbiome. Menstrual hygiene, contraceptive use, sexual intercourse, intimate partnership, diet and other factors showed finer-scale associations with the microbiome composition (explained variance 10.2%). This high-resolution mapping of the vaginal microbiome and its metadata in health provides a unique reference for follow-up case-control and intervention studies.

Keywords
Citizen science, lactobacilli, population cohort, vaginal microbiome

Collinsella aerofaciens as predictive marker of response to probiotic treatment in non-constipated irritable bowel syndrome

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Number

63

Themes

Host Microbe Interactions

Abstract

Probiotics are commonly used as an adjuvant treatment for irritable bowel syndrome (IBS), but there is currently no reliable guidance for selecting the appropriate probiotic for the different subtypes of IBS. In this study, we aimed to identify markers for recognizing non-constipated IBS patients who may benefit significantly from treatment with the probiotic strain *Lacticaseibacillus paracasei* DG (LDG). We conducted a post-hoc analysis of samples collected during a multi-center, randomized, double-blind, parallel-group, placebo-controlled trial, in which were randomized to receive twice daily capsules with at least 24 billion CFU of LDG for 12 weeks. The primary clinical endpoint was the composite response based on improved abdominal pain and fecal type. We investigated the fecal microbiome and serum markers of intestinal (PV1 and zonulin) functionality. In the probiotic arm, responders (25%) were different from non-responders based on the abundance of 18 bacterial taxa, including the family Coriobacteriaceae, *Dorea* spp., and *Collinsella aerofaciens*, which were overrepresented in responders. These taxa also distinguished responders (but not non-responders) from healthy controls. The probiotic intervention significantly reduced the abundance of these bacteria in responders but not in non-responders. Similar results were obtained for *C. aerofaciens* from the analysis of data from a previous trial performed on IBS with the same probiotic. LDG is effective for treating NC-IBS patients with a greater abundance of potential pathobionts, and *C. aerofaciens* emerges as a potential predictor of probiotic efficacy in IBS.

Keywords

probiotic, Lacticaseibacillus paracasei DG, abdominal pain, PV1

The genetic drift in *Lacticaseibacillus paracasei* DG over ten years of industrial production

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Number
65

Themes
Genetics and Genomics

Abstract
The commercialization of a probiotic strain assumes that all production lots are equivalent. However, the microbial biomasses of a probiotic are produced industrially in large quantities, repeatedly and continuously for years, and could be therefore potentially subject to genetic drift, which may affect its ability to confer health benefits to the host. In this study, we assessed the stability of *Lacticaseibacillus paracasei* DG (DSM 34154), a well-characterized commercial probiotic strain, by analyzing 8 isolates from different production lots over the past 10 years, an isolate from 8 years of subculturing in the lab, and the DG strain deposited in the DSMZ culture collection. We found that the genome organization of the *L. paracasei* DG strain remained largely unchanged, with all 10 isolates harboring the two plasmids present in the original strain and only a few point mutations compared to the reference genome of the DSMZ collection, none of which affected putative coding sequences. Moreover, phenotypic analyses of the 10 isolates, including resistance to simulated gastrointestinal transit, immunomodulatory capacity, adhesion to Caco-2 cell layer, fermentation profile, antibiotic resistance, and modulation of transepithelial electric resistance, revealed no significant differences. Overall, our study demonstrates that the isolates of the *L. paracasei* DG strain derived from industrial productions over the past 10 years are substantially equivalent in terms of genotype and phenotype. Similar quality control measures should be periodically conducted to ensure that probiotic properties and intended health benefits are preserved.

Keywords
probiotic, genetic drift, INFOGEST, Caco-2, TEER

Phenotypic and proteomic differences in biofilm formation of two *Lactiplantibacillus plantarum* strains in static and dynamic flow environments

Linda Huijboom

Number
67

Themes
Genetics and Genomics

Keywords
biofilm, flow, disinfectant, proteomics, cell wall

Abstract
Lactiplantibacillus plantarum is a Gram-positive bacteria capable of producing biofilms, which can increase the survival of this food-spoilage organism in the food chain. In our study, we compared two strains, WCFS1 and CIP104448, in their ability to produce biofilms both in a static and dynamic (flow) environment. The response to flow was strain dependent and resulted in a decrease of biofilm produced by WCFS1, but an increase for CIP104448. However, for both strains, the number of culturable cells in de formed biofilms increased under flow conditions. We further analysed the biofilm composition, structure and resistance to enzymatic and disinfectant treatments and found differences both between the two strains and between the two environments in which the biofilms are formed. Using proteomics, we investigated static supernatant, static biofilm and flow biofilm of both strains to determine underlying mechanisms important for the observed phenotypical differences, including increased disinfectant resistance.

Gas production by *Paucilactobacillus wasatchensis* WDCO4 is increased in Cheddar cheese containing sodium gluconate.

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Number
69

Themes
Microbial Communities
Fermentation and Metabolism, including protein transition

Abstract
Paucilactobacillus wasatchensis has been shown to cause gas defects in aged cheese through metabolism of galactose with production of CO_2. Previous data show gas production when no galactose is present. Metabolic modeling predicts *Pa. wasatchensis* WDC04 could metabolize gluconate with production of CO_2. Gluconate is often added to cheese to reduce calcium lactate crystal formation in aged cheese. To confirm gas production in Cheddar cheese, three replicate vats were produced using lactococcal starter with addition of WDC04. After milling, the curd was divided into six 10-kg portions with addition of salt, or salt with 0.5% galactose + 0.5% ribose, 1% sodium gluconate, 1% sodium gluconate + 0.5% ribose, 2% sodium gluconate, 2% sodium gluconate + 0.5% ribose. A control vat of cheese was produced without addition of WDC04. Cheeses were cut into 900-g pieces, vacuum packaged and stored at 12oC for 16 weeks. Bags were examined monthly for gas production. Results show an increase in gas produced during storage by adding galactose or gluconate to the curd, with the gluconate + ribose samples showing the highest. Based on calculations of total CO_2 in the cheese, the amount of galactose and gluconate added only account for about half of total gas production. Based on genome analysis, the excess gas could come from decarboxylation of amino acids. These results confirm that *Pa. wasatchensis* WDC04 can utilize gluconate to produce CO_2, and addition of gluconate to cheese is shown as another risk factor for unwanted gas production in aged Cheddar cheese.

Keywords
Cheddar cheese, Gluconate, Gas defect, NSLAB

Ecology of *Carnobacterium maltaromaticum* in freshwater environments

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Number
77

Themes
Microbial Communities
Bacteriophage and Antimicrobials

Abstract
This study explored the distribution, metabolic and antagonistic activity of *C. maltaromaticum,* isolated from different freshwater habitats in Denmark, intending to understand its ecology in this context. The results showed that *C. maltaromaticum* was widely distributed in freshwater environments although relatively rare in low pH environments. The isolates possessed a diverse and versatile metabolism, enabling maintaining functional capacities independent of habitat. The intraspecies competition among *C. maltaromaticum* isolates showed a low degree of interactions, with only a few strains exhibiting broad-spectrum inhibition activity. The frequency of bacteriocinogenic systems was low, with only one unmodified bacteriocin, piscicolin 126, found to be correlated with phenotypic antagonistic activity. Further, a bioinformatics approach showed that most potential bacteriocin gene complexes were not complete. Finally, one strain exhibited a broad inhibitory activity due to H_2O_2 production. Overall, this study demonstrated *C. maltaromaticum* as a generalist (nomadic) species with a constant presence in freshwater habitats independent of animal sources, since potential animal reservoirs were rare or absent due to low pH and the sampling period. Metabolic properties did not indicate a strong degree of adaptation to this habitat, and antagonistic activities appeared to play a minimal ecological role.

Kewords
Carnobacterium maltaromaticum; freshwater; metabolism; antagonism; bacteriocin; genomics

Mucosal immunization with *Lactiplantibacillus plantarum* surface-displayed recombinant SARS-CoV-2 epitopes induces adaptive and humoral responses

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Number
79

Themes
Host Microbe Interactions

Abstract
The Covid-19 pandemic has dramatically impacted the world. With restrictions lifted, many people are still left unvaccinated, and mucosal vaccines are the next in development to effectively fight the highly transmissible and pathogenic SARS-CoV-2 infection by establishing immunity and defense on the mucosal surface. For this application, oral and nasal vaccines have shown to have a more significant potential than parental vaccines by inducing both humoral and cellular immunity. To contribute to this work, we aimed to design mucosal vaccine vectors expressed by host lactic acid bacteria (LAB) carrying engineered plasmid constructs with a viral target peptide.

In the present study, using LAB as a mucosal vaccine vector is considered a promising alternative compared to other microorganisms because of its “Generally Regarded as Safe” status, potential adjuvant properties, and tolerogenicity to the host. This study determined the potential of *Lactiplantibacillus plantarum* expressing SARS-CoV-2 epitopes spanning the spike (S), membrane (ME), and envelope (E) proteins of SARS-CoV-2 in inducing adaptive and humoral immunity.

Oral administration of recombinant *L. plantarum* expressing SARS-CoV-2 epitopes increased the expression of IL-4, along with induced levels of TNF-α, interferon-gamma, and IL-10, specifically in S protein groups. At the same time, IL-6 seemed to be regulated during LPS-stimulated inflammation. Specific IgG and secretory IgA production were also detected against the S protein. This study suggests that *L. plantarum* is a potential vector that can

effectively deliver SARS-CoV-2 epitopes to intestinal mucosal sites and could serve as a novel approach for SARS-CoV-2 mucosal vaccine development.

Kewords
Mucosal Vaccine Vector, Immunoregulation, SARS-CoV-2, Host-Microbe Interaction

VAGINAL PROBIOTIC ON CAMEROONIAN PREGNANT WOMEN HIV POSITIVE: RESEARCH GAPS

PhD student KENFACK ZANGUIM MARIE JOSIANE, Sandra Condori, Sebastien Kenmoe, Esemu Livo, SARAH AHANNACH, Sarah Lebeer

Number
81

Themes
Microbial Communities

Abstract
INTRODUCTION: Research has demonstrated the positive effects of regular probiotic consumption on the gut microbiome. More recently, scientists have been exploring the potential of probiotics for improving vaginal health and determining the most effective and comfortable methods of using them. Research in the field of vaginal probiotics is still limited, particularly in Africa and specifically in Cameroon. Furthermore, the impact of vaginal probiotics on HIV-positive pregnant women has yet to be thoroughly investigated.

METHODOLOGY: This study was conducted in Yaounde, Cameroon among pregnant women in their second trimester, both HIV-positive and HIV-negative. For each participant, two vaginal swabs were taken at the mid-vaginal level. The presence of bacterial vaginosis (BV) was determined using Amsel's criteria and Nugent's score. Additionally, a questionnaire was administered to gather information on dietary habits, specifically in relation to vaginal probiotics and dairy products.

RESULTS: This study included a total of 55 pregnant women. We found that none of the women were familiar with vaginal probiotics and therefore did not use them. Regarding the results of BV based on Nugent's score, out of the 55 women, 18 were classified as BV-negative, 13 as BV-intermediate-positive, and 23 as BV-positive. Among the HIV-positive pregnant women, 7 out of 23 were BV-negative, 6 out of 23 were BV-intermediate-positive, and 7 out of 23 were BV-positive. Among the HIV-negative pregnant women, 11 out of 32 were BV-negative, 7 out of 32 were BV-intermediate-positive, and 14 out of 32 were BV-positive.

Kewords
vaginal probiotic, pregnant women, HIV

CovM, an accessory regulator of CovS in *Streptococcus salivarius*, mandatory for competence

Géraldine Houssa, Vande Capelle, Knoops, Rode, Hols

Number
83

Themes
Microbial Communities
Genetics and Genomics
Bacteriophage and Antimicrobials

Abstract
In *Streptococcus salivarius* (*Ssa*), competence is regulated at two levels, a proximal and a distal one. The distal regulation is governed by Two Components Systems (TCS) generally composed by a transmembrane histidine kinase (HK) and a cytosolic cognate response regulator (RR). In *Ssa*, 14 TCS were identified by *in silico* analyses, 13 complete TCS and 1 orphan HK-like protein named CovM (**Cov**S **M**odulator). The CovRS system is one of the 13 complete TCS, known to regulate virulence and required for growth under stress conditions in other streptococcal species. The system has also accessory regulators helping to adjust a specific cellular response to environmental stimuli. RocA which interacts with CovS in Group A Streptococcus, can modulate CovS kinase activity and favor virulence genes expression's repression.

RocA and CovM have a similar predicted 3D fold. Moreover, typical HK have a domain with a conserved histidine residue mutated in both proteins in a glutamine residue. Since we could not find any cognate RR to CovM and their homologies, we hypothesized a similar role to RocA: CovM would regulate CovS activity.

In this work, we showed that competence regulation required CovS presence in overexpressing/deleted mutants with a luminescence reporter strain P_{comR}-*luxAB*. RocA was shown to interact directly with CovS. Preliminary results seem to show that CovM needs its cytoplasmic part to play its role of CovS accessory regulator in opposition to RocA. Following this, we are trying to demonstrate a direct interaction between both proteins thanks to two approaches: co-precipitation and Split Nano-Luc technology.

Kewords
competence, regulation, histidine kinase, gram positive, streptococcus

Screening of natural *Lactococcus sp.* strains for persistence in the GIT and production of health-promoting metabolites

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Number
85

Themes
Fermentation and Metabolism, including protein transition
Host Microbe Interactions

Abstract
Besides lactobacilli, there are many other lactic acid bacteria strains, which exhibit health benefits and could be used as probiotics. Selected strains of *Lactococcus* genus that have been widely used in manufacturing biotechnological products and in food fermentation, were found to exhibit also health-promoting activities in *in vitro* and mice models of various human diseases.

As the first step of the selection for potential probiotics among a variety of lactococci strains, the ability to survive and persist in the gastrointestinal tract (GIT), as well as the biosafety should be considered. Thus, in this study we screened over eighty unique lactococcal strains from IBB PAS Central Collection, isolated from diverse food products, such as various types of raw milk, fermented milk products, kefir grains and sourdoughs, for their ability to withstand the unfavourable conditions encountered during their gastrointestinal passage, resist the acidic pH, tolerate bile salts, as well as adhere to the mucus. The safety properties of selected strains were evaluated based on haemolytic activity and antibiotic resistance profile. For the next step of the selection the ability to produce health-promoting metabolites, such as vitamins, lactate and short-chain fatty acids (SCFA) was tested using Bioscreen and mass spectrometry (MS) analysis.

The results of screening unveil that diverse beneficial properties among lactococci are strain-specific, and several selected strains have the potential to be used as probiotics.

This research was funded by The National Science Centre (NCN, Poland), under the grant number 2021/41/B/NZ9/02236.

Kewords
Lactococcus, probiotics, adhesion, stress resistance, vitamins, SCFA

Application of culture and molecular methods to study the microbial dynamics underlying the production of a naturally fermented milk

Ana Belén Flórez, Lucía Vázquez, Javier Rodríguez, Paula Rosa Suárez, Baltasar Mayo

Number
89

Themes
Microbial Communities

Abstract
The aim of this research was to study the succession of the three components of a microbial consortium driving milk fermentation in an artisanal naturally-fermented milk (NFM); i.e., *Lactococcus lactis* LA1, *Lactococcus cremoris* LA10, and *Lactiplantibacillus plantarum* LA30. In order to obtain a realistic snapshot of the microbial dynamics of the NFM, the growth of the three strains in milk was evaluated individually and in all possible mixes by combining culture-based and qPCR (specific target-gene of strain) methodologies. When cultured alone, *L. cremoris* LA10 reached maximum cell numbers of $6x10^8$ cfu/ml, while *L. Lactis* LA1 and *L. plantarum* LA30 reached, respectively, a cell density of 0.5 and 1.5 log units lower. The counts of *L. lactis* LA1 in milk were only increased in co-culture with *L. cremoris* LA10, whereas counts of the *L. cremoris* strain remained unchanged from those obtained alone. By the cultured-based approach, partial inhibition of *L. plantarum* LA30 by the two lactococci was observed at the beginning of the fermentation; however, this effect was not clearly detected by qPCR. Except for this result, culture and qPCR counts along fermentation agree well. During storage of the NFM at 4 ºC, *L. plantarum* LA30 counts remained stable for up to 25 days. In contrast, the viability of the two lactococci strains dropped drastically during storage, except in the co-cultures with *L. plantarum*. The knowledge acquired will help shed light on the complex relationships of the three strains in the consortium.

Kewords
Microbial consortium, naturally fermented milk, culture, qPCR

Fluorescence microscopy and microplate assays to study phage adsorption

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Number
91

Themes
Bacteriophage and Antimicrobials

Abstract
Virulent bacteriophages are known to be the most common cause of slow or incomplete milk fermentation, leading to low quality fermented products. To initiate infection, phages must first adsorb to their receptors on the bacterial surface. Studying phage adsorption is crucial to fully understand phage-host interactions and optimize starter cultures. Classical phage adsorption assays have been used for decades, but are notoriously time and materials consuming, as well as difficult to reproduce for some phage-host pairs.

Here, we develop a fluorescence microscopy assay for fast and simple analysis of viral adsorption, using phages of *Lactococcus lactis* and *L. cremoris* as a proof of concept. Using SYBR™ Gold and Nile Red as fluorescent markers for the phages and bacteria respectively, we were able to efficiently analyze the adsorption of five lactococcal phages on sensitive and resistant strains using fluorescence microscopy. We also develop a protocol to perform multiple phage adsorption assays in a microtiter assay. A 96-well plate was loaded with different lactococcal strains, and phages were added. After incubation and centrifugation of the microplate, the supernatants were spotted on a bacterial lawn of the host strain and phage plaques were counted.

As adsorption is essential for phage infection to begin, bacterial strains that are resistant to phages but still allow for phage adsorption indicate the presence of anti-phage mechanism(s). Fluorescence and microplate adsorption assays may be promising tools to efficiently test the adsorption of multiple phages to several strains simultaneously.

Kewords
Bacteriophages, adsorption, Lactococcus, fluorescence microscopy, 96-well plate

Unveiling the structure-function relationship of the broad spectrum salivaricin BlpK

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Number
93

Themes
Bacteriophage and Antimicrobials

Abstract
Streptococcus salivarius is a primo-colonizer of the human digestive tract, contributing to the homeostasis of the microbiome through the production of bacteriocins that reduce the colonization capacity of pathogenic bacteria. These interesting traits make this species useful for probiotic development. Among others, the HSISS4 strain has shown promising inhibitory activity against problematic pathogens including *Enterococcus faecium, Listeria monocytogenes* and *Staphylococcus aureus*. The inhibition capacity of this strain is the result of the concerted action of 6 different bacteriocins (BlpK, SlvV, SlvW, SlvX, SlvY et SlvZ). In this cocktail, the most potent and versatile peptide is BlpK, a linear class II bacteriocin with a mature sequence of 53 amino acids.

In this work, our objective was to investigate the action mechanism and structure-function relationship of BlpK. By utilizing computational tools such as AlphaFold and GalaxyHomomer, we developed a model for pore formation, which we subsequently confronted to an alanine scanning approach. Our findings revealed the involvement of three distinct groups of residues in stabilizing the monomer, facilitating oligomerization, and contributing to pore stabilization.

For the next step of this project, we are currently conducting a comprehensive screening using random mutagenesis to further explore the physicochemical properties allowed and/or required at each position of the bacteriocin. This approach will enhance our understanding of the mechanisms underlying the function of this highly potent bacteriocin.

Kewords
Streptococcus salivarius, Salivaricin, BlpK, Structure-function, Mutagenesis

The role of a specific probiotic formulation in women's health: *in vitro* and *in vivo* evaluation

Diletta Francesca Squarzanti, Patrizia Malfa, Franco Vicariotto, Elisa Viciani, Andrea Castagnetti, Laura Governini, Vincenzo De Leo

Number
95

Themes
Microbial Communities
Host Microbe Interactions

Abstract
Women throughout their life stages have peculiar health care needs, especially regarding their urogenital tract. This study aims to *in vitro* investigate the mechanism of action of a multi-strain probiotic composition containing *Lactiplantibacillus plantarum* PBS067, *B. animalis* subsp. *lactis* BL050, and *L. rhamnosus* LRH020 against the most common urogenital pathogens. In addition, the efficacy of this multi-strain probiotic complex has been tested in post-menopausal women.
The antimicrobial and antiadhesive properties of *L. plantarum* PBS067, *Bifidobacterium animalis* subsp. *lactis* BL050, and *Lacticaseibacillus rhamnosus* LRH020 were evaluated on vaginal and bladder epithelia infected with different urogenital pathogens (*Candida glabrata*, *Neisseria gonorrheae*, *Trichomonas vaginalis,* and *Escherichia coli*). Co-aggregation between probiotics and *Gardnerella vaginalis*, *E. coli*, and *Candida albicans* was also investigated.
Then, 50 post-menopausal women were enrolled in a clinical trial to evaluate the cytokines inflammatory pattern as infection marker and vaginal microbiota fluctuation after 28-day administration of the same probiotics tested *in vitro* (3B CFU/day; ISRCTN15737648). Vaginal wellbeing was evaluated by Vaginal Health Index (VHI).
In vitro results showed a strong inhibition of all pathogens tested. Probiotics co-aggregated with pathogens, reducing their growth and virulence. Additionally, clinical results showed an interesting decrease of menopausal symptoms and inflammatory cytokines (IL-6 and TNF-α).

After probiotic supplementation, the improvement of the VHI score, vaginal microbiota, and overall wellbeing, including vaginal health, was also observed.
These studies reveal the use of a combination of three specific probiotic strains as a new biological approach with significant evidence both *in vitro* and *in vivo.*

Kewords
Women, health, probiotics, microbiota, menopause, inflammation, cytokines

Streptococcus as a whole-cell catalyst for hydrolyzing lactose

Belay Tilahun Tadesse, Christian Solem

Number
97

Themes
Fermentation and Metabolism, including protein transition

Abstract
Streptococcus thermophilus is a fast-growing lactic acid bacterium (LAB) used in yoghurt and cheese manufacturing. We report that this bacterium can serve as an efficient cell catalyst for hydrolyzing lactose. When permeabililized by nisin we found that lactose can enter the cells and be hydrolyzed by endogenous lactase, whereafter galactose & glucose is expelled from the cells. To enhance the lactose hydrolyzing activity of *S. thermophilus*, we mutated a dairy strain and screened for variants with elevated β-galactosidase activity. Two isolates, ST30-8 and ST95, had 2.4-fold higher activity. Surprisingly, both strains were able to hydrolyze lactose when used as whole-cell lactase catalysts without permeabilization, and ST30-8 hydrolyzed 30 g/L lactose in 6 h at 50 °C using 0.18 g/L cells. Moreover, both strains hydrolyzed lactose while growing in milk. Genome sequencing revealed a mutation in L-lactate dehydrogenase, which we believe hampers growth and increases the capacity of *S. thermophilus* to hydrolyze lactose. Our findings will allow production of sweet lactose-reduced yoghurt without the use of costly purified lactase enzymes.

Kewords
Streptococcus thermophilus, lactase, whole-cell catalyst, sweetness

Effect of starter culture, fermentation and ripening on Gouda cheese flavour development in plant-protein based emulsions.

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Number
99

Themes
Fermentation and Metabolism, including protein transition

Abstract
Non-dairy cheese analogues (CA) that are commercially available are plant-based alternatives for cheese, usually made of vegetable oil, modified starch and various additives for flavour. The level of protein in these CA's is often low and to improve its nutritional value plant proteins could be used. The use of plant protein poses however some challenges such as the presence of off-flavours and their functionality in the matrix (such as the effect on texture).

In this study, different protein concentrates (pea, mung bean protein and casein), along with glucose, coconut oil and sodium citrate, were used to make an emulsion suitable for inoculation with lactic acid bacteria (LAB), both from plant en dairy origin and fermented for up to seven days. Strains were screened for their ability to reduce the pH of the matrix (<5) and to form a fermentation-induced gel.

Since the majority of flavours in traditional Gouda cheese are formed during ripening, LAB inoculated (pea and casein) protein-based matrices were stored at 13°C for up to 6 weeks to mimic Gouda cheese ripening conditions. Microbial and physical stability were tested over

time and after 6 weeks various physiochemical parameters were determined, like pH and volatile organic compound production.

In both fermented only and ripened samples, an increase in cheese-aroma's such as acetate, acetoin, butanediol, 3-methylbutanal and 3-methylbutanol during ripening was observed, as well as a decrease in off-flavours such as hexanal and 1-hexanol. LAB strains isolated from plant sources had on average the highest cheese aroma production.

Kewords
cheese analogue, plant-protein, LAB, fermentation, ripening, flavour

LAB as key tools for plant-based food fermentation: understanding their proteolytic activity and functionality in soy-based milk fermentation

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Number
103

Themes
Fermentation and Metabolism, including protein transition

Abstract
Climate change necessitates a reduction in the carbon footprint of food production. Consumers and the food industry share a demand for an increase in the variety of plant-based food products including fermented ones. However, producing good quality products is still far down the road. LAB, known for their wide vital role in food production has been leveraged as a tool to obtain various desired products in fermentation systems. LAB's long history of application, wide range of functionality including their intricately selective proteolytic activity, and GRAS (generally regarded as safe) status, this study aims to explore

and understand proteolytic characteristics of LAB to improve the fermentation of plant-based milk analogs. In this study, a total of 270 LAB strains isolated from various environmental and fermented food sources were screened using a colorimetric high-throughput assay. A total of 7 strains comprised of strains identified to be *Lactococcus lactis, Lacticaseibacillus rhamnosus, Streptococcus thermophilus*, were assessed to be putative proteolytic based on their acidification patterns relative to controls. Screening for presumptive genes using the draft genomes of the pre-selected strains was conducted using BLAST and the MEROPS peptidase database, identifying the presence of PII-type proteinases on their genomes. Proteolytic activity and hydrolysis patterns on fermented soy-based milk analogs were assessed using LC-MS/MS. Proteolysis across positive strains belonging to different groups showed distinct clustering of hydrolysis patterns in soy-based milk drinks with regards to their peptide length distribution and relative abundances of peptides derived from certain proteins from the soy drink.

Kewords
Plant-based milk, Lactic acid bacteria, proteinases, fermentation

Lactic acid bacteria selection to design efficient starter cultures for pulses fermentation

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Number
109

Themes
Fermentation and Metabolism, including protein transition

Abstract
To fight against global heating, it is advised by international authorities to limit consumption of animal proteins in developed countries and to switch to plant-based proteins.
Pulses are known to contain high concentration of proteins and then considered as an excellent protein source alternative compared to meat or milk proteins. Besides, pulses fermentation by lactic acid bacteria can provide a large range of diversity of taste and texture combined with microbial stabilization thanks to raw material acidification.

It is proposed to investigate the ability of 8 different lactic acid bacteria species (*Lacticaseibacillus paracasei, Lactiplantibacillus pentosus, Lactiplantibacillus plantarum, Lactococcus lactis, Levilactobacillus brevis, Limosilactobacillus fermentum, Pediococcus pentosaceus, Streptococcus thermophilus*) represented by 105 different strains, to acidify soy and pea preparations(1). The inoculation rate of the raw material is 1E6 cfu/mL and the temperature of fermentation is 37°C. The evolution of pH during fermentation is monitored by using online pH measurement device (Abscia from Absciss Instrumentation Scientifique).

In soy, *Streptococcus thermophilus* species (7 biodiverse strains) displays the shorter time to achieve pH 5.5. *Lactococcus lactis* strains (28 biodiverse strains) are rather close to *Streptococcus thermophilus*. In pea, the ranking for acidification between these two species is the opposite.

Results suggest that both lactic acid bacteria species are good candidates to ensure fast and robust acidification of pulses preparations.

Kewords
Plant-based, pulses fermentation, acidification

Regulation of carbohydrate metabolism in *Bifidobacterium breve* UCC2003 – Role of AraQ and MalR1 in degradation of complex carbohydrates.

Luiza Morawska, Anne De Jong, Ana Solopova, Francesca Bottacini, Oscar Kuipers, Douwe Van Sinderen

Number
111

Themes
Genetics and Genomics
Fermentation and Metabolism, including protein transition

Abstract
Bifidobacterium, a Gram-positive anaerobic bacterium that commonly resides in the human gastrointestinal tract, demonstrates remarkable versatility in utilizing various carbohydrates. Its ability to efficiently metabolize a wide range of carbohydrates is believed to play a significant role in its establishment and persistence within the gut of both adults and infants. However, so far, the control and utilization hierarchy of metabolic pathways of even simple sugars are not completely understood in bifidobacteria.

In silico genomes analyses revealed that bifidobacteria do not possess usual carbon catabolite repression regulators or their homologues. Instead, a presence of two LacI-type regulators, AraQ, and MalR1, was detected. Both transcriptional regulators (TRs) were shown to bind to identical promoter regions and their binding specificity to the DNA operator sites suggests that both TRs can act as repressors and activators of the bifid shunt genes.

In this study, we elucidate the role of AraQ and MalR1 in the control of central carbon flux in the bifidobacterial prototype strain, *Bifidobacterium breve* UCC2003. To understand the regulatory networks and the impact of TRs on carbohydrate metabolism, we performed comparative analysis of growth profiles and transcriptomic analysis of Δ*araQ*, Δ*malR1*, and Δ*araQ*Δ*malR1*, cultured in various carbohydrates. Our data suggests that AraQ and MalR1 act as global transcriptional regulators and regulate genes encoding for an array of sugar transporters, bifid shunt enzymes and secondary TRs. Moreover, we observed profound differences in growth of Δ*araQ*, Δ*malR1* and Δ*araQ*Δ*malR1* on malto-oligosaccharides, pointing on the essentiality of AraQ and MalR1 in starch-like carbohydrate utilisation.

Kewords
CCR, bifidobacteria, carbohydrate metabolism, transcription regulation

Comparison of multilocus and hsp60-sequence data analysis for subspecies determination of *Lactobacillus delbrueckii* isolates from raw milk

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Number
113

Themes
Genetics and Genomics

Abstract
Previously, we reported the genetic diversity of *L. delbrueckii* isolated from raw milk in Bulgaria and Japan at LAB13 (Bulagria: Urshev Z. et al., Japan: Tsuchihashi H. et al.). Multilocus Sequence Analysis (MLSA) using seven housekeeping genes (*fus*A, *gyr*B, *hsp*60, *ile*S, *pyr*G, *rec*A, and *rec*G) is a well-known method to subspeciate *L. delbrueckii*, but recently it is reported that the analysis using only *hsp*60 might be enough to estimate the subspecies of *L. delbrueckii* isolated in Japan. (Tsuchihashi H. et al., JDS, 2022).

The objective of this study was to confirm whether subspecies estimation using *hsp*60 analysis can be applied to *L. delbrueckii* isolated from raw milk in Bulgaria and to compare their distribution with that of isolates from Japan. In this study 31 and 10 strains isolated in 2019 and 2021, respectively, were used. The *hsp6*0 analysis was performed on all 41 strains

and MLSA was performed on 22 strains that showed a representative pattern by RAPD analysis.

Clustering using 7 genes (MLSA) or *hsp*60 sequence data both distributed the isolates from Bulgaria into clusters I and III (MLSA) and group1 and group3 (*hsp*60), representing the *lactis* or *sunkii* subspecies, respectively, in identical fashion, confirming the usefulness of *hsp*60 analysis for subspecies estimation of *L. delbrueckii*. No sharp distinction between the sugar utilization patterns of isolates clustered in the *lactis* and *sunkii* clusters was obtained. Among isolates from raw milk in Bulgaria these two subspecies were equally represented, while the cluster of *lactis* was predominant among isolates from Japan.

Kewords
hsp60, Lactobacillus delbrueckii, MLSA, raw milk

Genotypical and phenotypical lactic acid bacteria screening approach for the removal of antinutrients and off-flavours from plant-based materials

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Number
117

Themes
Genetics and Genomics
Fermentation and Metabolism, including protein transition

Abstract
The antinutrients and off-flavours present in plant-based foods are the major organoleptic and consumer acceptance drawbacks considered when developing plant-based dairy alternatives. Some lactic acid bacteria strains have demonstrated to encode for enzymes that are phenotypically able to degrade or remove those, through the action of fermentation. However, there are lacks on establishing bacteria, single and combinatorial, screening (SC) platforms that could optimize the starter culture development process for plant-based dairy alternatives. Here, it is shown a combination of genotypical and phenotypical screening methods to find out the optimal combination of lactic acid bacteria strains based on off-flavours, phenolic acids, saponins and trypsin inhibitor degradation through the fermentation of a pea, oat, and potato (POP) blend. The SC platform is

composed by five consecutive SC phases; genotypical SC, pH SC, colorimetric/growth SC, *in situ* fermentation SC and combinatorial SC. Specific LAB strains belonging to different LAB species such as *Lactiplantibacillus plantarum* and *Leuconostoc pseudomesenteroides* shown their promising capabilities to reduce specific unwanted compounds in POP blend.

Finally, this optimized SC platform will be significantly use in microbial screening purposes throughout starter culture development for plant-based yogurts, cheese, and other dairy and non-dairy fermented products.

Kewords
LAB, phenolic acids, off-flavours, saponins, trypsin inhibitors.

Lactic acid bacteria and ruminant methane mitigation

Dr William Kelly, Dr Yang Li, Dr Laureen Crouzet, Dr Sinead Leahy, Dr Graeme Attwood

Number
121

Themes
Genetics and Genomics
Host Microbe Interactions
Bacteriophage and Antimicrobials

Abstract
Methane (CH_4) resulting from enteric fermentation in ruminant livestock is a major source of agricultural greenhouse gases and several different interventions are being investigated to limit CH_4 emissions. The use of microbial cultures (particularly lactic acid bacteria, LAB) and their metabolites have been proposed as CH_4 mitigation approaches that could be used alone, or in combination, with other options. Lactate has an important role in ruminal metabolism and there is evidence from studies in cattle and sheep that bacteria involved in lactate production and utilization underpin low CH_4 emission phenotypes in ruminants. Although many rumen microbes produce lactate, it does not usually accumulate in the rumen because of the presence of a relatively limited number of cross-feeding bacteria that convert lactate to other SCFAs, particularly butyrate and propionate.

The aim of this study was to screen bacterial strains isolated from the rumen and from fermented or minimally processed foods for anti-methanogen potential. Bacteria screened included LAB, bifidobacteria, and the obligate rumen anaerobes *Kandleria* and *Sharpea* that

produce lactate as a fermentation end product. In silico screening revealed that several genomes contained gene loci associated with the production of bacteriocins and/or lanthipetides. Filtered culture supernatants were prepared from these strains and tested in triplicate for the ability to inhibit growth of the rumen methanogen *Methanobrevibacter boviskoreani* JH1^T in a micro-titre plate growth inhibition assay. A total of 30 strains (24%) showed more than 50% growth inhibition, with *Streptococcus equinus* and *Lactiplantibacillus plantarum* being the most effective.

Kewords
Rumen, enteric methane, methanogens, lactate metabolism, bacteriocins

Intra and inter-strain cross-activation between redundant SHP/Rgg quorum sensing systems in Streptococcus thermophilus

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Number
123

Themes
Genetics and Genomics

Abstract
Streptococcus thermophilus is widely used in the production of yoghurt and cheese. This bacterium has operons involved in the synthesis, post-translational modification and export of peptides called RAS-RiPPs (Radical SAM Ribosomally Post-translationally modified Peptides). *S. thermophilus* strains have accumulated between 0 and 7 operons. Our two model strains have 5 of these and 4 in common. These RAS-RiPPs may have antimicrobial activities. Quorum sensing controls the expression of these operons through transcriptional regulators of the Rgg family and autoinducible peptides called SHPs (Short Hydrophobic Peptides).

Our goal is to understand the regulation of the production of all RAS-RiPPs at the cellular level.

Each RAS-RiPP operon co-localises with a *shp*/*rgg* pair. We first used translational fusions with a luciferase reporter and confirmed that all operons were expressed. Then, we used *rgg* deletion mutants to identify which Rgg is involved. Some SHPs are very similar suggesting that cross-activation might occur. Therefore, we introduced a mutation that inactivated the natural production of all SHPs in the translation fusion strains and added synthetic SHPs in the medium to identify those able to induce the expression. Finally, we used mass spectrometry to look for the natural SHPs that are released in the culture medium. We observed for the first time that all the SHPs that are encoded in the genome are produced and secreted and confirmed cross-activation between SHPs.

In conclusion, it will be interesting to explore the biological relevance of this SHP interchangeability at the strain level and between strains.

Kewords
Streptococcus thermophilus, quorum sensing, Rgg, RRNPPA, cross-activation

Salt-Stressed Transcriptome Analysis of Staphylococcus equorum KM1031 Isolated from High-Salt Fermented Salted Seafood

Sojeong Heo, Do-Won Jeong

Number
125

Themes
Genetics and Genomics
Fermentation and Metabolism, including protein transition

Abstract
Staphylococcus equorum is a potential starter for Korean high-salt fermented foods because of its salt-tolerance and enzymatic activities that contribute to enhanced sensory properties of the food products. However, the mechanisms of salt tolerance of *S. equorum* are not fully understood. Here, RNA sequencing was performed on *S. equorum* strain KM1031 exposed to 7% NaCl (w/v) for 2 and 4 h to determine global gene expression changes. Salt pressure for 2 and 4 h resulted in significant differential expression of 4.8% (106/2,209) and 6.1% (134/2,209) of *S. equorum* KM1031 genes, respectively. Twenty-five core genes were differentially expressed on salt-treatment for both 2 and 4 h, seven of which were related to osmoprotectant uptake and synthesis. We analyzed the genome of strain KM1031 and identified osmoprotectant uptake (Opu) systems, potassium importers, sodium exporters, and the glycine betaine synthesis system. The RNA sequencing results indicated that the OpuD system and glycine betaine synthesis might play the main roles in the salt-tolerance of

strain KM1031. Finally, the results of RNA sequencing were validated by quantitative real-time PCR of likely salt stress-related genes. This transcriptomic analysis provides evidence regarding the osmotic stress responses of *S. equorum* strain KM1031.

Kewords
Staphylococcus_equorum, salt_pressure, transcriptome, jeotgal, OpuD, glycine_betaine

Strain typing with IR Biotyper

Ragna Tessin

Number
127

Themes
Genetics and Genomics

Abstract
With the use of novel technologies, an increasing number of bacteria have been identified, produced and used within the industrial sector over time. Subtle modifications in the genome of closely related strains can have a big influence on the bacterial capabilities. The ability to discriminate between bacterial strains at a precise level is of utmost significance for ensuring high quality processes and products in the industry. Currently, the most widely utilized methods for this purpose include Pulsed-Field Gel Electrophoresis (PFGE), Multi-Locus Sequence Typing (MLST), and whole genome sequencing (WGS). A spectroscopic technique known as Fourier Transform - Infra-Red (FT-IR) spectroscopy, specifically the IR Biotyper (IRBT) developed by Bruker®, has emerged as an expedient approach for strain typing. This method offers expeditious, accurate, and cost-effective outcomes. To assess its efficacy, more than 50 isolates of *Streptococcus thermophilus* were selected for analysis with the IRBT. Principal Component Analysis (PCA), Linear Discriminant Analysis (LDA), and dendrograms were employed as analytical tools. Successful discrimination among all isolates was achieved. Notably, two isolates that could not be differentiated using PFGE were effectively distinguished applying the IRBT. Nevertheless, challenges were encountered in

implementing the artificial intelligence (AI) function, which could potentially allow a faster and automated approach to strain typing.

Kewords
IR Biotyper, strain typing, phenotypic screening

Synergy between Bifidobacterium infantis LMG11588 and 6-HMO blend increases short chain fatty acid production in infants and toddlers ex vivo

Katja Johnson, Florac De Bruyn, Dominick Maes

Number
129

Themes
Host Microbe Interactions

Abstract
To enhance health benefits, a live microorganism can be co-administered with a specific substrate as a synergistic synbiotic. We assessed the synergies resulting from combining *Bifidobacterium infantis* LMG11588 and a blend of 6 human milk oligosaccharides (6-HMO). This synergy potential is relevant, since *B. infantis* prevalence in the infant gut is declining, especially in developed countries.

We have combined different groupings of *B. infantis* LMG11588 and/or a blend of 6-HMO in an *ex vivo* colonic simulation system seeded with fecal inocula from the same donors at

infant and toddler age. We followed how short chain fatty acid (SCFA) production and microbial community (16S rRNA seq) change and how the interaction between *B. infantis* and 6-HMO blend affects these outcomes. SCFA production and associated microbial dynamics serve as a proxy for presumptive *in vivo* health benefits.

When 6-HMO was administered, HMOs were partially consumed by the gut microbiota. However, HMOs were fully consumed for all infant donors when co-administered with *B. infantis*. This resulted in a meaningful increase in SCFA production for the *B. infantis* + 6-HMO combination compared to the summed production from individual ingredients (synergy). The increase in total SCFA concentration when supplementing *B. infantis* was approximately 60%. The synergistic benefit was maintained when adding *Bifidobacterium lactis* to the system, suggesting the mechanism is robust to additional probiotic supplementation. The synergistic synbiotic of *B. infantis* LMG11588 and 6-HMO could thus potentially maximize the health benefit potential related to colonic metabolite production and other biologically relevant activities from *B. infantis*.

Kewords
Bifidobacterium longum subsp. infantis, HMO, synbiotic, SCFA

Deciphering the Link Between Genetic Characteristics and Carbohydrate Utilization Phenotypes of Lactic Acid Bacteria Using Gene Trait Matching Analysis

Borowska Malgorzata, Colin Buttimer, **Ortensia Catalano Gonzaga di Cirella**, Colin Hill, Francesca Bottacini, Aylin W. Sahin, Elke K. Arendt, Aidan Coffey

Number
133

Themes
Genetics and Genomics

Abstract
The proven safety and extensive use of Lactic acid bacteria (LAB) in the food industry make them ideal candidates for functional food development. However, their diverse ability of utilising carbohydrates from botanical sources has been largely understudied. In this study, we combined phenotypic growth data in different carbohydrates with bioinformatic approaches using whole genome sequencing and comparative genomics. The genomic and phenotypic characteristics of four isolates of *Lacticaseibacillus paracasei*, 12 isolates of

Lactiplantibacillus plantarum, and 15 isolates of *Pediococcus pentosaceus* obtained from various sources were examined for their carbohydrate utilization abilities. A pool of 23 carbohydrates, including fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs), was used for phenotypic analysis. Through the application of Gene Trait Matching (GTM) analysis, a putative genetic cluster associated with ribose utilization has been identified in *L. paracasei*. In addition, it was demonstrated that the presence of intracellular β-fructofuranosidase does not always indicate short-chain fructan (FOS) biodegradation, while extracellular β-fructofuranosidase is consistently associated with long-chain fructan utilization (levan, inulin). This discovery contributes to our growing knowledge of LAB species and establishes a foundation for future genomic investigations aimed at unravelling the intricate connections between their diverse genetic characteristics and specific phenotypes.

Kewords
LAB, comparative, genomics, FODMAPs, GTM

Phenotypic study on 100 strains of Leuconostoc spp. for the selection of Mannitol-negative strains

Ueli Von Ah, Carlotta Sartori, Fanny Germanier, Anne Guisolan, Daniel Heine

Number
135

Themes
Fermentation and Metabolism, including protein transition

Abstract
Leuconostoc spp., especially *L. mesenteroides* subsp. *mesenteroides* and subsp. *cremoris* as well as *Leuconostoc lactis* are often found in cheese and used as flavour producing bacteria in starter cultures. However, they are even more important in plant-based fermentations such as Sauerkraut and Kimchi.

In this study we evaluated more than 100 strains of *Leuconostoc* spp. phenotypically using Biolog. The aim was to find strains with the ability to metabolize glucose, fructose and sucrose but lacking the ability to use mannitol as carbon source. 44 of the selected strains

showed this phenotypic trait if grown at 30 °C for 48 h. The goal for this selection was to use the strains for fermented products adding flavour and texture properties without loosing the properties of mannitol.

The whole genome sequence was available for a selection of the tested strains. For those, we combined the phenotypic data with the genome using Ductape in order to evaluate the correlation of the annotated pathways with the phenotype.

Kewords
Leuconostoc, LAB, phentoype, geno-phenotype, plant-based fermentations, flavour

Powerful selection of Lactic Acid Bacteria Strains With Improved rheological properties.

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Number
137

Themes
Fermentation and Metabolism, including protein transition

Abstract
Most antibiotics and antimicrobial agents target the bacterial cell envelope and interfere with the synthesis of peptidoglycan, membrane stability and permeability, and attachment of surface components. On this poster, we describe a powerful selection method developed to select for derivatives of lactic acid bacteria (LAB) with improved properties for dairy applications. Using inhibitory concentrations of several different cell envelope targeting

antibiotics and antimicrobial agents, we observed that a fraction of the selected resistant LAB isolates had improved rheological properties. To validate the efficacy of the method and to understand the mechanisms behind the improved rheology, we performed genetic and physiological characterization of several improved derivatives. The results revealed an unexpected diversity of genetic changes affecting other cellular functions than the targeted cell envelope. Here, we present the results of this work documenting the versatility of this powerful toolbox for strain development.

Kewords
antimicrobial agents, resistance, strain improvement, texture, LAB,

Acidification and growth rate measurements in plant based substrates using centrifugation-based clearing.

Patrick Janssen, Herwig Bachmann

Number
139

Themes
Fermentation and Metabolism, including protein transition

Abstract
Plant-based substrates are increasingly used for the production of dairy and meat analogs. Fermentation with selected organisms allows to overcome sensory challenges when working with those substrates. For the optimization of such fermentations the monitoring of cell growth is important. However, the turbidity of most plant-based suspensions creates difficulties when preforming optical density measurements. We have shown previously that centrifugation-based clearing allowed to measure growth rates and acidification in a high-throughput manner in skimmed-milk. (Douwenga et al, 2021). Here we apply a similar protocol to plant-based suspensions of soy, almond and oat protein which allowed us to obtain a clear supernatant. On this supernatant various lactic acid bacteria were cultivated. The results show a good correlations between the acidification rates when comparing plant-based suspensions and the corresponding cleared supernatant. In addition growth can be monitored directly through optical density measurements in the cleared suspensions. In combination with a fluorescent pH indicator this allows for the online high-throughput

determination of optical density and pH simultaneously, which is valuable for the optimization of plant-based fermentations.

Douwenga, Sieze & Janssen, Patrick & Teusink, Bas & Bachmann, Herwig. (2021). A centrifugation-based clearing method allows high-throughput acidification and growth-rate measurements in milk. Journal of Dairy Science. 104. 10.3168/jds.2020-20108.

Kewords
High-throughput screening, protein transition, method, optical density

Design of a chemically defined medium for water kefir

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Number
141

Themes
Microbial Communities
Fermentation and Metabolism, including protein transition

Abstract
In nature different microbial species often co-occur in close proximity. This allows interactions that range from antagonistic to mutualistic. An example for the co-occurrence of different microbial species in close proximity is water kefir, which is used across the world to ferment sugary water into a carbonated drink. In the kefir granule lactic acid bacteria(LAB), yeasts and acetic acid bacteria (AAB) are co-located. The lactic acid bacteria (e.g. ,*Lb. hilgardii* and *Lb.nagelii*) are responsible for the spatial structure of the granule by producing exopolysaccharide scaffolding. Other LAB and yeasts are suggested to share nitrogen compounds and vitamins. However, knowledge on the interactions that occur within the water kefir consortium is still limited. One of the complications that arise when studying the interactions between the consortium members with a systematic approach is the lack of a chemically defined medium for kefir propagation. Here, we design a chemically

defined medium for kefir propagation in a controlled laboratory setting. Especially adjustments in free nitrogen-and amino acid content were important for optimal granule growth, without disrupting the composition of the consortium. The results will allow more specific studies of the interactions in the consortium and open possibilities for the design of kefir-based synthetic microbial communities.

Kewords
chemically defined medium, microbial consortia, water kefir

Comparative genomic analysis of Periweissella and the characterization of novel motile species

Nanzhen Qiao, Julia Bechtner, Margo Cnockaert, Eliza Depoorter, Cristian Díaz-Muñoz, Peter Vandamme, Luc De Vuyst, Michael G. Gänzle

Number
145

Themes
Genetics and Genomics
Fermentation and Metabolism, including protein transition

Abstract
The genus *Periweissella* was proposed as a novel genus in the *Lactobacillaceae* in 2022. However, the phylogenetic relationship between *Periweissella* and other heterofermentative lactobacilli, and the genetic and physiological properties of this genus remain unclear. This study aimed to determine the phylogenetic relationship between *Periweissella* and the most closely related genera, *Weissella* and *Furfurilactobacillus*, by phylogenetic analyses and calculation of pairwise average amino acid identity. Targeted genomic analysis showed that fructose bisphosphate aldolase was only present in the genome of *Pw*. *cryptocerci*. Mannitol dehydrogenase was found in genomes of *Pw*. *beninensis*, *Pw*. *fabaria*, and *Pw*. *fabalis*. Untargeted genomic analysis identified the presence of flagellar genes in *Periweissella* but

not in other closely related genera. Phenotypes related to carbohydrate fermentation and motility matched the genotypes. Motility genes were organized in a single operon and the proteins shared a high amino acid similarity in the genus *Periweissella*. The relatively low similarity of motility operons between *Periweissella* and other motile lactobacilli indicated the acquisition of motility by the ancestral species. Our findings facilitate the phylogenetic, genetic, and phenotypic understanding of the genus *Periweissella* and its use in food fermentations.

Kewords
Periweissella, heterofermenter, phylogenetic relationship, carbohydrate fermentation, motility.

Anti-inflammatory effect of moonlighting protein, glyceraldehyde-3-phoshate dehydrogenase, secreted from Lactobacillus johnsonii

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Number
149

Themes
Genetics and Genomics
Host Microbe Interactions

Abstract
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), as a member of the moonlighting protein, possesses multiple vital biological functions. In our previous study, extracellular GAPDH in *Lactobacillus johnsonii* MG cells interacts with junctional adhesion molecule-2 (JAM-2) in Caco-2 cells and enhances tight junctions. However, the specificity of GAPDH toward JAM-2 and its role in the tight junctions in Caco-2 cells remain unclear.

In the present study, we assessed the effect of GAPDH on tight junction regeneration and explored the GAPDH peptide fragments required for interaction with JAM-2. GAPDH was specifically bound to JAM-2 and rescued H_2O_2-damaged tight junctions in Caco-2 cells, with various genes being upregulated in the tight junctions. To understand the specific sequences of GAPDH involved in JAM-2 binding and MG cell interaction, two peptides, namely ^{11}GRIGRLAF^{18} at the N-terminus and ^{323}SFTCQMVRTLLKFATL^{338} at the C-terminus, were purified and displayed good interactions with JAM-2. In contrast, the long peptide ^{52}DSTHGTFNHEVSATDDSIVVDGKKYRVYAEPQAQNIPW^{89} was predicted to bind to the bacterial cell surface.

Secretion of GAPDH, without secretion signal sequence, in specific lactobacilli via ATP-binding cassette (ABC) transporters was suggested. In the present study, the cell wall associated GAPDH level in MG culture medium was significantly reduced by adding of inhibitor for ABC transporter during fermentation. The secretion mechanism for GAPDH in MG remains unclear, while the involvement of transporter system will be discussed.

Keywords
GAPDH, JAM-2, Caco-2 tight junctions, ABC transporter.

Development of Spoilage Psychrotrophic Lactic Acid Bacteria in Ready-to-Eat Salad Under Cold Storage

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Number
151

Themes
Microbial Communities

Abstract
The consumption of ready-to-eat (RTE) foods as a convenience and fresh meal has been increasing in recent years. However, the quality and safety of RTE foods can be affected by spoilage bacteria, since they are not subjected to heat treatment. The objective of this study was to assess the effects of storage conditions on the quality of commercially produced mayonnaise-based salad in modified atmosphere packaging (MAP). The salads were kept under different temperatures (0, 2, 4, 6, and 8°C) for 7, 10, and 12 storage days to follow changes in the physicochemical and microbiological, and sensory profiles. The microbiota composition was determined by plating on the selective media (PCA, VRBG, MRS, and DRBC)

followed by the MALDI-TOF technique. The results revealed that lactic acid bacteria (LAB) belonging to the species *Lactococcus piscium*, *Carnobacterium maltaromaticum*, *Leuconostoc carnosum*, and *Leuconostoc gelidum* became dominant during storage. Their numbers increased from 10^6 to 10^9 cfu/g at the end of shelf-life, depending on storage temperature. The growth of LAB was accompanied by a decrease in pH and an increase in TTA from 6.31 to 4.44 and 1 to 2.9 respectively. The sensory quality deteriorated faster at high temperatures resulting in a sour and acidic odor on the 7th day, while the storage at 0 and 2°C preserved the salad quality for up to 10 days. In conclusion, psychrotrophic lactic bacteria are dominant spoilage microorganisms in RTE mayonnaise-based salad and can be effectively controlled by appropriate storage temperature.

Kewords
Ready-to-eat salad, MAP, spoilage bacteria, storage temperature

Seasonal impact on starter culture development in aged goat milk cheese

Vinícius Duarte, Beate Bjørgan, Siv Skeie, Davide Porcellato

Number
153

Themes
Microbial Communities
Genetics and Genomics

Abstract
Norway has a long tradition in the production of goat milk using mountain rangeland pasture during summer. However, little is known about how seasonal changes affect cheesemaking and cheese quality. In this study, we aimed to investigate the impact of goat milk seasonality on starter culture development through metataxonomic analysis and shotgun sequencing. Bulk milk was collected from four dairy farms in spring (A), at the first milkings on mountain pasture (B), midway in mountain pasture (C), and during oestrus (D) and further manufactured into a hard goat milk cheese. Every three months of ripening (until 12 months), cheese samples were collected, microbial DNA extracted, and Illumina 16S rRNA/metagenomic shotgun sequencing was carried out. Our results indicate that cheese from period D showed greater bacterial diversity/richness when compared to all other

periods. Cheeses produced from periods A and B showed a lower abundance of *Lactobacillus* and *Lactococcus* but were high in *Streptococcus*. On the contrary, cheese from periods C and D showed a greater abundance in *Lactobacillus* and *Lactococcus*, but less in *Streptococcus*. In total, four high-quality metagenome-assembled genomes were recovered from the different datasets and taxonomically annotated as *Streptococcus thermophilus*, *Lactococcus lactis*, *Lactococcus cremoris*, and *Lactobacillus helveticus*. Taken together, our results indicate that there is a remarkable effect of the season on starter culture development in cheese and further investigations are needed to evaluate why and how the same bacterial strains respond differently to milk collected from different seasons.

Kewords
Goat milk, goat cheese, metabarcoding, shotgun sequencing

The potential of indigenous lactic acid bacteria as starter culture in plant-based fermentations - assessment of technological properties

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Number
155

Themes
Fermentation and Metabolism, including protein transition

Abstract
Plant-based protein (PBP) sources have an incomplete protein profile on their own. However, their amino acid composition can complement each other to yield high-quality protein. Therefore, they represent attractive alternatives to animal protein. Yet, many PBP sources contain various antinutrients that can cause irritation and discomfort for the consumer, making them problematic as alternative protein sources. Bioprocessing methods

like fermentation are known to reduce antinutrients, and it is widely recognized that microorganisms naturally found in the raw material are suitable starter cultures in fermentation. The objective of this study was to establish and evaluate the fermentation properties of lactic acid bacteria autochthonous to cereals, pseudocereals, and legumes. Lactic acid bacteria (LAB) from spontaneous sourdough fermentation of legumes, pseudocereals and cereals were isolated and identified by culture-dependent methods, including genome sequencing. Twenty-two isolates, belonging to 6 different species, were screened for their acidification potential, proteolytic activity, exopolysaccharide (EPS) production, phytase activity, and fermentation pattern. Three isolates showing promising properties were further tested as single-strain starters in the fermentation of dough slurries. The three selected isolates belonged to the genera *Lactiplantibacillus*, *Pediococcus* and *Leuconostoc*. Two of these showed attractive fermentation properties such as good acidification, utilization of carbohydrates and production of organic acids and volatile aromatic compounds in dough slurries. One isolate showed poor growth and acidification ability in the dough slurries. The result of this study adds to the exploration of autochthonous microbes to become new starter cultures in fermentations of plant-based, protein-rich food.

Kewords
Cereals/Pseudocereals, Legumes, Autochthonous starter-culture, Technological properties

Chemotactic behavior of Ligilactobacillus agilis BKN88 against gut-derived substances.

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Number
157

Themes
Host Microbe Interactions

Abstract
ã€€A few members of lactic acid bacteria (LAB) display motility, and these LAB are presumed to utilize chemotaxis for survival in their own niche; however, the chemotaxis of motile LAB is poorly understood. Our research group has been studying *Ligilactobacillus agilis* BKN88 as a representative of motile commensals in animal guts and hypothesizes that this microbe exhibits chemotactic responses to gut-derived substances. Therefore, the aim of this study is to investigate chemotactic behavior of *L. agilis* BKN88 to better understanding its ecological traits. In order to identify substances involved in chemotaxis, an agar-drop assay was

performed. The results showed that BKN88 escaped from some organic acids, low pH, N-acetylneuraminate, bile acids (SC, SDC, STC), and was attracted to mucin. Five genes (mcp1 to mcp5), presumed to be chemotaxis receptors, were found in the genome of BKN88. The expression of each gene was confirmed by RT-PCR. A series of mutant strains, either expressing or lacking one of each MCP, were constructed. These mutans were subjected to the agar-drop assay to identify the specific MCP corresponding to each chemotactic substance. The results showed that MCP1- or MCP2-deleted strains had a weakened chemotactic response to SC, SDC, and STC, while MCP5-deficient strains had a weakened response to SC. The MCPs corresponding to other substances were unclear from the current results. Interestingly, BKN88 is relatively sensitive to acid and bile compared to other non-motile lactobacilli. This suggests that motile LAB may rely more on motility/chemotaxis to survive than on developing acid- or bile-resistance.

Kewords
lactobacillus, motility, chemotaxis, flagella

Lactococcal bacteriophages isolated from Gouda-type cheese-producing plant

Junhyeok Yu, Jennifer Mahony, Arjen Nauta, Martin Bonestroo, Douwe Van Sinderen

Number
159

Themes
Bacteriophage and Antimicrobials

Abstract
In the dairy industry, strains of *Lactococcus lactis* and *Lactococcus cremoris* are commonly used as mesophilic starters to facilitate milk acidification. However, phages that belong to the dominantly encountered genus *Skunavirus*, may cause interruption of this fermentation process through infection of these lactococcal starter strains. In the current study, samples including curd, whey, and ingredients (i.e., milk, starter, and whey cream) were collected from a Gouda-type cheese plant, and their skunaviruses were characterised. Using a panel of strains isolated from the starter culture, phages were identified and characterized by plaque assays and PCR. Three isolated skunaviruses (designated here as L1.1, Mm3.1, & L7.1) were characterized as genetically distinct as based on host range, thermal & biocidal resistance,

and genome sequence. Furthermore, phageome analysis of whey samples identified 8 genetically distinct skunaviruses, including the three aforementioned isolates, each encoding one or two distinct receptor binding proteins (RBPs). These RBP sequences allowed the prediction of the CWPS type of their host by phylogenetic analysis. The phageome composition through the production process was established by reads mapping of the RBP-associated sequences of the eight identified skunaviruses.

Kewords
Bacteriophage, Lactococcus, Phageome, Gouda cheese, RBP

Streptococcus thermophilus, a new bacterial chassis for the production of recombinant proteins

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Number
161

Themes
Genetics and Genomics

Abstract
Lactic acid bacteria have been used for a long time to produce fermented food. Thanks to their **GRAS** status and their beneficial effects on **health**, these bacteria also have

biotechnological interest**.** Today, advances in **synthetic biology** make them attractive for the production of useful biomolecules in various fields (biopharma, human nutrition…).

Streptococcus thermophilus is particularly interesting due to its short genome, its ability to grow fast, and its secretome containing a low number of proteins. These make it a good candidate for the **production of extracellular recombinant proteins**.
In our lab, recent work on *Streptococcus thermophilus* has revealed :
-(1) a strain without surface proteolysis
-(2) a peptide inducible expression system derived from Quorum Sensing

These two patented tools allow us to develop a **new bacterial chassis for protein production**, which should minimize **DownStream Processing** steps.
I showed that *S. thermophilus* was able to secrete 3 recombinant proteins (LytM, α-galactosidase, ManB) and 1 peptide (Kisspeptin). I also showed that the **strain without surface proteolysis** improves the quality of LytM production. Moreover, the **proteins are produced in an active form**.
Unlike common recombinant proteins producers, the optimization of ***S. thermophilus*** has not been addressed yet. The relatively low protein production level is mainly due to two bottlenecks: (i) the **growth level** and (ii) the **secretion efficiency.** Overcoming these problems would provide a bacterial chassis capable of producing recombinant protein of **high quality** and **high purity**.

Kewords
Synthetic biology, recombinant protein, secretion, Quorum-Sensing, surface-proteolysis

Good things come in small packages – delivery of vitamin K2 to human cells by extracellular vesicles from *Lactococcus cremoris*

Dr. Yue Liu, Eline Van Ophem, Anne Wiersma, Prof. Dr. Eddy Smid, Prof. Dr. Tjakko Abee

Number
165

Themes
Fermentation and Metabolism, including protein transition
Host Microbe Interactions

Abstract

Vitamin K2 is a lipophilic/hydrophobic vitamin accumulated in the membrane of certain bacteria species, and plays essential roles in human health as a carboxylation co-factor. However, the hydrophobicity of vitamin K2 forms poses challenges to their uptake by target cells of the human host to achieve desired biological function. In this study, lactic acid bacterium *Lactococcus cremoris* has been shown to secret bacterial extracellular membrane vesicles (EVs) that contain vitamin K2. When these EVs were applied to *in vitro* grown osteosarcoma cells, the carboxylation status of an important calcium-binding bone protein named osteocalcin, increased, indicating functional delivery of bioactive vitamin K2 by bacterial EVs. Notably, the efficiency of vitamin K2 delivery by EVs appeared higher than adding solvent-dissolved pure compounds at similar concentrations. Tests with pharmaceutical inhibitors also revealed that membrane fusion between *L. cremoris* EVs and human cells seemed to be the functional delivery route for EV-associated vitamin K2.

This study provides proof of principle that bacterial EVs are ideal vehicles to deliver lipophilic compounds like vitamin K2 to human cells. Research on EVs produced by bacteria that are key players in dairy/food fermentations, will promote the applications of bacterial EVs in efficient delivery of bioactive, nutritional compounds from the microbial origins to the human host, contributing to improved nutrition and conceivable health benefits.

Kewords
EVs, lipophilic vitamins, delivery vehicles, starter, health

CRISPR-Based Genome Editing in a Recalcitrant Bifidobacterium lactis Commercial Strain

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Number
167

Themes
Genetics and Genomics

Abstract
Bifidobacterium strains exhibit diverse health benefits, including infection prevention, irritable bowel syndrome alleviation, immune system enhancement, and antitumor effects, leading to commercial applications and formulations. However, challenges in manipulating bifidobacteria limit our ability to decipher the genetic basis for these effects, and their enhancement by engineering. This study aimed at generating knockouts in the recalcitrant

Bifidobacterium lactis Bl-04 using the endogenous Type I-G CRISPR-Cas system. To optimize transformation efficiency, we evaluated vectors with various replication origins and antibiotic resistance markers and tested diverse *Escherichia coli* strains (TG1, NEB 10-beta, and JM109) as intermediate plasmid hosts. CRISPR vectors encompassing a self-targeting spacer and flanking homologous arms were utilized for knockout generation. Results indicated that a vector backbone harboring the pBC1 origin of replication in combination with a chloramphenicol resistance marker enhanced transformation efficiency. Furthermore, *recA*+ *E. coli* strains (e.g., TG1) led to plasmid concatenation, hampering transformation efficiency. In addition, variation in targeting spacer efficiency was observed among different spacers. Notably, the use of 600bp flanking homologous arms facilitated efficient recombination without compromising transformation efficiency. In conclusion, the optimization of CRISPR-based genome editing vectors and the selection of suitable *E. coli* plasmid cloning hosts, along with considering methylation profiles, promoter design, homologous recombination efficiency, and scalable screening, are crucial factors for successful strain engineering. This approach provides a template framework for genome engineering in other non-model probiotic bacteria, unlocking opportunities for the development of next-generation probiotics.

Kewords
Bifidobacterium lactis, CRISPR, genome editing, transformation efficiency

Food matrix and disease prevention: *Lactiplantibacillus plantarum* fermentation as a tool to modulate bioactivity, digestibility and acceptability of pulse seeds

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Number
169

Themes
Fermentation and Metabolism, including protein transition

Abstract

The rise of noncommunicable diseases, driven by unhealthy diets, necessitates a reconsideration of food consumption as a preventive measure against diseases. Pulses, being rich in nutrients, fibers, and bioactive compounds, offer an ideal protein source for healthier and more sustainable diets. However, their consumption is hindered in Western countries due to low digestibility, off-flavours, and gastrointestinal discomfort.

In this study, we explored the potential of *Lactiplantibacillus plantarum* fermentation (0 to 72 hours) to enhance acceptability, digestibility, and bioactivity of pulses. The fermentation process significantly affected the seed-microstructure and led to de-glycosylation of phenolic compounds. We further examined peptides profile upon fermentation, *in-vitro* gastrointestinal digestion (INFOGEST), and transport across a Caco-2 cell monolayer by LC/MS/MS.

Fermentation of lentilf for 48-72 hours resulted in the formation of hydrophobic peptides and improved intestinal absorption. Moreover, exposure to fermented lentils led to over 30% increase in DPP-IV inhibitory activity in Caco-2 cells compared to unfermented lentils. Additionally, moderate fermentation of green lentils for 24-48 hours decreased the formation of flatulence-inducing raffinose-family oligosaccharides and improved the aroma profile by reducing grassy, beany off-flavours and forming pleasant aroma compounds.

Through the *ex-vivo* NuGUT continuous fermentation system, we observed that colonic fermentation of green lentils for 3 days increased the levels of beneficial commensal bacteria such as *Lactobacillus* spp. and *Roseburia* spp., which were retained when lentils were pre-fermented with *L. plantarum*.

Overall, our findings provide evidence that *L. plantarum* fermentation can effectively enhance the acceptability, digestibility, and bioactivity of pulses, encouraging their inclusion in daily diets.

Kewords

fermentation, L. plantarum, pulses, bioactivity, digestion, absorption

Isolation and typing of functional bacterial strains from fermented and unfermented complex plant-derived feed, food, and environmental samples.

Jolanda Lambert[1], Wim Engels[1], Marjo Starrenburg[1], Saskia Van Schalkwijk[1], Patrick Janssen[1], Eric Hester[1], Herwig Bachmann[1]
[1]*NIZO*

Number
171

Themes
Microbial Communities
Genetics and Genomics
Fermentation and Metabolism, including protein transition

Abstract

In a wide variety of traditional or novel plant based fermented food and probiotics applications, microorganisms such as lactic acid bacteria play an important role. Successful development of such applications therefore depends on the availability and functional properties of suitable microorganisms.

In this project, we investigated the microorganisms present in 127 complex fermented and unfermented plant-derived feed, food, and environmental samples, representing 95 different material types.

Determination of the bacterial composition of these samples by Illumina 16S amplicon sequencing revealed marked differences in the bacterial composition between fermented and unfermented substrates, while comprising 177 predicted genera in total.

Furthermore, 200 single strain isolates of the genera *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Pediococcus*, *Weissella*, and *Enterococcus* were obtained from the samples using various selective media, with 300 additional isolates expected from ongoing work. In addition, first assessments of functional properties such as bile and acid resistance of the isolates were performed using various screening methods. Currently, genome sequencing is ongoing; this information will be linked to functional properties through dedicated HMM modules developed previously.

In conclusion, the complex plant-derived samples and novel bacterial isolates obtained in this work can serve as a valuable source for screening and development of functional and potentially beneficial strains for food and probiotics applications.

Kewords

culture collection, probiotics development, upscaling, screening

TASTE OPTIMIZATION OF STEVIA EXTRACTS BY FERMENTATIVE MODIFICATION USING CO-CULTURES OF YEAST AND BACTERIA

MSc. Anna Zimmermann, Dr. Claudia Borgmeier, Dr. Esther Gabor, Dr. Guido Meurer

Number
173

Themes
Fermentation and Metabolism, including protein transition

Abstract
Stevia rebaudiana leaves have a sweet taste, which is based on the presence of steviol glycosides, that are 30 to 350 times sweeter than sucrose [1]. Due to their heat stability and lack of induction of glycaemic response, they are an attractive sugar substitute. However, applicability of water-based infusions with no further chemical processing is rather limited as they retain a bitter taste and a lingering liquorice aftertaste. Chemically extracted steviol glycosides with optional subsequent enzymatic modification for taste improvement are

therefore the current option of choice [2]. Especially the use of enzymes of recombinant origin, however, is met with scepticism by consumers [3].

In the present study, an alternative approach was pursued by applying co-cultures of bacteria and yeast for the fermentation of crude stevia infusion with the aim to transform an infusion with strong off-tastes into an ingredient delivering a clean sweetness profile in dairy products. We also addressed the occurrence of potential steviol glycoside modifications through microbial biotransformation. Via HPLC assessment. Surprisingly, no substantial changes in relative proportions of steviol glycosides were detected with the exception of specific increases identified for minor derivatives such as Rubusoside. Sensory evaluations on the other hand revealed substantial amelioration of the overall flavour profile in target beverages including low scores in artificialness and lingering as well as delivering cleaner sweetness and mouthfeel.

Kewords
Sweetener, Fermentation, Stevia, Co-cultures, Flavour, Dairy

Effect of divalent cations and carbohydrate concentration on phage-host interaction of dairy streptococci

Natalia Diaz-Garrido, Raphaela Joos, Ryan Cantwell, Douwe Van Sinderen, Jennifer Mahony

Number
175

Themes
Host Microbe Interactions
Bacteriophage and Antimicrobials

Abstract
Strains of *Streptococcus thermophilus* are widely exploited in thermophilic dairy fermentations where they contribute to the acidification, texture and/or flavour of products such as cheese and yoghurt. One the most persistent threats to consistent and successful fermentations are bacteriophages, which may infect the starter culture. In the present study,

we sought to evaluate the conditions under which phages of *S. thermophilus* infect their host bacteria optimally. To this end, we evaluated the impact of various divalent cations (calcium, magnesium and manganese) on plaquing efficiency, lysis-in-broth and host adsorption using representative members of four of the currently recognized five streptococcal phage groups. Despite its abundance in milk, our data suggests that calcium is not an explicit requirement for many of these phages. In the case of STP1 and SW16, calcium could readily be replaced by magnesium or manganese, indicating that these phages are more adaptable than SW13 and SW27 phages. Similarly, we evaluated the impact of varying concentrations of lactose on the plaquing and lysis efficiency of a selection of dairy streptococcal phages. This study confirms that the presence of divalent cations and the lactose concentration influence the phage infection process. This knowledge is critical to defining industrial processes to reduce the risk of phage infection and ensure product quality and consistency.

Kewords
Divalent Cations, Phage-Host interactions, dairy streptococcus

Nutritional requirements of the human vaginal isolate Lactobacillus crispatus in a chemically defined medium

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Number
177

Themes
Microbial Communities
Fermentation and Metabolism, including protein transition

Abstract
Lactobacillus crispatus is a gram-positive, homofermentative lactic-acid bacterium, commonly found in the human vagina (France, et al., 2022). *L. crispatus* dominance in the

vaginal microbiome is associated with reduced risks for unfavourable reproductive and sexual health outcomes, such as HIV infection (Gosmann, et al., 2017). In this study, the nutrient requirements and metabolic capabilities of *L. crispatus* have been studied by working towards a minimal chemically defined medium (CDM).

Omission experiments were performed for each component of the CDM with the human vaginal isolate *L*. *crispatus* RL10 (van der Veer et al., 2019). Growth and acidification were determined, by measuring the optical density at 600nm and pH in multi-well plates under anaerobic conditions.

L. crispatus RL10 proliferated on the CDM and acidified the medium to a pH around four. Magnesium, Tween80, potassium phosphate and some B-vitamins (riboflavin, nicotinic acid, Ca-(D)-pantothenate) were observed to be essential for growth. A purine and pyrimidine base or precursor were also required. The presence of adenine, despite the presence of other purines, especially improved the growth rate. Elimination of L-ascorbic acid, iron, uracil, guanine, thymidine, inosine, D-biotin, pyridoxine, alpha lipoic acid and thiamine in the redefined medium, resulted in the same optical density and acidification as on the complete CDM, albeit with a slower growth rate.

L. crispatus has a fastidious requirement for nutrients, suggesting to be highly dependent on the human host(ess). Understanding the need for certain nutrients adds to fundamental knowledge concerning commensal lactobacilli and it could aid in the development of therapeutics promoting their growth in the vaginal environment.

Kewords
Vaginal microbiome, Lactobacillus crispatus, nutrient requirements

Genome mining for genes related to bacteriophage resistance and its application

Thomas Janzen, Kosai Al-Nakeeb, Ditte Christiansen, Majken Bjerrum, Ronnie Machielsen

Number
179

Themes
Bacteriophage and Antimicrobials

Abstract
Infection by bacteriophages represents one of the main challenges during dairy fermentations. It is therefore an important task for producers of starter cultures to screen for lactic acid bacteria which are providing the best resistance towards infecting phages. For this we have developed a tool which enables the screening of bacteriophage resistance

related genes in genome sequenced strains of *Lactococcus lactis* and *Streptococcus thermophilus,* and consequent selection of promising candidates for food culture development. A database was established with more than 60 phage resistance related genes/gene clusters, including either

Genes coding directly for phage resistance like Abortive infection in *Lc. lactis* or CRISPR in *S. thermophilus (specific number of genes is used as quality criterion)* or

Genes coding for phage receptors like Pip in *Lc. lactis* or RGP or EPS in *S. thermophilus (absence and/or relevant mutations in these genes are the quality criteria)*

The tool was developed to detect absence/presence of genes, but also mutations within the genes leading to putative gene inactivation.

Approximately 2300 *Lc. lactis* and 1600 *S. thermophilus* genomes were analyzed for the presence/absence of specific gene clusters, respective mutations within genes essential for phage infection, and a statistical evaluation and rating was performed based on the analysis.

The outcome can be used to select strains for food culture development activities with a predicted superior phage resistance level and insensitivity towards our test phage collection.

Kewords
Streptococcus thermophilus, Lactococcus lactis, bacteriophages, phage resistance

Biodiversity of Bifidobacteria isolated from human microbiota in Inflammatory Bowel Diseases

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Number
181

Themes
Microbial Communities
Host Microbe Interactions

Abstract
Digestive inflammatory pathologies are a major public health issue with unclear etiology. Changes in the composition and functionality of the intestinal microbiota are associated with these pathologies, including the depletion of Bifidobacteria. We characterized the taxonomic diversity of bifidobacteria isolated from human intestinal microbiota at different stages of inflammatory bowel diseases (IBD) to address a possible correlation between the ecological origin of bifidobacteria strains and their taxonomy.

A total of 394 bifidobacteria were isolated from the intestinal microbiota of IBD patients (Crohn's disease and ulcerative colitis) and identified taxonomically by MALDI-TOF and whole genome sequencing. A particular high amount of *B. dentium* strains were isolated from microbiota of IBD patients (27% of isolated bifidobacteria). Other main species isolated were *B. adolescentis* (25%) and *B. longum* (17%). In ulcerative colitis, 39% of isolated bifidobacteria are *B. dentium* strains, with a majority of *B. dentium* isolated from patients in a remissive state of the pathology and 10% are *B. adolescentis*. In Crohn's disease, mainly *B. adolescentis* strains were isolated (31% of isolated bifidobacteria), particularly in patients in a remissive state. *B. dentium* strains were also high in Crohn's disease (23% of isolated bifidobacteria), but mainly in patients in an inflammatory state of the pathology. To conclude, this analysis allowed us to identify differences of bifidobacteria composition in gut microbiota in IBD patients.

High throughput methods are currently set up to characterize the functionality of all isolated bifidobacteria to address possible correlations between the functionality of isolated bifidobacteria and their origin.

Kewords
Bifidobacteria, microbiota, IBD

Establishing Genetic Tools for Industrial Lactic Acid Bacteria

Sailesh Malla, Jose Kiewiet, Anna Boguta, Paula Szymczak, Yixin Rong, Cedric Woudstra

Number
183

Themes
Microbial Communities
Genetics and Genomics

Abstract

We apply natural strain improvement approaches such as Adaptive Laboratory Evolution (ALE), direct selection strategies and mutagenesis and high-throughput selection for improving our industrial strains which are subsequently used to develop food cultures for application in various food-based products. However, establishing genetic engineering tools for industrial strains are equally important as those developed techniques can be applied to modify strains to gain further insight in their performance by conducting mode of action and in-depth characterization studies. Furthermore, the developed genetic tools can provide insights that help to guide next strain improvement strategies and support the protection and commercialization of our product strains.

Unlike laboratory model strains, genetic tool development for industrial strains can be highly challenging often demanding stepwise optimization for individual strains. As we have an extensive collection of lactic acid bacteria of which many have been industrialized, there is a strong drive for expansion of our toolbox for genetic engineering of industrial lactic acid bacteria. Besides continuously developing on the toolbox we are also improving the application of it to construct modified strains (expression, integration, deletion, introduction of point mutations, etc.), reporter strains and strain libraries.

Kewords
gene manipulation, GMO, Fluorescence reporter

Characterization of Lactobacillus-strains for vaginal women's health

Annalisa Visciglia, Marta Lo Re, Francesca Deidda, Carlotta Morazzoni, Angela Amoruso, Marco Pane

Number
187

Themes
Host Microbe Interactions

Abstract

Objective: Vaginal eubiosis is characterized by a beneficial lactobacillus-dominated microbiota. In contrast, vaginal dysbiosis, such as bacterial vaginosis and vulvovaginal candidiasis, is associated with an overgrowth of multiple pathogens, leading to increased risk of adverse urogenital and reproductive health outcomes. The aim of this work is to evaluate the antipathogen capability of four probiotic strains, *Lactobacillus acidophilus* LA02 (DSM 21717), *Lactobacillus crispatus* LCR04 (DSM 33487), *Limosilactobacillus fermentum* LF5 (DSM 32277) and *Limosilactobacillus fermentum* LF10 (DSM 19187), tested as viable and heat-treated strains.

Methods: Probiotic strains were initially discriminated through FTIR analysis by an IR Biotyper spectrometer (Bruker Optics-Daltonics GmbH) and phylogenetically classified through bioinformatic tools. Based on previous internal data demonstrating their potential antipathogen activity, the probiotic strains' inhibition of pathogens was tested by using two different eukaryotic models: VK2/E6E7 and 3D-model Reconstituted Human Vaginal Epithelium (RHVE). Pathogen survival and the effects of this competition on eukaryotic cells (including cell viability, damage, and cytokines response) were assessed. In the end, bioinformatic analyses of some genes related to vaginal wellness were performed.

Results: The probiotic strains, successfully characterized through FTIR analysis and phylogenetic classification, exhibited the capability to inhibit the growth of pathogens. Furthermore, both the viable and the heat-treated strains demonstrated significant cell protective effects.

Conclusions: This study enlightens the antipathogenic activity of selected probiotic lactobacilli, both in viable and heat-treated forms. These findings reveal the potential for these strains to serve as an excellent, non-invasive adjuvant therapy, and a potential prevention strategy for the treatment of vaginal infections.

Kewords

Probiotics, Vaginal microbiota, Heat-treatment, 3D model,

Assesment Of The Microbial Ecosystem Of The Greek PDO Cheese Anevato With Metagenomics

Dr Maria Govari, Dimitra Tsoliakou, Maria Gkerekou, Professor Panagiotis Skandamis, Professor John Kapolos, **Assistant Professor Konstantinos Papadimitriou**, Professor Marina Papadelli

Number

189

Themes
Microbial Communities
Genetics and Genomics

Abstract
Anevato is a PDO soft cheese produced in the Grevena region in Western Macedonia, Greece. It is made from sheep or goat milk or mixtures of them. The objective of this research was the estimation of the microbial populations of the Anevato cheese samples. The samples were provided by three different Anevato cheese producers, four by each producer. We performed culture-based microbiological analysis as well as shotgun metagenomics analysis for the characterization of the Anevato cheese microbiome. The main microbial populations found were lactic acid bacteria (LAB) like *Lactococcus lactis, Lactococcus raffinolactis, Streptococcus thermophilus, Lactobacillus helveticus, Streptococcus parauberis* and *Lactiplantibacillus plantarum*. Yeasts like *Kluyveromyces lactis* and *Saccharomyces cerevisiae* were also identified. In some samples *Enterobacteriaceae, Staphylococcus* spp., *Escherichia coli* and *Pseudomonas* spp. were also detected. Overall, Shotgun metagenomics were used for the characterization of the microbial ecosystem of Anevato cheese and the information obtained can be important for the understanding soft cheese production. Our analysis may also lead to the selection of novel starter cultures and the improvement of the quality of Anevato cheese.

This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call SUPPORT FOR REGIONAL EXCELLENCE (MIS 5047289).

Kewords
metagenomics,cheese, LAB, yeasts,cheese production, microbiome

Development of effective method to improve survival of *Lactobacillus paragasseri* OLL2716 in yogurt

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Number
191

Themes

Fermentation and Metabolism, including protein transition

Abstract

Lactobacillus paragasseri OLL2716 (LG21) has been reported to have suppressive effects on *Helicobater pylori* infection and to improve gastric conditions by clinical human trials. In these trials, subjects ingested yogurt containing viable LG21. Although the number of viable LG21 is the key factor for these functions, it has been confirmed that viable LG21 gradually decreases during cold storage of yogurt. The objective of this study is to develop a method to improve survival of LG21 in yogurt during cold storage.

Yogurt containing LG21 was manufactured by adding LG21 to ingredients together with *L. bulgaricus* and *S. thermophilus*, which mainly contribute to fermentation. We hypothesized that stress imposed on LG21 during fermentation might affect the decrease in survival rate during cold storage. To confirm this hypothesis, we changed the timing of LG21 addition from 'before yogurt fermentation' to 'late in yogurt fermentation'. As a result, we found a significant improvement of the survival rate of LG21 during cold storage by just changing the timing of LG21 addition.

In general, bacteria in the stationary phase are known to be more stress tolerant than those in the logarithmic growth phase. We suggest that LG21 added before yogurt fermentation is likely to shift from the stationary phase to the logarithmic growth phase, whereas LG21 added late in fermentation remains in the stationary phase during cold storage, which could contribute to the increased survival rate.

Kewords

Lactobacillus paragasseri, yogurt fermentation, survival, growth phase

Microbial diversity in table olive brines assessed through next generation sequencing and culture-based approaches

Anastasios Tsoungos, Dimitrios Pavlidis, Konstantinos Panousopoulos, Marina Papadelli, John Kapolos, Konstantinos Papadimitriou

Number

193

Themes
Microbial Communities

Abstract
Table olive fermentation has been extensively studied over the years and the evolution of the microbiota during the process has been well documented. However, the knowledge regarding the microbial diversity at the retail level is limited. In this work, a total of nine commercially produced brine samples from three cultivars, namely cv. Kalamata, cv. Konservolia, and cv. Halkidiki were obtained from different markets and analyzed for their microbiological and physicochemical properties. Lactic acid bacteria (LAB) and yeasts were enumerated and 10% of the colonies were randomly selected, isolated and purified. Rep-PCR and RAPD-PCR were used as molecular tools for typing and differentiation of the bacterial and yeast isolates, respectively. Further, total DNA was extracted directly from the olive brines and was subjected to shotgun metagenomics for the identification of the representative microorganisms at the species level. *Lactiplantibacillus pentosus* was the most abundant species in natural black cv. Kalamata and cv. Konservolia olives, while LAB were barely detected in green cv. Halkidiki olives that were dominated by yeasts. Microbial isolates were identified by MALDI-TOF/TOF, which confirmed the dominance of *Lactiplantibacillus pentosus* in the two natural black olive samples (cv. Kalamata & cv. Konservolia) and the scarcity of LAB in cv. Halkidiki green olives. The findings suggest that table olive brines available at retail have the potential to serve as a valuable source of microbial isolates for controlled fermentations.

Kewords
lactic acid bacteria, table olives, shotgun metagenomics

SkinbacTM Beauty: Exploring the Potential of Biotics in Skin Health

Giovanni Deusebio, Annalisa Visciglia, Annalisa Bissone, Daniele Zogno, Serena Allesina, Angela Amoruso, Marco Pane

Number
195

Themes
Host Microbe Interactions

Abstract

Objective: Recent evidence highlights the positive benefits of incorporating biotics as skincare ingredients to promote skin health. This study aims to comprehensively evaluate safety and efficacy of Skinbac™ strains *in vitro* and *in vivo*, to uncover their potential in promoting skin health.

Methods: Heat-treated strains, *L. plantarum* Skinbac™SB01 and *B. lactis* Skinbac™SB05, collectively known as Skinbac™ Beauty, were assessed. *In vitro* experiments were conducted using Normal Human Epidermal Keratinocytes (NHEK). Safety was determined by MTT and LDH assays, while efficacy was evaluated by measuring aquaporin3, claudin1 (CLND1) and occludin (OCLN) expression, the reduction of Reactive Oxygen species (ROS), the modulation of cytokines (e.g. TNF-α, IL-6, IL-8 and IL-23), and pathogen inhibition. Skinbac™ Beauty was then incorporated as unique active ingredient in a face cream, and its effects were studied *in vivo*, analyzing skin deep hydration, roughness, elasticity and density.

Results: i)*In vitro*: Skinbac™ Beauty effectively increased aquaporin3 (+22% vs untreated cells, $p<0.05$), CLDN1 (+9% vs damaged cells, $p<0.05$) and OCLN (+5% vs damaged cells) expression, reduced ROS levels (-32% vs untreated cells, $p<0.05$), inhibited growth and biofilm formation of *Staphylococcus aureus*, and modulated key cytokines TNF-α, IL-6, and IL-8.

ii)*In vivo*: Statistically significant improvements were observed in skin deep hydration, elasticity and density.

Conclusions: The results of this study demonstrate that Skinbac™ Beauty possesses beneficial properties for the skin, including the maintenance of barrier regulation and integrity, and moisturizing, antioxidant, and antipathogen effects. These findings suggest the potential of Skinbac™ Beauty in promoting overall skin health.

Kewords
Probiotics; Heat-Treated Strains; Skin Health; Skin Microbiome

Processing variations for mahewu, an indigenous LAB fermented maize beverage suggest use of sorghum and millets to replace maize

Mrs Sakile Kudita, dr.ir Sijmen Schoustra, Dr. Juliet Mubaiwa, prof.dr. Eddie Smid, Dr. Anna Alekseeva, dr.ir. Anita Linnemann

Number
197

Themes
Microbial Communities

Abstract
Lactic acid bacterial fermentation is a common traditional maize processing technology in Sub-Saharan Africa. However, the long-term sustainability of maize-based food systems is under threat from climate change, hence the increased interest in sorghum and millets as maize alternatives in popular local foods. This study surveys processing practice variation and resultant microbial community composition variations in mahewu, a traditional lactic acid fermented maize beverage from Zimbabwe, seeking if potential exists for replacing maize with sorghum and millet as base raw material. A cross-sectional survey with 124 respondent from five districts was conducted using focus group discussions and personal interviews; and mahewu samples collected from each respondent to profile microbial community composition (amplicon sequencing) and aroma profiles (GC-MS). Although Mahewu is produced using the same processing steps across the country, variation exists in the base ingredients used for the porridge cooking step. Maize was the most preferred although sorghum and pearl millet were also used. The study has shown that it is possible to substitute maize with sorghum or millets in the production of mahewu. The effects of such a substitution on the microbial composition of mahewu will soon be determined. Our study will inform both the scientific community as well a policy makers on avenues of climate resilient food system transformation and the potential role of LAB in enabling use of different raw materials for processing of traditional cereal based fermented foods.

Kewords
Microbial communities, spontaneous fermentation, cereals, climate change

The sweet-cold spot: Impact of Sugars, Temperature and Bacteriocins on the Production of Exopolysaccharides (EPS) by sausage spoilage *Leuconostoc mesenteroides*

Miguel Fernandez de Ullivarri, Anala Gopalakrishna Bhat, Sterre De Vries, Sanjana Laobangdisa, Lorraine Draper, Matthew McCusker, Janneke Wijman, Eelco Heintz, Colin Hill, Saurabh Kumar

Number

199

Themes

Fermentation and Metabolism, including protein transition
Bacteriophage and Antimicrobials
Genetics and Genomics

Abstract

Exopolysaccharides (EPS) synthesis by *Leuconostoc mesenteroides* in meat products can result in undesirable textural changes, leading to compromised sensory attributes and reduced consumer acceptability by imparting a slimy texture to processed meats, altering their appearance and mouthfeel. we studied the influence of nutritional, physicochemical and antimicrobial factors on EPS/slime production by *L. mesenteroides* isolates from industrial meat sausages.

Four isolates were studied: KS273, KS279 (High EPS producers), KS276 and KS277 (Low EPS producers). The impact of Temperature (8-37 °C), Sugar type (glucose, sucrose, fructose) and sugar concentration (0-5%) in M17 medium on EPS production was evaluated, as well as the impact of Nisin A and Lacticin-3147 on EPS/Slime production.

The coordinates for optimal and minimal EPS production were the same for all the isolates, being (Sucrose; 5%; 8°C) and (No Sugar; 0%; 37 °C), respectively. In agreement with previous reports, EPS production was positively correlated to sugar concentration, and sucrose was more triggering than glucose or fructose. In contrast to previous studies, low temperature (8 °C) was a key triggering factor for EPS production by the four strains, inducing KS273 and KS279 to produce it even without added sugar. Regarding bacteriocins effect, both Nisin and Lacticin delayed the bacterial growth and the slimy phenotype of all isolates by 3-5 days, compared to untreated samples.

These results indicate that sugar type, concentration, temperature and addition of bacteriocins are relevant factors that could help to modulate the EPS production and reduce the slimy spoilage of meat products by *L. mesenteroides.*

Kewords

Leuconostoc, Sausage, Exopolysaccharides, Slime, Sugar, Temperature, Bacteriocins

Microbial diversity in lactic acid bacteria coming from traditional animal rennets

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Number
201

Themes
Microbial Communities

Abstract
Several traditional cheeses in the Peloponnese region of Greece are still produced by the addition of animal rennets to the milk. Apart from active enzymes, this kind of rennets may transfer lactic acid bacteria (LAB) to milk. The aim of the study was to assess the microbial ecology of different animal rennets used to produce cheeses in industrial and/or domestic scale. The rennets were analyzed microbiologically for mesophilic and thermophilic rods and cocci in MRS and M17 agar plates, respectively. From each sample 20 random colonies were isolated and identified through MALDI-TOF/TOF. The MRS counts ranged from <1.0 to 6.50 logCFU/g, while the counts on M17 were always lower, across all samples. Species identification highlighted heterogenicity among the different rennets. *Enterococcus faecium, Lactiplantibacillus plantarum, Levilactobacillus brevis* were isolated from almost all the samples. From traditional rennets utilized for industrial cheese production *Lactiplantibacillus paraplantarum, Streptococcus macedonicus*, *and Leuconostoc mesenteroides*, were frequently isolated. Unique species in rennet samples were *Lactobacillus delbrueckii* subsp. *lactis*, *Latilactobacillus curvatus*, *Weisella cibaria and Weisella confuse,* and *Enterococcus faecalis.* Domestically prepared rennets used in the production of homemade cheeses, exhibited less LAB species diversity probably because a single stomach was utilized for rennet preparation. They were characterized by *Pediococcus pentosaceus,* and *Limosilactobacillus reuteri*. The findings are promising to assess the isolates for their potential technological traits.

This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call SUPPORT FOR REGIONAL EXCELLENCE (MIS 5047289).

Kewords
metagenomics, rennet, traditional, cheese, coagulation, milk

Amino acid decarboxylation by *Paucilactobacillus wasatchensis* as a potential cause of gas defects in aged Cheddar cheese

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Number
203

Themes
Microbial Communities
Fermentation and Metabolism, including protein transition

Abstract
Paucilactobacillus wasatchensis (WDC04) is a non-starter lactic acid bacteria that is linked to late gas defects in Cheddar cheese. Recent research has shown that this organism has the capability to produce CO_2 from a 6-carbon sugar. However, WDC04 has still been known to produce splits and cracks in Cheddar cheese in the absence of these sugars. In cheese production trials, more CO_2 is released than can be accounted for by added carbon substrates. One possible source of gas production could be the decarboxylation of free amino acids with the formation of biogenic amines. Putrescine and cadaverine, the decarboxylation products of arginine/ornithine and lysine respectively, have been detected in cheese inoculated with WDC04 and genes for this conversion have been found in the genome. To test for gas production, 100-700 mM of lysine, arginine or ornithine was added to carbohydrate restricted MRS containing 2% Oxyrase and inoculated with WDCO4 and incubated at 30°C. Growth curves were measuring by turbidity over 72 h and gas production using Durham tubes. Growth and gas production was only seen in samples containing ornithine and lysine with the addition of ribose (1%). The only source of ornithine in cheese is from the conversion of arginine using the ADI pathway from the microorganisms present. A common Cheddar cheese starter, *Lactococcus lactis*, has the ability to convert arginine to ornithine under carbohydrate starvation and further culture-based analysis is currently being conducted to determine if the starter culture contributes to gas production by *Pa. wasatchensis* WDC04.

Kewords
Non-starter LAB, Cheddar Cheese, Gas Defects

Metagenomic profiling of microbial diversity in plant-based meat alternative products

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Number
205

Themes
Microbial Communities

Abstract
Plant-based meat alternatives are popular due to their health benefits, ethical considerations and environmental sustainability. These products are designed to mimic the taste, texture, and appearance of traditional meat but are made from plant proteins such as soy, peas, wheat gluten and mycoprotein derived from filamentous fungi Fusarium venenatum. Some products undergo a fermentation process with specific microbes which enhances the product preservation, flavours and nutritional value. Metagenomic approach helps to characterize the microbial diversity and identify dominant microbial taxa, detect pathogens and explore metabolic capabilities. Understanding the microbiome is critical for maintaining product quality and safety during storage.

16S and ITS metagenomics focuses on sequencing and analysing the rRNA gene, a highly conserved region in the genome of bacteria and fungi respectively. The workflow of metagenomics typically involves DNA extraction, amplification of rRNA gene by using V3 and V4 primers for bacteria and ITS1 and ITS2 primers for fungi, sequencing libraries are prepared by incorporating sample specific barcodes and adaptors into the PCR amplicons, the libraries are sequenced using Illumina MiSeq and subsequent bioinformatics analysis to classify the taxonomic composition of microbial community.

Bacterial taxa such as Paeniglutamicibacter, Glutamicibacter, Agrilactobacillus, Latilactobacillus, Lactiplantibacillus and Corynebacterium were dominant taxa found in products indicating the presence of diversity of microbial community. Metagenomics is a culture independent approach which provides a holistic view of the microbial community by capturing the entire genetic material present in the sample. It allows better understanding and identification of microbes at species or even strain level.

Kewords
Metagenomics, plant-based meat alternatives, bacterial fungal community

Unintended fermentation in maize ogi production technology: the impact of technological variations

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Number
207

Themes
Microbial Communities
Fermentation and Metabolism, including protein transition

Abstract
Traditional food fermentation can result in products of variable characteristics due to uncontrolled production conditions and variations in processing technology. Our study investigated how fermented maize starch called "*ogi*" is traditionally produced using different technologies, for the preparation of "*akpan"*, a yogurt-like street food widely consumed in Benin. Among the steps that differentiate the processing technologies and may affect *ogi* characteristics, the steeping duration of maize grains after heat treatment was critical, as it significantly impacted, simultaneously, the physicochemical properties, microbial counts and microbial composition of maize starch. Combining the variations in steeping duration with the kneading of ground maize before filtration or not, groups of *ogi* processing technology were found for which starch samples of very low pH values (3.2 - 4.0), high lactic acid content (6.6 - 11.8 g/L) and ethanol (1.2 - 1.8 g/L) were obtained already before the actual fermentation step generally applied and known to turn maize starch into *ogi*. These groups of technology applied a common steeping time of 12 - 24 h and kneading before filtration, or a long steeping time of 44 – 72 h with or without a kneading. Results suggest a hidden fermentation in the aforementioned processing technologies, while the intentional fermentation later induced similar organic acids and ethanol contents in *ogi* samples, but variable free amino acids, volatile organic compounds as well as fungal composition. Understanding how this non-intentional fermentation take place request attention for an effective control of the fermentation step in *ogi* production to obtain desirable metabolites.

Kewords
Starch, processing technology, lactic fermentation, microbiota, metabolites

Leuconostoc spp. in the rind of soft smear cheese: a contribution to food safety

Alexandra Roetschi, Florian Gschwend, **Emmanuelle Arias-Roth**

Number
209

Themes
Microbial Communities

Abstract
The association between hypervirulent *Listeria monocytogenes* and dairy products has been demonstrated recently. Smear cheese types in particular support the growth of *Listeria monocytogenes* in the rind. In this study, we investigated the inhibitory properties of the microflora of a PDO artisan soft smear cheese produced from thermized milk.

Fresh cheeses produced in nine dairies were collected and inoculated on their surface with *Listeria innocua*, a surrogate of the pathogenic species. Rind samples were monitored for *Listeria* counts. Rind microbiomes were analyzed by amplicon sequencing and gave a total 567 ASVs assigned to four phyla, 64 genera and 106 species. In particular, *Leuconostoc* spp. were negatively correlated to *Listeria* counts throughout ripening. Inhibition of *Listeria* was observed *in vitro* for 5 out of 10 *Leuconostoc mesenteroides* strains isolated from the cheese rind. This inhibition was associated with plasmid encoded mesentericin Y genes in the genomes of the strains. Challenge tests were conducted with a subset of mesY positive *Leuconostoc* strains inoculated in the vat milk together with the starter and 0.5 cfu/ml *Listeria*. Efficacy of treatments was evaluated based on a qualitative detection in 25g rind at the end of shelf life. Addition of mesY positive *Leuconostoc* had a significant impact *in situ*, with 100% positive loaves for the control and 25% positive loaves for the *Leuconostoc* treatment. Next to the putative production of mesentericin Y, monitoring of biochemical parameters suggests that the co-metabolism of residual glucose and citrate by *Leuconostoc* is likely to have contributed to the protective effect.

Kewords
Listeria monocytogenes, smear cheese, amplicon sequencing, Leuconostoc

Combining *Oenococcus oeni* and *Lactobacillus plantarum* for malolactic fermentation in red wine

Emilie Sloth, Gregoire Boullion, Denise Felix da Silva, Rikke Dollerup Bech, Isabella Westergaard

Number
211

Abstract

The lactic acid bacteria (LAB) species *Oenococcus oeni* is the dominant species highly adapted to perform malolactic fermentation (MLF) in a wine environment. However, other LAB species can also be present and perform MLF such as the species *Lactobacillus plantarum.* Even though these species have adapted to sustain the stressful conditions of the wine environment, namely low pH, high ethanol concentration and presence of sulphur dioxide (SO_2), direct inoculation of starter cultures can result in either slow or failed MLF. This challenge highlights the need for robust starter culture solutions to ensure a stable vinification process.

This study aimed to investigate the effect of a combined solution, composed of *O. oeni* and *Lb. plantarum* strains, on the MLF rate for red wine production. Two experimental designs were performed where single starter culture solutions were compared to combined solutions under various inoculation schemes. The progression of MLF was followed using enzymatic assay kits.

In a reverse-inoculation scheme, where the bacterial culture is added 24 hours prior to addition of *Saccharomyces cerevisiae*, the MLF rate was markedly improved with the combined culture solutions, of *Lb. plantarum* (Viniflora® NoVA) with either *O. oeni* (Viniflora® CH11, Viniflora® CH16 and Viniflora® Oenos) compared to the single strain starter cultures. After 6 days of fermentation, the L-malic acid content was measured at 1.12 g/L for a combined culture compared to ~1.80 g/L and 1.34 g/L for Viniflora® CH11, Viniflora® CH16, Viniflora® Oenos and Viniflora® NoVA, respectively. The results suggest that depending on the inoculation protocol, a combined starter culture solution could result in a more robust MLF compared to a single strain culture.