Preface to the 11th Symposium on Lactic Acid Bacteria
Egmond aan Zee, The Netherlands
August 31 to September 04, 2014

A warm welcome to Egmond aan Zee for the 11th Symposium on Lactic Acid Bacteria. The theme of this meeting is “Embarking on 30 more years of LAB research” and that is exactly what this Symposium intends to do: to enthuse especially the younger generation of LAB researchers to keep up the excellent work performed in the field. Looking back 30 years [YES, indeed, although we celebrated 30 years of LAB research at LAB10, in fact this meeting is exactly 30 years after LAB1 ;-)] to when this series of Symposia started, it is incredible how much progress the field has made and one can only look forward and wonder what the LAB science will look like 30 years from now. Hopefully, many of the young scientists who are here now (for the first or even already for a second time) will be there and will have been part of the exciting developments ahead and will have shaped that future. It is this incentive that started the series of LAB Symposia in the first place and that we will maintain in future LAB Symposia.

In this Poster Abstract Book you will find well over 300 poster abstracts, of which over 20 have been selected for short oral presentations in the plenary part of the meeting as well as during 2 Thematic Sessions. Several more will be presented in poster flashes. It is the LAB11 Organising Committee’s firm belief that the importance and viability of the field of LAB research, both the fundamental and the application-oriented research, are reflected in these posters, which are the first indicators of how the field will develop.

Because of their importance in allowing researchers to communicate directly on exciting new findings, concepts and ideas and to contact colleagues with different specializations, all of which are paramount to scientific development, the posters will be on display during the whole Symposium.

We really hope you will enjoy the meeting and are looking forward to hosting and meeting you at LAB11!

On behalf of the LAB11 Organising Committee,

Jan Kok

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Egmond aan Zee, The Netherlands, August 31 to September 4, 2014

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SL 01

Comparative Genomics of *Bifidobacterium thermophilum* RBL67 reveals novel potential colonization factors

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The genome of *Bifidobacterium thermophilum* RBL67 contains 1845 CDSs. A MegaBLAST of the CDSs against 46 bifidobacterial genomes revealed 162 CDSs unique for RBL67. Most homologous (86% of the CDSs) were found in *B. thermacidophilum*, corroborating the close relationship between the two bifidobacteria. The percentage of homologous found in other bifidobacterial ranged from 61 to 70%. 677 CDS were found in all bifidobacterial strains, potentially representing the core genome of the genus.
A presence-absence matrix of genes was constructed and subsequently clustered. This allowed identification of genes with a similar occurrence in the genus *Bifidobacterium.* Genes encoding for pili formation were present in all strains, but 6 strains harbored a second set of pili-genes. Remarkably, these genes are encoded in four distinct loci of the RBL67 genome, although they have a clear functional and phylogenetic relationship. Further an Internalin-like protein was found in *B. breve*, *B. angulatum*, *B gallicum*, and RBL67. Internalins are listerial proteins that bind to human E-cadherin and the presence of such proteins in bifidobacteria suggests involvement in adhesion. The Internalin-like proteins contained a highly conserved C-terminal of 130 amino acids. This C-terminus is also found in proteins of RBL67, *B. breve* and in enterococci, streptococci, and lactococci. Most of the proteins possessing this C-terminus are uncharacterized, but some have domains that are involved in the extracellular biology of bacteria, like a murein transglycosylase, a transpeptidase, and the already mentioned internalin. This suggests that the C-terminus is involved in the extracellular biology. Extracellular expression of the RBL67 proteins in *Lactococcus lactis* resulted in irreversible clumping of cells, suggesting that the conserved C-terminus is a cell walls binding domain.

SL 02

Genomic analysis of 80 *Oenococcus oeni* strains and connecting the genome with winemaking properties

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Oenococcus oeni is the main member of the lactic acid bacteria family used for malolactic fermentation (MLF) during winemaking. This bacterium is well adapted to harsh wine conditions including low pH, high ethanol concentrations, and limited nutrient availability. MLF is conducted in all red, and numerous white and sparkling base wines; its principal role being to reduce acidity and augment wine aroma and flavours.
Previously we have sequenced 11 *O. oeni* strains, to bring the total number of sequenced strains to 14. Analysis of these genome sequences showed that there was a distinct core genome, shared by all *O. oeni* strains, and highlighted that the scope of genetic variation between strains (strain specific sequences) was about 33% of the genome. In terms of relatedness, two of the strains differed considerably from the rest, and this was found to concur with previous MLST typing studies. In addition, there was found to be a very close evolutionary relationship between the strains isolated from Australian spontaneous wine fermentations.
In order to better understand the extent of genetic diversity within *O. oeni* a further 80 strains have been sequenced. Analysis of relatedness of the 94 sequences showed three major clusters of genetically related strains; the most distant cluster, previously not observed, included a group of seven strains.
A subset of the 80 sequenced *O. oeni* strains is being phenotyped for tolerance to wine stresses (e.g. high ethanol concentration, low pH and low temperature) in red and white wines. Data from this work will be mapped to the genomic datasets to identify genetic determinants of wine relevant traits.

SL 03

Targeted deletions using CRISPR-Cas via homology-directed repair

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Genetic tools have been developed in lactic acid bacteria for functional analysis of key genes conferring probiotic or starter culture activity. Among these are transposition/insertion element mutagenesis, plasmid integration by conditional replication, gene replacement via homologous recombination, and recombineering. Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR associated genes (cas) encode an RNA-guided bacterial immune system that targets DNA for sequence-specific nucleolysis. CRISPR-Cas systems have been broadly applied for genome editing in model eukaryotic systems but few bacterial species. Here, we present a novel genome editing method that employs CRISPR-Cas chromosomal self-targeting in combination with homology directed repair. We show that the active and orthogonal CRISPR1 and CRISPR3 systems in *Streptococcus thermophilus* can be used for targeting the *lacZ* locus. Guidance of each Cas9 endonuclease to induce a double stranded DNA break (DSB) within *lacZ* was accomplished through plasmid-based expression of CRISPR arrays containing a 30 bp sequence with identity to the N-terminus of *lacZ*. A linear homologous template identical to regions flanking the target site, but deficient in the features needed for Cas9 cleavage was co-transformed with the targeting plasmids to perform genome surgery. Double-crossover events resulting in a designed 65-bp frameshift deletion were observed at high frequency in clones which employed the template for repair of chromosomal injury following a DSB. Mutation of the *lacZ* gene resulted in loss of β-galactosidase activity. Therefore, chromosomal targeting by Cas9 followed by homology directed repair was effectively and efficiently exploited for designed genome editing in *S. thermophilus*, without the need for plasmid integration, counterselection, or extensive screening. This open new avenues for use of CRISPR-Cas9 systems as platforms for high throughput functional genomics in bacteria.

SL 04

Flavor development in cheese: a food-omics approach

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This study aimed to investigate the performance of starter cultures with respect to flavor and taste development in different cheeses and during ripening to provide leads for future product development. During ripening fat, proteins and lactose are metabolized which has a direct effect on the taste, flavor and texture of cheese. Different compound classes such as amino-acids and small peptides, aldehydes, sulphur compounds, volatile acids, sugars, lipids and alcohols are important for flavor and taste development and their concentration varies from cheese to cheese and during ripening. To cover the broadest possible range of compound classes an untargeted food-omics based analytical platform was developed, combining three different and complementary analytical methods to analyze the volatile and non-volatile metabolites that may be related to the flavor and taste of cheese. The non-volatiles were analyzed by NMR spectroscopy to determine a complete chemical fingerprint, and a GC-FID/MS method in combination with oximation and silylation. All volatiles were analyzed by an SPME-GC-FID/MS method. All cheeses were also examined by a trained sensory panel and multivariate data-analysis was used to model the mouthfeel, flavor and odor attributes as a function of the relative metabolite concentrations. This poster focuses on the development of flavor and taste in different cheeses during ripening, and on the identification of metabolites that discriminate the cheeses in terms of mouth feel, flavor and odor.

SL 05

***In vivo* imaging of infrared fluorescent protein-expressing lactic acid bacteria in mice**

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Lactic acid bacteria (LAB) are administered orally as probiotics or recombinant protein delivery vehicles with an aim to elicit beneficial health effects.The knowledge of interactions of bacteria with intestinal tissues, bacterial passage through the gastrointestinal tract and potential transfer of bacteria to other organ systems is important in explaining the mechanisms of action of probiotics. Such studies usually require sacrifice of animals at designated time points. *In vivo* imaging represents an attractive noninvasive alternative which enables continuous monitoring of LAB in living animals. In vivo imaging has already been demonstrated by the use of luminescence-based approach.We have shown that in vivo imaging can also be performed with the use of fluorescence in infrared part of the spectrum. We have prepared a codon-optimized gene for infrared fluorescent protein (iRFP) and expressed it in *Lactococcus lactis*, *Lactobacillus plantarum* and *Escherichia coli*. Functional expression of iRFP was achieved by the addition of biliverdin or hemin to the growth medium. We have optimized iRFP expression conditions and confirmed its spectral properties. Healthy mice were orally administered with different concentrations of iRFP-expressing LAB and imaged with Ivis Spectrum *in vivo* imaging system.The comparison of intestinal localization and persistence of different bacteria was made in order to optimize the method.We have demonstrated the suitability of iRFP for *in vivo* fluorescent imaging of lactic acid bacteria. We have also confirmed the superiority of iRFP as a reporter protein for lactic acid bacteria that enables sensitive measurement of fluorescence directly in the bacterial culture.

SL 06

The transcriptome landscape of *Lactococcus lactis* MG1363: regulatory RNAs uncovered

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RNA sequencing has recently revolutionized transcriptome analyses. As a result, novel non-coding RNAs have been discovered in several organisms and subsequent studies have revealed an increasing number of new regulatory mechanisms at the level of RNA. Here we present the transcriptome map of *Lactococcus lactis* MG1363 using directional RNA-seq on a 5'-enriched cDNA pool. This genome-wide map shows transcription start sites and operon structures of previously annotated genes. In addition, 67 new ORFs were uncovered and the annotation of 20 genes was corrected. The data was manually mined for novel non-coding RNAs that potentially play a regulatory role in *L. lactis.* Nearly 200 *cis*- and *trans*- encoded RNAs, and 113 putative riboswitches were identified using this approach. During starter culture production and fermentation, *L. lactis* is exposed to a constantly changing environment. Fluctuations in temperature, osmolarity, pH and nutrient availability cause significant stress to cells. Previous studies using DNA microarray and proteomics technologies have identified genes and proteins involved in the various environmental stress responses in *L. lactis.* In this study we used directional RNA-seq to uncover stress-specific regulatory RNAs after a short period of exposure to a variety of different stressors.
The role of a non-coding RNA located in the 3'UTR of ArgR, the regulator of e.g. the *arc*-operon in *L. lactis*, is currently being examined. This small RNA, called ArgX, shares a homology with the Shine Delgarno sequence of *arcC1.* Results on this potential dual repressor *argRX* will be presented.

SL 07

Cyclic-di-AMP levels are controlled by GdpP and DacA and affect salt stress resistance in Lactococcus.
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Lactococcus lactis is a commonly used starter culture bacterium in cheese making and therefore experiences a number of different stressors (e.g. heat, osmotic, acid) affecting cell growth and survival. In previous work we found that high temperature incubation of *Lactococcus* leads to the formation of spontaneous mutations in the *gdpP* gene which leads to heat resistance and in some cases salt hypersensitivity. The *gdpP* gene (also known as *yybT* and *llmg_1816*) encodes a membrane bound protein cyclic-di-AMP (c-di-AMP) phosphodiesterase and therefore down-regulates the level of the stress signaling molecule c-di-AMP. In a screen for salt resistant suppressors of the *gdpP* mutant, we found destructive mutations in the *dacA* gene, encoding the membrane bound c-di-AMP synthase, were very common. Over 40 independent nonsynonymous mutations, frameshift mutations or DNA insertions in the *dacA* gene (*llmg_0448*) were identified. Nonsynonymous mutations were found in and near the three predicted N-terminal transmembrane helices and also in the enzymatic domain. Levels of c-di-AMP in the *gdpP* mutant were significantly higher than the *gdpP/dacA* suppressor double mutants but were undetectable in wild-type. Autolysis of the *gdpP* mutant was slower than wild-type while the *gdpP*/*dacA* mutant autolysed faster, indicating changes in cell wall structure. From our results it can be concluded that the GdpP and DacA proteins regulate levels of the signaling molecule c-di-AMP which in turn regulates salt resistance in *L. lactis.*

SL 08

Genetic determinants of reutericyclin biosynthesis in *Lactobacillus reuteri*
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Reutericyclin is a unique tetramic acid derivative produced by *Lactobacillus reuteri* (1, 2). Reutericyclin is bactericidal against most gram-positive bacteria, including *Clostridium difficile* (3). This study aimed to determine the genetic determinants of reutericyclin biosynthesis. Comparative genomics of reutericyclin-producing strains with other *L. reuteri* identified a genomic island with a GC content 5 - 10 % lower than the average of *L. reuteri.* The 14 ORFs include genes coding for a nonribosomal peptide synthetase (NRPS), a polyketide synthase (PKS), homologues of *phlABC*, and putative transport and regulatory proteins. RtcNRPS is composed of a condensation domain, an adenylation domain likely specific for D-leucine, and a thiolation domain. RtcPKS is composed of a ketosynthase domain, an acyl-carrier protein domain, and a thioesterase domain. The products of *rtcPhlABC* may acetylate the tetramic acid moiety produced by RtcNRPS and RtcPKS to form reutericyclin. Mutants with deletions in *rtcNRPS* or *rtcPhlABC* did not produce reutericyclin but remained reutericyclin-resistant. Deletion of genes for transport and regulatory proteins eliminated reutericyclin resistance. Homologues of RtcNRPS and RtcPKS are found in *Bacillus* and homologues of RtcPhlABC are found in *Pseudomonas.* However, the combination of the two is exclusive to *Streptococcus mutans*, *L. plantarum* and *L. reuteri*, indicating that the genes in these organisms share a common evolutionary origin. *S. mutans*, *L. reuteri*, and *L. plantarum* colonize the upper intestinal tract of animals (1, 4, 5). The occurrence of the biosynthetic genes in these organisms suggests that reutericyclin and related compounds might contribute to their competitiveness in those habitats.
(1) ISME J 4:377. (2) Appl Microbiol Biotechnol 64:326. (3) J Antimicrob Chemother 66:1773 (4) Arch Oral Biol 20: 171 (5) Int Dairy J 16: 1018

SL 09

Quantitative analysis of growth rate dependent energy metabolism in lactic acid bacterium

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Fluctuating environmental conditions cause alterations in the specific consumption rate of substrates which determine the maximal energy production of a microorganism. Current study was dedicated to unravel the molecular mechanisms behind previously observed 30% increase in growth efficiency of *Lactococcus lactis* with the fivefold increase in specific growth rate (μ). Therefore, μ dependent steady state data for absolute proteome and transcriptome together with intra- and extracellular metabolic fluxes were analyzed using metabolic modeling. Additionally, protein turnover rates for individual proteins were measured at steady state conditions for two specific growth rates and energetic burden was calculated.
In the background of decreased consumption of amino acids and more homolactic fermentation pattern, minor changes, mainly increase of translation apparatus, were observed in quantitative proteome with the increase of μ. Higher metabolic fluxes at higher μ values were achieved by an average 3.5-fold increase in apparent catalytic activities of enzymes and ribosomes (μ 0.5 vs 0.1 h^1), indicating prevalently post-translational regulation of cells. Individual protein turnover rates were measured for approximately 75% of the total proteome. On average, protein turnover increased by sevenfold with the fivefold increase in μ.We found that protein turnover costs alone account for 38% and 47% of the total energy produced at μ 0.1 and 0. h^1, respectively, and gene ontology groups Energy metabolism and Translation dominated synthesis costs at both growth rates studied. These results reflect the complexity of metabolic changes that occur in response to changes in environmental conditions and signify the trade-off between biomass yield and the need to produce ATP for maintenance processes.

SL 10

***Lactobacillus helveticus* bacteriophages portrait with an in depth look at the phage resistance**

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Physiological and genetic studies of phages attacking lactic acid bacteria are accumulating in the last decades because phage infections are still a cause of serious economic losses for the dairy industry. We report the essential data on an extensive characterization of phages of *Lactobacillus helveticus*, an emerging probiotic, long and widely used in dairy fermentation. Twenty-one *L. helveticus* bacteriophages, isolated from different cheese whey starters, were phenotypically and genetically characterized. A high biodiversity was evidenced both by host range tests and molecular (RAPD-PCR, restriction enzyme analysis) typing. One-step growth curve experiments evidenced that phages were active and virulent. Thecomplete genomic sequence of the dairy *L. helveticus* ΦAQ113 bacteriophage was determined. ΦAQ113 showed a genome structure composed of classical modules for DNA replication/regulation, DNA packaging, head and tail morphogenesis, cell lysis and lysogeny. Additionally, ΦAQ113 displayed phylogenetic similarities with two phages that belong to the gut species *Lactobacillus gasseri* and *Lactobacillus johnsonii*, supporting the increasing consideration of *L. helveticus* as health-promoting organism. Considering the arising probiotic potential of this species, host resistance mechanisms against phages were studied, by comparing the genome sequences of the phage sensitive strain CNRZ 892 and a resistant derivative. Genetic and microbiological tests were performed and suggested the presence of two possible phage resistance mechanisms, i.e. the inhibition of adsorption and the Type I restriction/modification system. Genetic determinants of CRISPR systems, which are involved in phage resistance of other species, were searched and sequenced in 18 *L. helveticus* strains. Thirty-four CRISPR loci were identified and classified on the basis of repeats family and Cas proteins. Physiological tests, however, ruled out the involvement of CRISPR arrays in the resistance to phages.

SL 11

Sodium reduction of bread through glutamate conversion in sourdough fermentation by *L. reuteri*

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NaCl contributes to the taste and the texture of bread; therefore, reduction of NaCl in bread is challenging. In other foods, umami-tasting glutamate allowed salt reduction without compromising the taste (1). However, the effect of glutamate on the taste of bread is unclear. This study investigated the effect of sourdough on the taste of bread. Sourdough was fermented with strains of *L. reuteri* accumulating glutamate or g-amino-butyrate (GABA) as alternative end products of glutamine metabolism. Sourdough was also fermented with GABA-accumulating *L. reuteri* 100-23 and the glutamate-accumulating mutant *L. reuteri* 100-23D *gadB* (2). Wheat bread was produced with 6% sourdough and 1 and 2% NaCl, respectively. A consumer panel (n=40) ranked the salty taste of sourdough bread produced with *L. reuteri* equal to reference bread with 1.5% NaCl but higher than reference bread with 1.0% NaCl. Bread crumb produced with *L. reuteri* 100-23 or 100-23D *gadB* was differentiated by a consumer panel (n=40) in a triangle test. The taste profile of the bread crumb with 1 or 2% NaCl was also evaluated by a trained panel (n=9). Sourdough bread tasted more umami but not more salty than reference bread. The difference in glutamate concentrations between sourdough breads were equal to its taste threshold (0.03%), nevertheless, the taste difference between *L. reuteri* 100-23 and 100-23D *gadB* was attributed to umami. Volume and texture of the sourdough bread were comparable to reference bread. In conclusion, the use of sourdough fermented with glutamate-accumulating lactobacilli allowed reduction of NaCl without adverse effect on other taste- or quality attributes.

(1) J Food Sci 49:82 (2) Microb Cell Fact10, S8 (Presentation at LAB10)

SL 12

Nutraceutical potential of *Lactobacillus reuteri*: selenoprotein release demonstrated by a comprehensive proteomic study

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Selenium (Se) is an essential dietary trace element for humans and microorganisms. It is called “essential toxin” since it is fundamental at low concentration but toxic at higher levels. Se assumption depends on diet supply and its deficiency is associated with various chronic diseases such as oxidative stress, cardiovascular diseases and some kinds of cancer. It generally may be considered an anticarcinogenic and antioxidant agent. The use of Se-enriched lactobacilli is an innovative approach to counteract selenium-deficiency: microorganisms, grown in presence of inorganic (toxic) forms of selenium, are able to convert it into more bio-available organic forms, introducing it into proteins, called selenoproteins.

In this study a comparative proteomic approach has been used on different cellular districts (*in toto* proteome, envelope enriched fraction, extracellular proteome) and pI ranges (4-7 and 6-11) of the probiotic *Lactobacillus reuteri* Lb2 BM DSM 16143. Furthermore, LA ICP-MSI analyses were used in order to understand the effects of Se on the strain and to determine in which proteins and specific amino acids it is inserted.

From these analyses emerged the ability of the strain to metabolize Se and to fix it into selenocysteine in 7 cytosolic proteins. Se also induced the up-regulation of the sugar metabolism and a certain degree of stress. Two of these SeCys-containing proteins were also detected in the extracellular environment being useful for nutraceutical applications, since they allow to release Se at human gut level also before cell lysis.

This *L. reuteri* strain is a good candidate to be used as nutraceutical supplement since it combines its probiotic features with its ability to generate organic bio-available Se forms.

SL 13

Insight into the mechanisms of cobalamin production and its regulation in *Propionibacterium freudenreichii* by comparative genomics and proteomics

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Propionibacterium freudenreichii, a member of the class Actinobacteria, is traditionally used as a secondary starter culture in manufacture of Emmental-type cheeses. In addition to its role in flavour development and in the formation of “eyes”in the cheese, *P. freudenreichii* is claimed to exert multiple beneficial effects, including suppression of undesirable microbes in food and displaying promising probiotic properties. However, probably the most important feature of this organism is its ability to produce appreciable amounts of cobalamin, a natural form of Vitamin B_{12}.
In this project multiple strains of *P. freudenreichii* are studied using phenotypic characterisation, as well as, genomic, proteomic and molecular biological approaches. The amounts of cobalamin produced in various growth media are assessed by UPLC-UV method in order to identify strains with different cobalamin biosynthetic capabilities. Selected strains displaying differing phenotypic profiles are subjected to genome sequencing in a comparative genomics approach, and to comparative proteome studies using 2-dimensional gel electrophoresis (2DE) and liquid chromatography-mass spectrometry (LC-MS/MS) for identification of differentially expressed proteins under various growth conditions. Identified key enzymes involved in the so-far uncharacterised part of cobalamin biosynthesis are characterized in vitro after cloning and overexpression of the genes in *E. coli* and purification of the proteins by biochemical tools. As a result, we hope to elucidate the dynamics behind the biosynthesis of cobalamin in *P. freudenreichii* and improve the understanding of this very capable and interesting, yet still poorly understood organism.

SL 14

***Lactobacillus gasseri* CP2305 and Brain-Gut Interaction**

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Gut microbiota play a role in the regulation of the HPA axis reactivity and have been suggested to influence stress-related disorders over the lifespan. Although bidirectional communication between the brain and the gut has long been recognized, little is known about how gut microbiota affect neuronal pathways and CNS signaling systems. In this study, we examined the effects of *Lactobacillus gasseri* CP2305 (CP2305) on psychological state in healthy humans and gene expression in the HPA axis-related tissues of rats.
The aim of this study was to elucidate the underlying mechanism for beneficial effects of CP2305.
Sleepquality, psychological state, and brain blood flow in healthy volunteers were measured to assess beneficial effects of CP2305. Autonomic nerve activities, plasma stress-related hormones, and gene-expression in various tissues were measured in rats.
Regular administration of either alive or dead CP2305 for 4 weeks significantly improved sleep quality and abdominal complaints in healthy volunteers. A significant alteration of cerebral blood flow was also observed after the CP2305 intakes. An oral administration of CP2305 into rats enhanced vagal afferent nerve activities, while suppressing the adrenal sympathetic nerve activity. Pre-feeding of alive or dead CP2305 for 3 weeks significantly prevented the reduction of pelvic nerve activities after an administration of CRF into jugular vein and acute diarrhea caused by an intra-peritoneal injection of CRF. Microarray and pathway analyses demonstrated that CRF administration changed a group of genes preferentially related to inflammatory responses in the hippocampus, adrenalglands, and colon. Pre-adiministration of CP2305 significantly prevented these CRF-induced changes in gene expression.
Our results suggest that *Lactobacillus gasseri* strain CP2305 may suppress stress-associated symptoms through modulating brain-gut interaction.

SL 15

Lifestyle of lactobacilli in murine forestomach biofilms

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Lactobacilli form stable biofilms on the non-glandular epithelium lining the upper gastrointestinal tract of several vertebrates and are used as starter cultures in food fermentations. *Lactobacillus reuteri*, a prominent member of those biofilms, is also a common starter culture of sourdoughs. In order to investigate the lifestyle of lactobacilli in rodent forestomach biofilms in more detail , we applied metatranscriptomics to analyze gene expression (assessed by mRNA) and community composition (assessed by rRNA) in C57BL/6 mice.
Lactobacillales were major biofilm inhabitants followed by *Clostridiales.* All detected *Lactobacillus* speciesbelonged to the subgroups of *L. johnsonii/acidophilus* or *L. reuteri.*
To identify mRNA transcripts specific for the forestomach, forestomach and hindgut metatranscriptomes were compared. Gene expression of the biofilm microbiota was characterized by high abundance of transcripts related to glucose and maltose utilization, peptide degradation and amino acid transport. *In vitro* fermentations showed that members of the *L. johnsonii/acidophilus* group preferably utilized glucose, while the *L. reuteri* group preferred maltose pointing at resource partitioning in the murine forestomach. The forestomach microbiota transcribed genes encoding extracellular proteins involved in adhesion (e.g. MucBP, extracellular glycansucrases) or related to glutathione synthesis enhancing oxidative stress resistance. Various pathways related to metabolite formation (urea degradation, arginine pathway, γ-aminobutyrate) and cell wall modifications (DltA, cyclopropane-fatty-acyl-phospholipid synthase) contributed to increased acid tolerance. Interestingly, several of those pathways were previously also associated with the competitiveness of *Lactobacillus* species in cereal fermentations.
In summary, this study shed light on lifestyle lactobacilli in the murine forestomach. Results obtained here might also be relevant for similar lactobacilli biofilms in birds, pigs, horses and humans.

Abstracts of posters, presented at the 11th Symposium on Lactic Acid Bacteria Egmond aan Zee, the Netherlands, August 31 to September 04, 2014

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A001

Typing *Streptococcus thermophilus* strains isolated from Turkish yoghurts by PFGE and RAPD PCR

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The genetic diversity of 59 strains of *Streptococcus thermophilus*, 55 among them isolated from Turkish traditional yoghurts, were studied. Randomly amplified polymorphic DNA (RAPD) and pulsed field gel electrophoresis (PFGE) have been used. Three random primers, XD9, M13 and OPI-02 MOD, and two restriction enzymes, *SmaI*, *ApaI*, widely used for typing *St. thermophilus* strains, according to literature, were applied. For each experiment's result and combined numerical analysis of PFGE and RAPD-PCR results, dendrograms were performed by UPGMA method via Gel Compar II 6.5 software. Multi-Dimensional Scaling (MDS) analysis was, also, applied via XLSTAT (Addinsof) software. The typeability, reproducibility, discriminatory power and congruence were studied for these techniques. Interpreting PFGE results according to Tenover *et al*. (1995) criteria was included. Adjusted Rand and Wallace indices were calculated in order to evaluate the correlation between PFGE and RAPD results. The objective was to detect the best typing option for *Streptococcus thermophilus* strains. Further discrimination was achieved with combined numerical analysis of the results of RAPD-PCR assays, PFGE assays and both methods together. There is a good congruence between combined numerical analysis of RAPD-PCR and PFGE results. Poor correlation was detected according to Tenover *et al.* (1995) criteria.PFGE was determined as good predictor of RAPD according to Wallace's index. Using OPI-02 MOD primer seems valuable RAPD-PCR method for typing *St. thermophilus* strains regarding to its typeability, reproducibility, discriminatory power and correlation with PFGE. These two last parameter may increased by combining results of OPI-02 MOD primer with other primers. Which is easier, Faster and less expensive for typing *St. thermophilus* strains, particularly, large number of new isolates.

A002

Comparative genomics of the *Bifidobacterium breve* taxon

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Background.
Bifidobacteria are commonly found as part of the microbiota of the gastrointestinal tract (GIT) of a broad range of hosts, where their presence is positively correlated with the host's health status. In this study, we assessed the genomes of thirteen representatives of *Bifidobacterium breve*, which is not only a frequently encountered component of the (adult and infant) human gut microbiota, but can also be isolated from human milk and vagina.
Results.
In silico analysis of genome sequences from thirteen *B. breve* strains isolated from different environments (infant and adult faeces, human milk, human vagina) shows that the genetic variability of this species principally consists of hypothetical genes and mobile elements, but, interestingly, also genes correlated with the adaptation to host environment and gut colonization. These latter genes specify the biosynthetic machinery for sortase-dependent pili and exopolysaccharide production, as well as genes that provide protection against invasion of foreign DNA (i.e. CRISPR loci and restriction/modification systems), and genes that encode enzymes responsible for carbohydrate fermentation. Gene-trait matching analysis showed clear correlations between known metabolic capabilities and characterized genes, and it also allowed the identification of a gene cluster involved in the utilization of the alcohol-sugar sorbitol.
Conclusions.
Genome analysis of thirteen representatives of the *B. breve* species revealed that the deduced pan-genome exhibits an essentially close trend. For this reason our analyses suggest that this number of *B. breve* representatives is sufficient to fully describe the pan-genome of this species. Comparative genomics also facilitated the genetic explanation for differential carbon source utilization phenotypes previously observed in different strains of *B. breve*.

A003

The effect of modified atmosphere gas composition on the role of lactic acid bacteria in meat spoilage as determined by MALDI-TOF MS

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Modified atmosphere packaging (MAP) is widely used to extend the microbiological shelf-life of meat. Carbon dioxide inhibits the growth of microorganisms while oxygen is required for the formation of oxymyoglobin to maintain colour of red meats, and nitrogen is used as supporting gas. Typically poultry meat has been packaged in a low O_2 or even in CO_2/N_2 atmosphere as this atmosphere is most inhibitory to spoilers and oxymyoglobin is not relevant in white meat. Typical members of the spoilage microbiota have been described as *Lactococcus*, *Carnobacterium* and *Enterococcus*. However, recently the common poultry meat MAP contains a high O_2 level, namely to inhibit the growth of upcoming *Campylobacter sp.*. We investigated the spoilage of poultry meat in high O_2 MAP (80% O_2 / 20% CO_2) at 4° and 10°C to find a shift of the spoilage microbiota as compared to low O_2 MAP. Over a period of two weeks the total bacterial counts were determined and the composition of the spoilage microbiota was identified by MALDI-TOF MS. It was possible to statistically monitor a wide range of different spoilage bacteria at species and biotype level. *Brochothrix*, *Carnobacterium sp*. and *Pseudomonas sp*. were the main genera found after 8 days at 4°C and 10°C, with different kinetics and predominance of species. These results indicate that high O_2 MAP leads to a major change in the composition of spoilage microbiota from anaerobic/aerotolerant lactic acid bacteria to (facultatively) aerobic bacteria. As a consequence spoilage of poultry meet in high O_2 MAP follows different kinetics, spoilage metabolite formation, and determination of shelf-life needs a critical review.

A004

Detailed characterization of *Lactobacillus helveticus* strains aimed at identifying new industrial starters

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Lactobacillus helveticus is an important food associated species traditionally used in the manufacture of Swiss type cheeses and long-ripened Italian cheeses and it is also acquiring an increasing interest as probiotic. The overall goal of this study was to acquire knowledge on the biodiversity of 88 *L. helveticus* strains from the CRA-FLC collection. For the initial screening, properties useful for a dairy application (such as growth conditions, sugar fermentation profile, milk acidification, phage sensitivity) were selected. Most strains required addition of whey to the medium for optimal growth. A more detailed characterization was carried out on 11 strains. Acidification analysis in milk showed a high diversity between the 11 strains with only four strains that did not reach pH 3.5 after 20 h of incubation at 40°C. *L. helveticus* strains showed a wide range of behaviours at the different conditions of growth tested; extreme conditions were disadvantageous for almost all the strains, i.e. growth in the presence of 3% and 4% of NaCl at 40°C or growth in anaerobiosis at 30°C for 6 hours. We observed optimum growth under aerobic conditions at 40°C and with no salt in the medium. Main amino acids produced were serine, proline and glutamic acid; two strains out of 11 were able to assimilate sucrose, maltose and trehalose. Four strains stimulated the growth of *Propionibacterium freudenreichii* as shown by an increased final cell mass of about 50% -100% after 48 h of incubation in whey. The strains did not appear lysogenic after induction with Mitomycin C. The demonstrated phenotypic biodiversity between *L. helveticus* provides useful information for strain selection and starter culture design in fermented dairy products.

A005

Microbial dynamics in Norwegian fermented milks during storage

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Five commercial fermented milks were screened for microbial development during storage. Three of these products, yogurt, buttermilk and a probiotic fermented milk, were produced using a commercial starter while the other two product, kefir and a traditional fermented milk (Tettemelk), were produced using a traditional technology. Lactic acid bacteria (LAB) communities were monitored by quantitative PCR (qPCR) coupled with microbial species identification performed by DGGE. The use of a photoreactive DNA binding dye allowed the distinction of dead and live cells in the product and their quantification. During storage, the number of living cells declined in a different manner according to product. Some LAB species were identified only among the living cells microbiota while other microbial species were not detected as living cells towards the end of the storage period. Most of the species detected as dead microbiota were not related to the starter culture used and were probably related to the original microbiota of the milk. The study highlighted most of the microbial changes occurring in some Norwegian traditional and commercial fermented milk.

A006

MICROBIAL BIOINFORMATICS: Making sense of your genome sequence data

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What's next after sequencing and assembly of your bacterial genomes ?
What does all your genome sequence data mean ?
Which genes are responsible for differences in phenotypes ?
What are the genetic differences between strains ?
MICROBIAL BIOINFORMATICS provides scientific advice and custom services in bioinformatic analysis of microbial genomes. We can assist with identification of RNA- and protein-encoding genes on the genome (chromosome and plasmids), and the prediction of their functions, using a variety of bioinformatics tools and databases. We can compare your sequenced genomes with each other, and with published genomes (templates) in databases. In-depth information is obtained on conserved (core) genes and gene order, variable genes, phylogenetic relations and evolution. Species-specific or strain-specific genes and gene clusters are identified and their functions predicted.
In recent years we have performed contract research on a strictly confidential basis for industry, academia and research organizations. Our studies have focussed on the comparison and in-depth genome analysis of typically 30-60 strains of various lactic acid bacteria, including *Streptococcous thermophilus*, *Lactococcus lactis*, *Lactobacillus plantarum* and *Lactobacillus paracasei* [1].
MICROBIAL BIOINFORMATICS is a spin-off of the Center for Molecular and Biomolecular Informatics (CMBI), Netherlands Bioinformatics Centre (NBIC), Top Institute Food and Nutrition (TIFN), and Kluyver Centre for Genomics of Industrial Fermentation.
For more information see: www.microbial-bioIT.nl
[1] Smokvina T, Wels M, Polka J, Chervaux C, Brisse S, Boekhorst J, van Hylckama Vlieg JE, Siezen RJ. (2013) ***Lactobacillus paracasei*** **comparative genomics: towards species pan-genome definition and exploitation of diversity.** PLoS One. Jul 19;8(7):e68731.

A007

Metabolic profiling of anaerobic and respiratory cultures of *Lactobacillus plantarum* C17

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Lactobacillus plantarum is a fermentative lactic acid bacterium (LAB) used in the production of many fermented and functional foods. Recently, it has been demonstrated that respiratory metabolism results, in this species, in the expression of a phenotype with enhanced technological and stress response properties.
We investigated the effect of anaerobiosis (AN) and respiratory promoting conditions (RS; 30% dissolved O_2, hemin and menaquinone) on the growth, O_2 uptake, activity of O_2-related enzymes (pyruvate oxidase, POX; NADH oxidase, NOX; NADH peroxidase, NPR), metabolic profile (^{1}H-NMR spectroscopy) and oxidative stress response (catalase, tolerance of H_2O_2 and menadione) of L. plantarum C17 (wild-type) and its natural oxidative stress-tolerant mutant C17-m58, using chemostat (D=0.07 h^{-1}, pH 6.5, 35°C) cultivations in chemically defined medium CDM. Respiratory conditions impaired biomass production in CDM, compared with cultivations in the complex Weissella medium broth (WMB). Activities of POX, NOX and NPR, O_2 consumption and robustness to oxidative stresses were higher in C17-m58.
Significant amounts of formate and acetate were found in AN cultures of wt and mutant strains (^{1}H-NMR analyses), confirming the activation of anaerobic pyruvate formate lyase-acetate kinase (PFL-ACK) pathway in *L. plantarum*. High concentrations of pyruvate, instead, were measured in RS supernatants, indicating a reduced functionality of POX *in vivo* (35°C) and the need to clarify the regulation and control of O_2 metabolism. Adipate was also found in respiratory samples, probably because of lipid oxidation by O_2 or H_2O_2. Adipic acid, used as gelling agent in foods, is chemically synthesized by benzene or benzene-derivative, with possibly release of toxic compounds. Since no biotechnological process has been yet developed for its production, this trait could be investigated in respiring LAB.

A008

The pangenome of the *Bifidobacterium bifidum* taxon

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Bifidobacteria are human gut commensals bacteria that are perceived to exert health-promoting activities. In this study, we analyzed the Pan-genome of *Bifidobacterium bifidum*, which is a key member of human gut microbiota of infants. *In silico* analysis of the genome sequences of 15 *B. bifidum* strains isolated from different environments (infant stool, adult intestine and feces of adult and infant) display a highly syntenic structure of their chromosomes as well as a considerably large set of conserved genes representing the core genome sequences of this taxon. Genetic variability within the genomes of *B. bifidum* species resulted to be associated to genetic loci encoding host-microbe interaction structures such as sortase dependent pili as well as genetic systems providing protection against invasion of foreign DNA (i.e. CRISPR loci and restriction/modification systems). Growth experiments on different carbohydrates followed by transcriptomics analyses confirmed the *in silico* data and supported intriguing and unique saccharolyitic features of this bifidobacterial taxon like the utilization of host-glycans.

A009

Redesign the taxonomy of the genus *Bifidobacterium* through genomic-based analyses

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The *Bifidobacterium* genus encompassing 48 recognized taxa, which have been isolated from different ecosystems. However, the currently taxonomy of bifidobacteria is hampered by the relative paucity of genotypic data so far available. Here, we renew the taxonomy of this genus of microorganisms using genome-based approaches, which highlighted several inconsistences. In particular, we display the high genetic relatedness occurring between many *Bifidobacterium* taxa that did not support the status as separate species. The results here presented are based on Average Nucleotide Identity analysis involving the genome sequences for each type strains harboring the 48 bifidobacterial taxa as well as phylogenetic analysis of the predicted core genome of the *Bifidobacterium* genus. This study highlight that the availability of complete genome sequences allow the reconstruction of a robust bifidobacterial phylogeny than a single gene-based sequence comparison, discouraging the designation of new bifidobacterial taxon without a genome-based validation.

A010

Optimization of chemically defined medium for the study of anaerobic and respiratory growth in *Lactobacillus plantarum* and *Lactobacillus casei*

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Lactic acid bacteria have complex nutritional requirements being auxotrophic for several amino acids (AA), nucleotides and vitamins. *Lactobacillus plantarum* and *L. casei* can synthesize most AA, except the branched-chain amino acids.
We verified that the chemically defined medium CDM, used in several *L. plantarum* studies (Teusink et al. 2005, Appl Environ Microbiol 71, 7253-7262; Wegkamp et al. 2010, Lett Appl Microbiol 50, 57-64), did not support aerobic (O_2) and respiratory (O_2, heme and menaquinone) growth of *L. plantarum* and *L. casei*. The effect of Tween 80 (as a source of unsaturated fatty acids, uFA), L-alanine, L-asparagine, L-aspartate, L-proline and L-serine, missing in ordinary CDM, on the anaerobic (AN) and respiratory (RS) growing cells of *L. casei* N87 was investigated in a factorial design. Supplementation with Tween 20, 40 and 60 (saturated fatty acids, sFA) were also evaluated. Results revealed that when both Tween 80 and AA pool were missing in the substrate L. casei was unable to grow in RS. The presence of Tween 80 did not affect AN cultivation but dramatically improved RS growth, suggesting specific oleic acid requirement in *L. casei* in RS condition. Asn+Pro+Ser (related to TCA cycle and pyruvate precursor pathways) was the best AA combination to stimulate RS cells. Tween 40 and 60 completely inhibited AN and RS growth because of high sFA concentration. Composition of membrane FA (degree of unsaturation and length of FA chains) in AN and RS cells, grown with or without Tween 80, was analyzed by Gas-Chromatography (GC).
The optimized CMD could be suitable for comparative studies of metabolic pathways in other respiring lactobacilli.

A011

Genomic origin of biochemical diversity of *Lactobacillus casei* strains used in Swiss cheese production

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Lactobacillus casei (*L. casei*) is a gram positive bacterial species that is used in various industries and in particular in dairy production. *L. casei* has a big natural habitat diversity that includes gastrointestinal and reproductive tracts of vertebrates, milk, plant material, and meat environments. The Agroscope culture collection comprises more than thousand *L. casei* strains which were isolated mainly from dairy products for decades. For many of these strains, phenotypic features were determined by biochemical methods which showed a high diversity.
To determine the genomic basis of the phenotypic diversity of *L. casei*, we sequenced the genomes of 40 strains from the Agroscope culture collection using an Illumina HiSeq2000 instrument. We determined the pan and the core genome which comprises 6,059 and 1,800 genes, respectively. Interestingly, 1,785 genes of the dispensable genome have not been described before in *L. casei*. Possible sources for the large dispensable genome are horizontal and vertical gene transfer. In our study, we found that horizontal gene transfer is the main source of these genes. We also determined clusters of orthologous genes including other bacterial species and found that the majority of genes originated from horizontal transfer is related to *Lactobacillus rhammusos* (76 %) and other *Lactobacillus* species (16 %).
Furthermore, we carried out a phylogenetic analysis that included genomes of all published *L. casei* strains from different habitats. We found that *L. casei* strains from cheese production do not exclusively cluster with strains used in dairy production, but also build several clusters with strains from various other habitats such as human blood or corn silage.

A012

Genetic diversity in *Lactobacillus fermentum* strains assessed by multi-locus sequence typing

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Lactobacillus fermentum is a Gram-positive species of bacterium in the genus *Lactobacillus*. It is commonly found in fermenting animal and plant material. To probe evolution and phylogeny of *Lactobacillus fermentum*, 209 *Lactobacillus fermentum* strains were examined by multi-locus sequence typing (MLST). The strains included isolates from fermented congee (n=61), sourdough (n=1), and traditional fermented milk (n=147) from different geographical locations. The MLST scheme, based on the analysis of twelve housekeeping genes, such as *dnaA*, *pepN*, *pyrG*, *groEL*, *recA*, *clpX*, *uvrC*, *dnaK*, *murC*, *ropB*, *murE* , and *pepX* were developed. The 12 targeted loci were successfully amplified and sequenced for all strains. Phylogenetic analysis showed that all 209 strains belonged to 7 subgroups, including 72 sequence types using the Bionumerics 6.0 and eBURST V3. Subsequently, the nucleotide sequence alignments for all gene fragments from unique STs were used to infer clonal relationships with ClonalFrame 1.1. ClonalFrame identifies regions that are likely to have arisen by homologous recombination and accounts for them when reconstructing the clonal genealogy. The results clearly showed that most of the strains isolated from fermented congee, pickles, sourdough, and traditional fermented milk formed different subgroups, respectively. These results indicate the strains isolated from the different ecological niches have experienced different evolution. The *Lactobacillus fermentum* population analyzed in this study demonstrated both a high level of phenotypic and genotypic diversity, as well as specificity to different ecological niches.

A013

Phenotypic and genotypic assessment of *Lactobacillus plantarum* strains isolated from foods & intestines

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Twenty strains of *Lactobacillus plantarum* isolated from asian fermented foods, infant feces and shrimp intestines were evaluated for their phenotypic and genotypic characteristics. Variations of phenotype among strains were found in term of their sugar fermentation, adhesion ability and antibacterial activity. The ability of adhesion and sugar consumption within closely related of *L. plantarum* strains correlated with sources of isolation. Five strains isolated from infant feces showed the highest adhesive capacity, while L*. plantarum* strains isolated from fermented foods showed the largest capacity to utilize different sugars. All strains showed an effective inhibition against many pathogenic bacteria. The genes involving in adhesion (*mup, mabA, msa*) were found to be present in all *L. plantarum* strains. The presence of genes located in the *plnABCD, plnEFI* and *plnGHSTUVW* operons were detected by PCR and found to be common among *L. plantarum* strains. However, the *pln* genes encoding plantaricin EF, plantaricin I, plantaricin J, and plantaricin NC8 were absent in all infant isolates. All strains of *L. plantarum* isolated from fermented foods and shrimp intestines harbored more and different kinds of *pln* genes in their chromosome higher than those isolated from infant feces. Repetitive PCR (rep-PCR) fingerprinting technique demonstrated genotypic diversity among all *L. plantarum* strains, and revealed the distinctive phylogenetic position of the infant as compared to the strains isolated from fermented foods and shrimp intestines. Therefore, these phenotypic and genotypic data may support knowledge of niche adaptation and functional properties of *L. plantarum* strains. Comparative genome analysis of the strains is in progress as to provide a basis for future gene-trait matching and functional approaches.

A014

***Lactobacillus casei* group effective identification**

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Lactobacillus casei, L. paracasei and *L. rhamnosus* form a closely related taxonomic group (*Lactobacillus casei*-group) within the facultatively heterofermentative lactobacilli. Some strains belonging to these species have been used for long as probiotics in a wide range of products, and they commonly represent the dominant species of nonstarter lactic acid bacteria in ripened cheeses, where they contribute to flavor development.
The close genetic relationship among *L. casei*-group species, and the related starter cultures, hinders the development of an adequate selective identification method useful to understand the role played by each microbial population in the matrix and the routine biochemical tests often fail to discriminate among closely related species occurring in the same ecological environments, like inside ripened cheeses.
The aim of this study was a comprehensive analysis of 75 cheese isolates previously identified as *L. casei* group, combining multiple approaches. Alternative proposed culture media for *L. casei*-group selective growth were tested, giving poor discrimination among strains. Furthermore, an existing species-specific PCR based on the 16S rRNA gene was adapted to laboratory conditions and applied on isolates. They were analyzed with more than one primer pair in order to avoid misidentification due to possible cross-reactions. This approach allowed the identification of the majority of the strains, but still leaving some of them with an uncertain identity. Finally, a new species-specific multiplex PCR assay was developed based on a universal protein encoding gene sequence. Primers were designed to rapidly distinguish *L. casei*, *L. paracasei* and *L. rhamnosus* strains based on amplicon size. In conclusion, multiple molecular methods were applied and critically evaluated based on their ability to clearly identify the collection of *L. casei*-group isolates.

A015

An insight into the microbiota of exotic fruits and flowers of Northern Argentina: molecular identification of lactic acid bacteria

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Lactic acid bacteria (LAB) are naturally present in a wide variety of niches. Because of their beneficial properties, LAB are used as starter cultures, as probiotics, and in the production of interesting compounds. Exploring the LAB biodiversity of virgin niches may lead to the isolation of unique strains or species with interesting technological and/or health-promoting properties. A great diversity of exotic fruit trees exist in the Northern region of Argentina, constituting an interesting source of LAB. The present study aimed to isolate and identify LAB from fresh fruits and flowers from Northern Argentina. Guava (yellow and pink varieties), papaya and passion fruits as well as passion fruit flowers were aseptically collected. LAB isolation was performed by culture-dependent techniques, using MRS medium for LAB and FYP medium for fructophilic LAB. One hundred and ninety Gram-positive, catalase-negative isolates were obtained; identification was assayed by RAPD-PCR fingerprinting (M13b and XD9 primers) or rep-PCR fingerprinting analysis [(GTG)5 primer] and numerical clustering. Molecular identification of representative isolates of each cluster was performed by partial sequencing of 16S rDNA. Among the fruit isolates, *Enterococcus hirae* (1 strain), *Ent. casseliflavus* (4), *Ent. faecium* (1), *Weissella minor* (9), *Lactococcus lactis* subsp. *cremoris* (1), *Leuconostoc mesenteroides* subsp. *mesenteroides* (3), *Leuc. pseudomesenteroides* (16), *Lactobacillus brevis* (6), *Lb. plantarum* (1) were identified, whereas *Ent. faecalis* (1), *W. cibaria* (2), *Lc. lactis* subsp. *lactis* (1) and *Leuc. mesenteroides* subsp. *mesenteroides* (2) were found in flowers. The results suggest that various LAB species naturally inhabit many exotic fruits and flowers, among which the most abundant strains belonged to the *Leuconostoc* and *Weissella* genera. Further studies are needed to evaluate the potential health-promoting or industrially-relevant properties of the strains.

A016

A genome-based approach to clarify the taxonomic placement of *Leuconostoc fallax*

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The species *Leuconostoc fallax* was first isolated in 1991 from sauerkraut. According to phenotypic data, DNA-DNA homology values and analysis of the similarity of 16S rRNA coding sequences, it was ascribed to genus *Leuconostoc* although with a peripheral position with respect to the other species (Martinez-Murcia and Collins, 1991).
More recently, additional data collected by Chelo *et al*. (2007) and de Bruyne *et al*. (2008), focusing on phylogenetic analyses, based on different molecular markers (i.e. *dnaA*, *gyrB*, *rpoC*, *dnaK*, *atpA* and *pheS* gene sequences), questioned this placement, as *L. fallax* was found to be less atypical as a *Leuconostoc* than what was thought before. In the last years, a number of genome sequences have been made available for the majority of *Leuconostoc* species, including *L. fallax* (Nam *et al*., 2011), although not those of the type strains.
From this starting point, the aim of the present study was the investigation of the evolutionary history of *Leuconostoc* species, and the definition of an improved taxonomy of the family *Leuconostocaceae*.
Evolutionary rates of the different genes used as molecular markers were compared to understand the discrepancies among previous placements, and a more consistent taxonomic position of *L. fallax* was defined by collecting and integrating phenotypic, ecological and genomic data.
This was also an excellent case study to prove the value of genome-based comparative taxonomic analyses, which, in the near future, are expected to provide good explanatory hypotheses at different taxonomic levels, with also important consequences on procedures of strain naming and identification.

A017

A new distinctive operon for stress response in *Oenococcus oeni*: expression in strain PSU-1 and evolution of the genetic structure in the genus *Oenococcus*.

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Oenococcus oeni represents the LAB used to conduct malolactic fermentation in wine because of its resistance to many environmental stresses, e.g., pH, alcohol typical of this niche. Harsh environmental conditions can be seen as evolutionary pressure; therefore studies on stress responses in *O. oeni* might also provide insights on the genetic content and evolution of the species. In a previous study, Stefanelli *et al.* (2011) had carried out a transcriptomic analysis for strain PSU-1 in response to four different stress conditions. Data revealed the differential expression of a putative operon of three genes in three stress conditions (exposure for 6 h to pH 3.5, pH 3.5-10% ethanol, and 42°C), and included a transcriptional regulator (OEOE_0878), and two genes with opposite orientation (OEOE_0877 and OEOE_0876). The aim of the present study was to deepen the knowledge on the expression of such putative operon in the same conditions of the microarray and to investigate its distribution in other LAB. Upregulation of the operon was confirmed by RT-qPCR, and co-transcription of OEOE_0877 (putative permease) and OEOE_0876 (hypothetical protein) was assessed. *In silico* analyses and PCR screenings revealed that this operonic structure was present in a collection of 38 *O. oeni* strains, including the sequenced strains and 24 isolates from Italian wines, and the operonic structure appeared to be present only in the genus *Oenococcus*, although homologs were found in other bacteria. The presence and sequence conservation of the stress-responsive operon in *O. oeni* strains could indicate its importance in the stress response of the species, although further studies are required to clarify its biological role in such peculiar bacterium.

Stefanelli *et al*. (2011). Poster D024. LAB10 Symposium

A018

Polyphasic genotyping of *Lactobacillus gasseri* using rep-PCR and CRISPR

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Lactobacillus gasseri are commensal microorganisms native to the gastrointestinal and genital tracts of humans and are considered to have a beneficial influence on immunomodulation and gastrointestinal health. Previous genotyping studies using traditional methods such as PFGE revealed high diversity among *L. gasseri* strains. Here, we report polyphasic genotyping of *L. gasseri* using a rep-PCR based DiversiLab system in tandem with comparative analysis of CRISPR loci. Rep-PCR amplifies intervening sequences between randomly distributed repetitive elements in the genome such that each strain is represented by a barcode pattern, reflecting differential amplicon sizes. The CRISPR-Cas system incorporates invasive DNA segments as novel spacers, providing a record of shared and divergent evolutionary exposure to bacteriophages and foreign plasmids. CRISPR arrays thus constitute hypervariable and fast evolving genetic loci suitable for typing. In this study, 17 *L. gasseri* strains tested by rep-PCR were differentiated into distinct groups. All L. gasseri strains produced a barcode pattern from rep-PCR, whereas only six of the 17 *L. gasseri* contained repeat-spacer arrays. The diverse and unique spacers found in these six strains imply that there may be functional CRISPR-Cas systems in *L. gasseri*. Therefore, in highly related strains of *L. gasseri* containing a CRISPR array, comparison of spacer content could be utilized as a fine-tune typing tool, in addition to revealing the evolutionary path of strains.

A019

Elucidation of potential health risks and global phylogeny of *Streptococcus infantarius* dairy variants in contrast to clinical strains through MLST and the assessment of pathogenic traits

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Streptococcus infantarius subsp. *infantarius* (*Sii*) is part of the *Streptococcus bovis* / *Streptococcus equinus* complex (SBSEC) associated with colonic cancer and infective endocarditis worldwide. *Sii* dairy variants are highly prevalent in East (EA) and West African (WA) traditional fermented dairy products (FDP). Comparative genomics of EA *Sii* revealed dairy adaptations paralleling those of Streptococcus thermophilus, suggesting a fermentative role in FDP. The taxonomic heritage questions the safety of *Sii* and possibly traditional FDP for human consumption.
A novel multi locus sequence typing (MLST) scheme was developed to investigate the evolutionary relationship between SBSEC, food-associated and clinical *Sii* strains (n=50) based on 10 housekeeping genes. Strain-specific adhesion to intestinal extracellular matrix molecules and intestinal epithelial cell lines Caco2, HT29 and HT29-MTX including invasion and inflammatory potential (NF-kB activation) were assessed.
Sii strains were grouped into two main MLST clusters (I) and (II) distinct from other SBSEC. Cluster (I) comprised clinical (IA1), EA dairy (IA2), WA dairy (IB) isolates. Cluster (II) comprised WA dairy (IIA) and Asian dairy isolates (IIB). EA isolates (IA2) showed more prevalent adhesion to fibrinogen, fibronectin, collagen I and mucin than WA (IIA) or clinical isolates (IA1). Contrary, the inflammatory potential was significantly more prevalent and acute in clinical and WA isolates than EA isolates. No significant invasion of tested cell lines was observed, suggesting that this might not be a main pathogenicity mechanism for Sii.
Conclusively, evolution of *Sii* seems to advance in several main strain lineages with clear differentiations between EA and WA. The later displayed higher diversity and inflammation potential and thus potentially increased health risks, requiring regional-specific interventions and epidemiologic studies to assess the pathogenic potential of *Sii* in traditional African FDP.

A020

Study of Orf14 from *Lactococcus lactis* phage bIL76 and other *lactococcal* single-stranded DNA binding proteins shows their archaeal origin

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Single-strand-nucleic acid binding (SSB) proteins are identified in all domains of life (Eukaryotes, Archaea, Bacteria) and viruses. It was suggested that prokaryotic, archaeal and eukaryotic SSBs all descended from a common ancestor polypeptide with a single OB-fold via gene duplication and recombination events. Yet, bacterial SSB proteins, as other proteins involved in DNA replication, clearly differ from those found in Archaea and Eukaryotes. It was proposed that the corresponding genes in phage genomes were transferred from bacterial hosts. Recently, new SSB proteins encoded by virulent lactococcal bacteriophages (Orf14$_{bIL67}$-like proteins) have been identified and characterized structurally and biochemically.
This study aimed at determining the phylogenetic relationships between Orf14$_{bIL67}$-like proteins and other SSBs. Results of large scale phylogenetic analysis and pairwise sequence comparison of SSB proteins from different phyla show that Orf14$_{bIL67}$-like proteins form a distinct, self-contained and well supported phylogenetic group connected to archaeal SSBs. This stands in remarkable contrast to other phage SSBs. Functional studies demonstrate that, despite the structural and amino acid sequence differences to bacterial SSBs, Orf14$_{bIL67}$ protein complements the conditional lethal *ssb-1* mutation of *Escherichia coli*.
Here, we identified for the first time a group of phage-encoded SSBs, which are clearly distinct from their bacterial counterparts. All methods support the hypothesis that the Orf14$_{bIL67}$-like SSBs of lactococcal phages constitute a new protein family within the SSB superfamily. Results of our study suggest that unlike other phages, the virulent lactococcal phages carry *ssb* genes that were not acquired from their hosts, but transferred from an archaeal genome and adapted to phage multiplication in bacteria. This finding indicates that bacterial phages can also contribute to the horizontal gene transfer between Bacteria and Archaea.
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A021

Diversity of LAB populations associated to chicha, a traditional maize-based fermented beverage from Northwestern Argentina assessed by pyrosequencing and culture-dependent molecular identification

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Chicha is a traditional maize-based alcoholic beverage from Andean regions which manufacturing process is a household and communal activity. In this study, samples from two *chicha* productions were obtained from local producers at Maimará (*chicha* M) and Tumbayá (*chicha* T) each of them exhibiting particular production procedures. Presumptive lactic acid bacteria (LAB) (146 *chicha* M, 246 *chicha* T) recovered from samples at 10 production steps were identified by ISR-PCR and RAPD-PCR profiles, species specific PCR and 16S rRNA gene sequencing. In *chicha* M, most of the isolates belonged to *Leuconostoc* genus (*Lc. mesenteroides* and *Lc. lactis*) although *Lactobacillus*, *Weissella* and *Enterococcus* were also present. In contrast, *chicha* T exhibited the presence of *Enterococcus* and *Leuconostoc* while *E. faecium* was the most representative species during fermentation. As a complementary approach, *chicha* samples were analyzed by pyrosequencing of the V3-V5 region in the 16S rRNA gene to obtain asnapshot of the global bacterial population. Results revealed that 12 and 13 families were present in *chicha* M and T, respectively; among them nine families were detected in both productions. Regarding LAB, six genera were identified from both *chicha* productions: *Enterococcus*, *Lactococcus, Streptococcus, Weissella, Leuconostoc*, *Lactobacillus* whereas *Pediococcus* was only detected in *chicha* M. Identification at species level showed that while *Lb. plantatrum, Lb. rossiae, Lc. lactis* and *W. viridescens* predominated in samples of *chicha* M, *E. ratti* and *Lc. mesenteroides* were the most abundant species in *chicha* T. Results from this study provided information on this unknown microbial genetic reservoir and confirmed the survival of LAB during alcoholic fermentation, a first crucial stage in determining their role and significance.

A022

Molecular characterization of *Lactobacillus delbrueckii* subsp. *bulgaricus* phages

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Phages infecting *Lactobacillus delbrueckii* subsp. *bulgaricus* were isolated from whey samples obtained from industrial fermentation environments. These phages were fully characterised using several appproaches: (1) physical characterisation through host range analysis and electron microscopy (2) phage population dynamics assessment through genomics (3) identification of structural proteins through mass spectrometry and (4) transcriptome analysis by Northern Hybridization and RT-PCR. The phages display a great deal of similarity to previously published group b *Lb. bulgaricus* phages c5, LL-Ku and phiLdb with a region of divergence within the region predicted to encode the tail structural components of ϕLdb17 and ϕLdb25A. This region may represent a functional insertion element with a potential role in host range determination or extension. The transcriptome of a group b phage was determined for the first time revealing two transcripts E1, consisting of the replication module and L1, encoding the morphogenesis module and lysis cassette. Primer extension was used to determine the transcription start sites and locate the promoter consensus sequences of these transcripts.

A023

Whole genome sequences of forty-three bacteriophages infecting *Streptococcus thermophilus*

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Streptococcus thermophilus is one of the most widely employed lactic acid bacterial species in the production of fermented foods worldwide. Akin to all bacteria, these dairy bacteria are prone to phage attack as phages are ubiquitous in nature and indeed in the dairy processing environment. Until recently, phages infecting *S. thermophilus* have been classified into two groups based on their mode of DNA packaging i.e. *cos* phages are those which possess cohesive ends and *pac* phages are those which employ the “headful”mode of DNA packaging. In 2011, a third group of *S. thermophilus* phages, named the 5093-like phages, were identified based on their unique genetic content. In the present study, the whole genome sequences of forty-three bacteriophages infecting a total of eighteen strains of the dairy bacterium *S. thermophilus* were determined. The genome sizes of these phages ranged from approximately 30.3 to 38.1 kilobase pairs (kbps) and showed a high degree of conservation with previously and currently sequenced *cos* and *pac* phages, with some divergence observed in many cases within the early gene cluster. In addition to the *cos* and *pac* phages, additional members of the 5093-like group as well as representative members of a previously undescribed fourth group were also identified. The modular arrangement of each genome corresponded quite well with those of previously published *S. thermophilus* phage genomes. The genomes were also analysed for novel genetic elements to identify components that may contribute to the evolutionary success of these phages in the dairy processing environment.

A024

Comparative genomics of over 50 *Lactococcus lactis* strains

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Previously, comparative genome hybridization (CGH) was used to provide preliminary insight into the genomic diversity of *L. lactis* ssp *cremoris* and ssp *lactis* strains isolated from a variety of niches around the world [1]. Limitations of CGH were that only genes were identified with high sequence identity to the genes of reference *L. lactis* genomes (IL1403, KF147, SK11) and *L.lactis* plasmids (from NCBI) on the array. We have now sequenced total DNA isolated from the same set of *L. lactis* strains, using Illumina technology (Hiseq2000, 100bp paired end), performed *de novo* assembly and automatically annotated using the RAST server, followed by manual curation. These genomes were compared with each other and with 10 circular *L. lactis* reference chromosomes (5 ssp *cremoris* and 5 ssp *lactis*) and their 24 plasmids from the NCBI database. The genomes ranged in size from 2.3-2.9 Mb and encoded 2200-2900 predicted protein sequences. This allowed us to determine the core genome and pangenome of the *L. lactis* species, and to find unique genotypic characteristics of each strain, and of the ssp *cremoris* vs ssp *lactis* strains. A total phylogenomic tree now shows the relatedness of different strains. The ssp *cremoris* genomes are found to contain many more pseudogenes than the ssp *lactis* strains, and generally these pseudogenes are conserved between different ssp *cremoris* isolates.
[1] Siezen RJ, Bayjanov JR, Felis GE, van der Sijde MR, Starrenburg M, Molenaar D, Wels M, van Hijum SA, van Hylckama Vlieg JE. Genome-scale diversity and niche adaptation analysis of *Lactococcus lactis* by comparative genome hybridization using multi-strain arrays. Microb Biotechnol. 2011 May;4(3):383-402.

A025

936-type phage evolution in a processing environment: Insights from whole genome sequencing

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The 936-type phages are the most frequently isolated phage species in global dairy facilities utilising *Lactococcus lactis* starter cultures. Despite extensive efforts to control phage proliferation, these phages continue to negatively impact cheese production in terms of final product output and monetary return. Twenty 936-type phages were isolated from whey samples of Gouda-type cheese fermentations performed in 2009 and 2010, and an additional twenty-two 936-type phages were then isolated from 2014 whey and whey cream samples. The above-mentioned phages were subject to genome sequencing using 454-pyrosequencing and Pacbio platforms.
Comparative genomic analysis revealed three groups among the phage isolates based on differential factors associated with the structural module: those (1) encompassing a gene specifying a neck passage structure (NPS), (2) harbouring a gene encoding a putative fibre accessory protein and (3) containing both of the afore mentioned genes. While several studies show that 936-type phages are highly conserved, particularly in their structural modules, this study has revealed novel elements highlighting the evolutionary trends of these viruses. For example, several phages were found to encode one or more genes specifying a putative orphan methyltransferase within the packaging genes and/or replication module. Using PacBio sequencing and heterologous expression studies, these MTases were found to protect phage DNA from endonuclease activity, a finding not previously observed among these phages. This highlights the adaptability of the 936-type phages which may account for the difficulty in controlling this species in dairy fermentations.

A026

Proteomic analysis of secreted cell surface proteins in S-layer and non-S-layer forming species of the *Lactobacillus acidophilus* complex

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The *Lactobacillus acidophilus* complex is a clade of homologous Gram-positive, lactic acid bacteria including *L. acidophilus*, *L. helveticus*, *L. crispatus*, *L. amylovorus*, *L. gallinarum*, *L. delbrueckii* subsp. *bulgaricus*, *L. gasseri*, and *L. johnsonii*. Although these bacteria are closely related, they have varied ecological lifestyles ranging from dairy and food fermentations, to allochthonous probiotics, and autochthonous commensals of the host gastrointestinal tract. Bacterial cell surface components play a critical role in the molecular dialogue between bacteria, and their interaction with the intestinal mucosa. Notably, the *L. acidophilus* complex bacteria can be split based on their ability to produce S-layers, which are semi-porous, crystalline arrays of self-assembling, proteinaceous subunits found as the outermost layer of the bacterial cell wall. Based on previous data regarding the identification of S-layer associated proteins (SLAPs) in *L. acidophilus*, we employed a proteomic analysis of secreted surface proteins of the S-layer forming and non-S-layer forming bacteria of the *L. acidophilus* complex. Using a modified LiCl extraction protocol coupled with LC-MS/MS, we have proteomically identified the various extracellular proteins and SLAPs of the *L. acidophilus* complex, including anticipated annotated cell surface proteins, as well as conserved hypothetical proteins of unknown function. Analyses of these data highlight the proteomic complexity and differences of the cell surface of probiotic lactobacilli and reveal the potential for SLAPs to mediate intimate interactions with the intestinal mucosa. This opens new avenues for the selection of effective probiotics, and the engineering of immunomodulatory bacteria.

A027

Phenotypic and genomic diversity of *Lactococcus lactis* strains isolated from non-dairy niches

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Industrial strains of *Lactococcus lactis* are widely held to have evolved from non-dairy niches. Indeed, non-dairy environments remain a rich source of *Lactococcus* strains. Such strains have diverse metabolic properties rarely found in dairy isolates and evidence to support their impact on flavour development in dairy fermentations is mounting. A bank of L. lactis strains from sources including plant and vegetable material were phenotypically and genotypically compared to well-known dairy strains. Interestingly, considering that few ssp. *cremoris* strain are encountered outside the dairy environment, many of these isolates were confirmed as ssp. *cremoris* by 16S rRNA sequencing. Multi-locus sequence typing using concatenated sequences from seven genes (*atpA*, *pheS*, *rpoA*, *pepN*, *pepX*, *bcaT*, 16S rRNA gene) revealed two distinct clusters which separated subsp. *cremoris* and *lactis*, with non-dairy isolates grouping amongst dairy strains. Isolates were examined for resistance to antibiotics, growth in the presence of heavy metals and the ability to ferment different carbohydrate substrates. Non-dairy strains showed similar minimum inhibitory concentration (MIC) profiles for antibiotics to the dairy strains examined whereas a strain-to-strain variation was found for sensitivity to metal ions. The non-dairy strains also exhibited wider carbohydrate fermentation profiles in comparison to dairy lactococci. Three strains, isolated from grass (DPC6860), baby corn (DPC6853) and the bovine rumen (DPC6856), were selected for genome sequencing and comparative genome analysis revealed specific differences between dairy and non-dairy strains in terms of plant-derived sugar utilisation, heavy metal transport and resistance, bacterial defence mechanisms (resistance to antibiotics, bacteriocins and bacteriophage), among others. This study sheds further light on the diversity of *L. lactis* from outside the dairy environment, their route to domestication and their potential application in dairy fermentations.

A028

Isolation of lactic acid bacteria from Northwest Argentinean pseudocereal flour and grains. Evaluation of B group vitamin production

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The pseudocereals quinoa (Chenopodium quinoa) and amaranth (sp.) are ancient Andean crops with high nutritional value; they contain elevated concentrations of proteins, and different levels of vitamins and minerals. However, many of these compounds are altered or removed during milling, processing or cooking. Lactic acid bacteria (LAB) are widely used as starter cultures for the fermentation of different foods. LAB are usually auxotrophic for several vitamins although some strains have the ability to synthesize B vitamins, suggesting that the use of adequately selected strains could increase the concentration of these vitamins in fermented foods and their nutritional value. The aim of this study was to evaluate the LAB microbiota of quinoa and amaranth flour and grains of Northwestern Argentina (NOA) and determine their capacity to produce B vitamins, folate (B9) and riboflavin (B2). LAB were identified by biochemical and molecular methods. More than seventy strains of LAB were isolated and identified as belonging to the species *Lactobacillus* (*L.*) *pentosus*, *L. rhamnosus, L. sakei* and *L. plantarum*. Intra-, extracellular and total concentrations of B2 and B9 were determined using microbiological methods. Of the total tested strains, most grew on the B2 free medium and produced different levels of this vitamin (between 0.08 and 285 ng / ml), Almost 90% of the isolated LAB grew in synthetic medium without B9 and produced between 14 and 80 ng / ml.The results obtained put in evidence that native LAB isolated from quinoa and amaranth from NOA, produce significant levels of vitamin B2 and B9, indicating their potential to be included as starter cultures in pseudocereals containing food preparation with higher nutritional value.

A029

Comparative genomics analysis of industrial *Lactococcus lactis* strains

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Lactococcus lactis is a lactic acid bacterium widely used in the dairy industry for the production of diverse cheeses. Several decades of meticulous microbial selection have provided large collections of strains with appropriate technological attributes such as fast milk acidification, improved bacteriophage resistance, and desired aroma production. The objective of this study was to link specific phenotypes to the genetic content of select strains following whole genome sequencing. Genome sequences were generated for 24 *L. lactis* subsp. *cremoris* or subsp. *lactis* industrial strains using 454 or Illumina pyrosequencing. Following raw reads assembly with Newbler or NGen, contig ordering was performed using Mauve against the closest complete genome, and a basic structural and functional annotation was produced with RAST. Proprietary bioinformatics tools based on BLAST were developed for the classification of all coding sequences (CDS) into gene families. The 24 draft genomes ranged in size between 2.29 and 2.80 Mb, and were organized into 70 to 722 contigs, reflecting a varying content of repeated sequences, notably insertion sequences. The number of predicted CDS varied between 2,517 and 3,134 per genome, slightly above gene contents observed in complete genomes. In most draft genomes, putative plasmid-based contigs could be detected, although this prediction of plasmid nature is not trivial. Overall, 97,261 CDS were classified into 6,542 gene families (pan-genome), showing a core-genome of approx. 1,700 families for each subspecies. In contrast, the number of strain-specific gene families was almost double in subsp. *lactis* compared to *cremoris*. Our data indicate a significant strain-to-strain genetic diversity, in agreement with already observed physiological distinctive features, paving the way for further genomic analyses.

A030

Milk adaptation and pathogenic potential among members of the *Streptococcus bovis* / *Streptococcus equinus* complex

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The *Streptococcus bovis*/*Streptococcus equinus* complex (SBSEC) consists of species commonly found in the gastrointestinal tract (GIT) of herbivores. Certain members of the complex like *Streptococcus macedonicus* and *Streptococcus infantarius* are frequently isolated from traditional fermented foods, mostly of dairy origin. However, some species of this complex, like *Streptococcus gallolyticus* and *Streptococcus pasteurianus* are known pathogens of humans involved in a range of diseases including endocarditis, menengitis, bacteremia, colon cancer etc. Analysis of the genomeof *S. macedonicus* strain ACA-DC 198 isolated from traditional Greek Kasseri cheese revealed important traits of adaptation to the dairy environment. The strain contains a significant percentage of potential pseudogenes indicating that it may have evolved through genome decay processes. *Streptococcus macedonicus* has an extra gene cluster for lactose and galactose metabolism unique among the SBSEC members and a typical proteolytic system required for casein hydrolysis. Furthermore, we found evidence in the genome of *S. macedonicus* suggesting HGT (horizontal gene transfer) events with potential donors *Lactococcus lactis* and *Streptococcus thermophilus*. Perhaps the most pronounced among these HGT events is the presence in *S. macedonicus* of pSMA198 which belongs to the lactococcal pCI305/pWV02 family of plasmids. Our analysis suggests that pSMA198 may have been acquired by *S. macedonicus* from *L. lactis*. Analogous traits have been reported for *S. infantarius* suggesting that at least these two strains present adaptations to the dairy environment among SBSEC. Finally, we were able to identify a number of potential virulence factors (VFs) within the SBSEC members whose presence varied among species of the complex. *Streptococcus macedonicus* and *S. infantarius* miss some of the VFs present in *S. gallolyticus* suggesting a diminished pathogenic potential for the two species.

A031

Preliminary characterization of potential probiotic lactic acid bacteria isolated from raw milk and indigenous locally produced dairy products in Saudi Arabia

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Available data on characterization of beneficial bacteriocinogenic bacteria 'probiotics' in food products of dairy origin in Saudi Arabia appear still little if none. Therefore, aims of this study were to isolate, preliminary characterize and assess the probiotic potential and antibacterial activity levels of some lactic acid bacteria (LAB) isolated from raw milks and indigenous dairy products locally produced in Saudi Arabia. Six raw milk and indigenous locally produced dairy samples were obtained from local markets in Jeddah, Saudi Arabia. Isolation of LAB was carried out by plating onto de Man Rogosa Sharpe (MRS) agar. The plates were incubated at 37°C for 24-48h in anaerobic jars using AnaeroGen system. Isolates were randomly picked up from plates, purified by streaked twice on fresh MRS plates. LAB identification was carried out by phenotypic methods, i.e. colony morphology, cell morphology, motility, Gram staining and production of cytochrome oxidase and catalase. Hemolytic activity by the production of different types of hemolysins was determined by plating actively growing cells of tested strains onto Columbia blood agar. A clear zone on blood agar plates was considered a positive result. Antimicrobial sensitivity was determined by the agar diffusion method using six antibiotics selected as representative of different classes of antimicrobial agents. Based on zones of inhibition, a qualitative report of “susceptible”, “intermediate”or “resistant”could be determined for each bacterial strain tested. Acid and bile tolerance were studied and bacterial viability was assessed by enumeration on MRS agar. Thirty three LAB were isolated from six raw milk and fermented dairy product samples (raw cow milk, raw gaot milk, cow cream, cow laban, goat madheer and cow yogurt).

A032

ACE-Inhibitory activity and technological potential of lactic acid bacteria isolated from Greek traditional yogurt and fermented milk samples

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Fermented dairy products are generally considered to be beneficial for human health as they contain live lactic acid bacteria frequently exhibiting biofunctional features. In the present study, 55 lactic acid bacteria were isolated from three traditional Greek yogurt samples and five fermented milk samples, in order to assess their ability to produce bioactive peptides with inhibitory activity against the angiotensin-converting enzyme (ACE) as well as their technological potential. Strain typing was performed by rep-PCR while identification at species level was done by 16S rDNA sequencing. Acid production potential was determined by measuring the pH in milk cultures after 6 h of growth; proteolytic activity by growth on milk agar plates and the *o*-phthaldialdehyde spectrophotometric assay; peptidolytic activity using spectrophotometric assay with Gly-Pro-p-nitroanilide as substrate; lipolytic activity by growth on tributyrin agar plates; citrate metabolism by growth on Simmons citrate agar plates; exopolysaccharide production by growth on ruthenium milk agar plates. Finally, ACE-I activity was evaluated using a spectrophotometric assay with N-Hippuryl-His-Leu hydrate as the substrate. Among the 33 lactobacilli and 22 cocci examined, several *Lactobacillus delbrueckii* subsp. *bulgaricus*, as well as *Streptococcus thermophilus* strains have been identified, along with other thermophilic, homofermentative species. It was also demonstrated that many strains exhibit promising technological features. Moreover, a number of them were selected for their ACE-I activity, and the isolation and identification of the respective bioactive peptides is currently in progress.

A033

Characterization of lactic acid bacteria isolated from crop of *Phormia regina* (Meigen)

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Many insect species are inhabited by large and diverse microbial communities resident in their digestive system. Lactic acid bacteria (LAB) are found as commensals within humans, insects and animal. In this work we describe the isolation and characterization of LAB associated to the crop of *Phormia regina*, commonly known as the “black blow fly”. The crop is a diverticulated structure, consisting of a duct and a bilobed sac which diverts ventrally and comes off from the foregut just anterior to the cardia. Its ability to store considerable amounts of liquid nutrients that may be in short supply makes this organ essential to the Diptera. Insects were obtained from colonies of *P. regina* maintained in the insectary of the Laboratory of Phisiology of the University of Cagliari, Italy. Dissection of the insects was carried out following the method described by Youdeowei (1974). The crop of each fly was aseptically removed and homogenized in 1 ml of sterile 0,9% saline. In total, more than 30 presumptive LAB isolates with Gram-positive and catalase-negative properties were obtained from the samples using MRS agar incubated in anaerobic conditions at 30 °C. The LAB counts varied from 3.9 to 4 log cfu/crop. The strains were identified at the species level by physiological tests, biochemical characteristics, carbohydrate fermentation patterns, 16S rRNA gene sequencing and Rep-PCR. *Lactobacillus* was the most prevalent genus, *L. sakei* and *L. plantarum* being the main species detected. *Leuconostoc mesenteroides* was also frequently isolated. This is the first study that describes the presence of LAB associated to the alimentary tract of this fly species.

A034

Known and assumed potential of the Agroscope culture collection

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The Agroscope Institute for Food Sciences (IFS) has got a long tradition for both the development and production of bacterial cultures used in milk and meat products. In 1908 already, cultures were delivered to Swiss cheese makers to ensure a fast and standardised acidification of the vat milk during cheese production, thus guaranteeing safe products. The Agroscope culture collection has been established in the course of the 20th century by systematically isolating bacteria from milk products and cheese production plants known for excellent cheese quality.
Today, the Agroscope culture collection consists of more than 10.000 strains encompassing approximately 300 species. The majority of strains are lactic acid bacteria (LAB). Preliminary investigations suggest that most of the collection’s LAB are free from acquired antibiotic resistance (AbR) genes since the bacteria have been collected before the massive use of antibiotics in animal husbandry. Furthermore, from results of whole genome sequencing a high intraspecific diversity can be expected.
The Agroscope culture collection is the backbone for the development of customized starter and non-starter cultures for various applications in food fermentation and biotechnology, especially in milk products. According to their specific characteristics, the strains of the collection are used in the frame of applied projects in fields as varied as flavour formation during cheese ripening, protective cultures or cultures for proof of origin, as well as in more fundamental research on the pangenome.
The vast diversity and versatility of the Agroscope culture collection makes it a precious tool with high potential for future biotechnological developments.

A035

Species-level identification in mixed populations of lactic acid bacteria by multiplexed PCR

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In this study, we succeeded in differentiating 19 species of Lactic Acid Bacteria including *Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus reuteri, Lactobacillus gasseri, Lactobacillus acidophilus, Lactobacillus helveticus, Lactobacillus rhamnosus, Lactobacillus casei, Lactobacillus paracasei, Lactobacillus bulgaricus , Lactobacillus salivarius, Bifidobacterium animalis* subsp. lactis, *Bifidobacterium bifidum, Bifidobacterium longum* subsp. *infantis, Bifidobacterium longum* subsp. *longum, Bifidobacterium breve, Lactococcus lactis, Streptococcus thermophilus*, and *Pediococcus pentosaceus* by means of *pgi* gene sequence comparison. The *pgi* gene was extracted from publically and privately held genomes of the species above (n=205) and aligned by UPGMA clustering. Full-length sequences of approximately 1700 bps were considered for inference to phylogenetic trees, with regions of polymorphism within each species utilized for primer design, targeting a single species. All primer sets were then tested against a range of 7-10 individual isolates of each species. A multiplex PCR protocol for the simultaneous detection of these 19 species of bacteria was optimized into four reactions. The sizes of the amplicons permitted the unambiguous identification of strains. Additionally, the nineteen target species were tested in several combinations of strains with a minimum population of 10^5 CFU and a maximum load of 10^{10} CFU of all other possible strains combined. By exploiting the polymorphic regions of the *pgi* gene, a species-specific set of primers has been developed to identify a single species or used in a multiplex assay to rapidly detect any number of 19 species. The clear distinction validates the approach of using the *pgi* gene as a phylogenetic-taxonomic marker for these closely related species. This method offers a rapid and reliable means of identification of lactic acid bacteria species important in the food and dietary supplement industries.

Section B: Genetics and Physiology

B001 **Anti-inflammatory effect of *Lactococcus lactis* strains containing a eukaryotic DNA vector coding for interleukin 10 for the prevention of inflammatory bowel diseases**
M. Zurita Turk, F.A. Lima, A.C. Santos, C. Prosperi, P. Mancha Agresti, V. Pereira, B.M. Souza, M. de Azevedo, C. Rocha, V. Azevedo, S. Leclercq, A. Miyoshi

B002 **Aerobic glucose metabolism of *Lactobacillus brevis*: improved biomass and the oxygen dependent ATP-yielding pathway from lactate to acetate**
J. Kong, L. Zhang, T. Guo

B003 **Transcriptional analysis of *Lactobacillus brevis* BSO 464 grown in degassed and pressurized beer.**
A. Bergsveinson, R. Ziola

B004 **Genome sequence and transcriptome analysis of a meat spoilage lactic acid bacterium *Lactococcus piscium* MKFS47**
M. Andreevskaya[1], P. Johansson[2], P. Laine[1], R. Rahkila[2], E. Jääskeläinen[2], L. Paulin[1], J. Björkroth[2], P. Auvinen[1]

B005 **A typical signal sequence-independent export mechanism of elongation factor Tu in *Lactobacillus reuteri***
K. Nishiyama, Y. Yamamoto, T. Mukai

B006 **The novel *Lactococcus lactis* protein LcpI interacts with LCP-family proteins and facilitates secretion of LysM-containing autolysin**
S. Kulakauskas, J. Armalyte, G. Buist, S. Mesnage, S. Furlan, S. Chat, P. Courtin, O.P. Kuipers, J. Kok, P. Serror, M. Chapot-Chartier

B007 **Transcript fate of overexpressed membrane proteins in single *Lactococcus lactis* cells**
L.A. van Gijtenbeek, B. Poolman, A.M. van Oijen, J. Kok

B008 **Potential of *Lb. plantarum* IBB3036 and *Lb. salivarius* IBB3154 for use as probiotics in animals**
J. Zylinska, R.K. Gorecki, J.K. Bardowski

B009 **The cooperative behavior of *Streptococcus thermophilus* urease in the yogurt consortium**
D. Mora, M.C. Remagni, S. Arioli

B010 **Bimodal expression of the *Lactococcus lactis* arginine deiminase operon**
J. Siebring, M.J. Hoogeboom, J. Kok

B011 **Acidocin B produced by *Lactobacillus acidophilus* M46 is a circular bacteriocin**
M.J. Van Belkum, J.Z. Acedo, C.T. Lohans, John C. Vederas

B012 **Functional analysis of the N-terminal region of endolysin Lyb5 encoded by *Lactobacillus fermentum* temperate bacteriophage fPYB5**
T. Guo, J. Kong, C. Zhang, W. Liu, S. Wang

B013 **Role of peptidoglycan endopeptidases of the NlpC/P60 family in *Lactobacillus plantarum***
M.C. Duchêne, R.T. Rolain, B. Hallet, P. Hols

B014 **Identification of sirtuin homolog enzymes of *Lactobacillus paracasei***
H. Atarashi, S. Ikehara, N. Tanaka, Y. Shiwa, H. Yoshikawa, S. Okada, A. Endo, T. Miyaji, J. Nakagawa

B015 **Identification of bifidobacterial promoters that are suitable for transposon mutagenesis in bifidobacteria**
M. Sakanaka, S. Tamai, Y. Hirayama, A. Yokota, S. Fukiya

B016 **The influence of lactic acid bacteria strains on the antifungal resistance mechanisms of *Candida albicans* strains**
C. Sarbu, T. Vassu, I. Stoica, L. Marutescu, C. Chifiriuc, I. Nedelcu, D. Pelinescu

B017 **Transposon mutagenesis in *Bifidobacterium breve*: construction and characterization of a Tn5 transposon mutant library in *Bifidobacterium breve* UCC2003**
L. Ruiz, M. O'Connell-Motherway, N. Lanigan, D. van Sinderen

B018 **Draft genome sequence and annotation of *Lactobacillus plantarum* strain Lp90 isolated from Apulian (Italy) wine**
G. Spano, G. Caggianiello, A. Lamontanara, L. Orrù, V. Capozzi, V.M. Vania, B. Renckens, J.R. Bayjanov, S. van Hijum, L. Cattivelli

B019 ***Lactococcus lactis* metabolism and gene expression during growth on plant tissues**
L. Golomb, M.L. Marco

B020 **Genomics of the genus *Bifidobacterium* reveals species-specific adaptation to the glycan-rich gut environment**
C. Milani, G.A. Lugli, S. Duranti, F. Turroni, F. Bottacini, M. Mangifesta, A. Viappiani, L. Mancabelli, D. Mora, N. Segata, R. Barrangou, A. Margolles, D. van Sinderen, M. Ventura

B021 **Motility of *Lactobacillus curvatus* NRIC 0822: Novel evidence of flagellum-mediated motility outside the *Lactobacillus salivarius* clade**
F.J. Cousin, S. Lynch, H.M.B. Harris, A. Mccann, D.B. Lynch, T. Irisawa, S. Okada, A. Endo, P.W. O'Toole

B022 **Expression of prophage-encoded endolysins contributes to autolysis of *Lactococcus lactis***
G. Buist, G.R.R. Visweswaran, D. Kurek, M. Szeliga, O.P. Kuipers, J. Kok

B023 **Comparative genome analysis reveals strains specific gene clusters involved in exopolysaccharides biosynthesis in *Lactobacillus plantarum***
G. Spano, G. Caggianiello, D. Fiocco, M.P. Arena, P. Russo, L. Orrù, A. Lamontanara, S. van Hijum, V. Capozzi

B024 **Investigation of factors affecting aerobic and respiratory growth in *Lactobacillus casei* N87**
T. Zotta, A. Ricciardi, R.G. Ianniello, G. Iacomino, G. Pannella, E. Parente

B025 **Systematic phenome analysis of *Lactococcus lactis* deletion mutants in genes coding for hypothetical transcriptional regulators.**
P. Oskolski, K. Szatraj, A. Koryszewska-Baginska, J. Bardowski, T. Aleksandrzak-Piekarczyk

B026 **Isolation and characterization of oxidative stress-resistant lactobacilli mutants promoting their survival during low-temperature storage**
Y. Yamamoto, R. Sasaki, T. Mukai

B027 **Impact of fluorescent labeling and flow cytometric cell sorting on viability and vitality of *Lactococcus lactis***
G. Hansen, C. Lindvald Johansen, L. Jespersen, N. Arneborg

B028 **Biofilm formation by *Lactobacillus plantarum* WCFS1 under static and dynamic conditions**
M.D. Fernández Ramírez, M.N.N. Nierop Groot, E.J. Smid, T. Abee

B029 **Identification of Restriction-Modification systems of *Bifidobacterium animalis* subsp. *lactis* CNCM I-2494 by SMRT sequencing and associated Methylome analysis**
M. O'Connell Motherway, D. Watson, F. Bottacini, A. Clark, R.J. Roberts, J. Korlach, P. Garault, C. Chervaux, J.E.T. van Hylckama Vlieg, T. Smokvina, D. van Sinderen

B030 **Molecular characterization of inulinase genes of newly isolated Bulgarian *Lactobacillus* strains that metabolize prebiotic carbohydrates**
P. Velikova, K. Petrov, P. Petrova

B031 **Cross-talk mediated by Rgg-peptide cell-cell communication systems from *Streptococcus salivarius***
J.T.R. Mignolet, L. Fontaine, P. Hols

B032 **The physiological role of two secondary amino acid transporters, SERP1 and SERP2, in *Lactococcus lactis***
E.E.E. Noens, H. Trip, N.L. Mulder, J.S. Lolkema

B033 **Synthesis of alanine and 2-aminobutyrate by an aminotransferase in *Pediococcus acidilactici***
S. Irmler, R. Portmann, B. Guggenbühl, H. Berthoud

B034 **Detection of *Lactobacillus parabuchneri*, a potential spoilage organism, in milk and cheese**
S. Irmler, D. Wüthrich, R. Bruggmann, E. Eugster, D. Wechsler, H. Berthoud

B035 **High efficiency *Lactobacillus casei* electrotransformation**
L. Welker, E. Hughes, J.R. Broadbent

B036 **Construction and characterization of prophage-free derivative of the prototype *Lactococcus lactis* subsp. *lactis* IL1403 strain**
P. Langella

B037 **Monod revisited: Bet-hedging during bacterial diauxic shift**
A. Solopova, J. van Gestel, F.J. Weissing, H. Bachmann, B. Teusink, J. Kok, O.P. Kuipers

B038 **A novel type of peptidoglycan-binding domain highly specific for amidated D-Asp cross-bridge, identified in *Lactobacillus casei* bacteriophage endolysins**
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S. Balzaretti, S. Arioli, V. Taverniti, D. Mora, S.D. Guglielmetti

B001

Anti-inflammatory effect of *Lactococcus lactis* strains containing a eukaryotic DNA vector coding for interleukin 10 for the prevention of inflammatory bowel diseases

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Interleukin-10 (IL-10) is one of the most important anti-inflammatory cytokines involved in the intestinal immune response and its absence is related to inflammatory bowel diseases (IBD). However, oral treatment with IL-10 is limited due to its extreme sensitivity and survival in the gastrointestinal tract, and systemic treatments are hindered by IL-10's short half-life and undesirable side effects. In this context, the aim of this work was to evaluate the anti-inflammatory effect of two *Lactococcus lactis* (LL) strains: LL MG1363 (wild-type) and LL MG1363 FnBPA [capable of delivering a eukaryotic expression vector (pValac) coding for murine IL-10 to eukaryotic cells] as a new strategy for the prevention of IBD in two mouse models [Dextran sulfate sodium (DSS)-induced and Trinitrobenzenesulfonic acid (TNBS)-induced]. The pValac-*IL-10* plasmid was firstly constructed and its functionality was confirmed by evaluating the expression and secretion of IL-10 by transfected eukaryotic cells. Mice from the DSS and TNBS models received, by gavage, bacterial supplementation (10^9 UFC/mouse/day) of LL MG1363 (wt), LL MG1363 (pValac-*IL-10*), LL MG1363 FnBPA (wt), LL MG1363 FnBPA (pValac-*IL-10*) or saline solution (control and DSS or TNBS groups). On sacrification day, large intestines were removed, visually inspected for macroscopic evaluation and prepared for histological evaluation, cytokine and secretory IgA analysis. Overall, mice from the pValac-*IL-10* groups showed statistically lower damage scores in their intestines (both at macroscopic and microscopic levels), higher IL-10 and lower TNF-α, IL-6 and IL-17 levels (DSS assay), and decreased IL-17 and IFN-γ levels (TNBS assay), compared to mice from the control and wt-groups, showing that administration of these two LL strains containing the pValac-*IL-10* plasmid was effective in the prevention of inflammation in two murine models of IBD.

B002

Aerobic glucose metabolism of *Lactobacillus brevis*: improved biomass and the oxygen dependent ATP-yielding pathway from lactate to acetate

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The facultative anaerobe *Lactobacillus brevis* generates more acetate and a higher biomass under aerobic growth than anaerobic condition, which is economically beneficial to the commercial starter culture production. But the detailed metabolic pathway from pyruvate to acetate in the aerobic condition has yet to be fully understood. In this study, the metabolisms of *L. brevis* ATCC 367 under static and aerobic conditions were determined, and revealing the conversion from lactate to acetate after glucose exhaustion under aerobic condition. To investigate the metabolic pathway from pyruvate to acetate, the pyruvate dehydrogenase gene (*pdh*) or pyruvate oxidase gene (*pox*) of *L. brevis* ATCC 367 was knockout, respectively. The results indicated that the pyruvate dehydrogenase played the dominant role in the acetate production under aerobic condition. Moreover, gene expression analysis and enzymatic measurement further confirmed the induction of oxygen on the pyruvate oxidase and the suppression of glucose on the pyruvate dehydrogenase and pyruvate oxidase, in which stimulated the oxygen dependent ATP-yielding pathways though the pyruvate dehydrogenase from lactate to pyruvate, subsequently to acetate conversion. Therefore the mechanisms of high biomass in the aerobiosis of *L. brevis* were clarified.

B003

Transcriptional analysis of *Lactobacillus brevis* BSO 464 grown in degassed and pressurized beer

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Beer is a harsh medium to support bacterial growth, given the presence of antimicrobial compounds such as ethanol and hops and inhospitable levels of oxygen, carbon dioxide and nutrients. Despite these conditions, there are bacteria capable of establishing growth in beer, thereby causing spoilage. Beer-spoiling organisms (BSOs) are frequently lactic acid bacteria, however, the ability to spoil beer is an isolate-specific trait, not a species-conserved ability. This indicates a high degree of genetic specialization in BSOs, however, all potential genetic adaptations involved have yet to be fully characterized. Recently, our laboratory has gathered evidence, which suggests the presence of pressure and dissolved carbon dioxide (dCO_2) is a strong selective pressure for rapid BSOs. Utilizing the sensitive technique of RNAseq, we have determined the difference in transcriptional activities of the most virulent BSO within our culture collection, *Lactobacillus brevis* BSO 464 (Lb464), during its growth in pressurized and non-pressurized beer. We next examined the behavior of specific pressure-induced transcripts under pressure conditions in several BSOs of varying beer-spoilage ability using droplet digital PCR (ddPCR) to correlate elevated expression of pressure- and dCO_2- specific transcripts with increased virulence in beer. These results were analyzed within the context of several meta-genomic comparisons performed in our laboratory of BSOs and non-BSOs, in order to reveal the shared genetic elements contributing to increased beer-spoilage ability. Several interesting insights have been gained into the main genetic and physiological responses that these beer-spoilage organisms must employ to circumvent the various stresses in the beer environment.

B004

Genome sequence and transcriptome analysis of a meat spoilage lactic acid bacterium *Lactococcus piscium* MKFS47

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Lactococcus piscium is a psychrotrophic lactic acid bacterium (LAB) that has been shown to be either food-preserving or food spoilage-associated, depending on the individual strain properties and growth circumstances. The presented strain, MKFS47, causes spoilage in a variety of cold-stored packaged meat products, leading to formation of buttery and sour off-odours. Nevertheless, the quantity of this bacterium is often underestimated, when the standard mesophilic enumeration technique is applied. Our study presents the complete genomic sequence of *L. piscium* MKFS47 that will be indispensible for the understanding and explaining its physiology and spoilage potential, as well as for the genomic manipulations and phylogeny studies. In addition, time-course RNA-seq based transcriptomic data were analyzed for the pure culture of *L. piscium*. The genome consists of only one chromosome with 2289 CDSs encoded. Comparative genomic analysis was performed with 28 annotated genomes from *Lactococcus* genus, it revealed three distinct clades within this group. Constructed pathway database shows that *L. piscium* contains genes for glycolysis, biosynthesis of almost all amino acids and catabolism of wide range of carbohydrates, including many plant-specific. In addition, genes involved in the production of spoilage substances were determined. Many functions that are present in the genome, such as nucleoside utilization, adhesion, bacteriocins and hydrogen peroxide production, may be beneficial for the survival in meat environment. Transcriptome analysis revealed that genes, involved in sugars and glycerol catabolism, putrescine formation, glycogen metabolism and stress protection, are highly upregulated, while downregulated pathways include the biosynthesis of cell wall, branched-chain amino acids and nucleotides, as well as oligopeptide transport. Noteworthy, this study is a first whole transcriptome report of a spoilage LAB, combined with the genome analysis.

B005

A typical signal sequence-independent export mechanism of elongation factor Tu in *Lactobacillus reuteri*

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Our recent study showed that the elongation factor Tu (EF-Tu), located on the cell surface of *Lactobacillus reuteri* JCM1081, binds to sulfated carbohydrates of mucins. Several moonlighting proteins such as EF-Tu, which lack a typical N-terminal export signal sequence, have been found to be localized outside the cell in *Lactobacillus*. However, the mechanism underlying the N-terminal signal sequence-independent export of these cytoplasmic proteins is largely unknown. The aim of this study is to identify the EF-Tu secretion signal and characterize its export pathway. We found that the secretion of EF-Tu is affected by sodium azide, which is known to be a SecA inhibitor. The *secY::cm*^{r} mutant of *L. reuteri* JCM1081 showed little export of EF-Tu, compared to the wild-type strain. Moreover, the secretion of EF-Tu was not mediated by membrane vesicle budding and autolysis. These results strongly suggest that Sec pathway was directly or indirectly involved in the secretion of EF-Tu. Furthermore, using partial deletion of plasmid-encoded *ef-tu* genes, we could identify the 35-amino-acid C-terminal region in EF-Tu that contributes to its secretion. The replacement of several hydrophobic amino acid residues in the C-terminal sequence with glycine strongly interfered with export of EF-Tu without abolishing the intracellular expression, indicating that these hydrophobic amino acids are essential for the export of EF-Tu. We conclude that, in *L. reuteri*, the secretion of EF-Tu depends on this C-terminal sequence containing certain hydrophobic amino acids and that this sequence contributes to the Sec-dependent export processes.

B006

The novel *Lactococcus lactis* protein LcpI interacts with LCP-family proteins and facilitates secretion of LysM-containing autolysin

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Bacterial autolysins are important for cell growth, division and separation. The major autolysin AcmA of *Lactococcus lactis* is composed of a signal peptide that is followed by a glucosaminidase active site and a peptidoglycan-binding domain that contains three LysM modules. In a transposon mutagenesis screen we identified the gene *lcpI*, inactivation of which strongly diminished the secretion of AcmA. The *lcpI* homolog of the Gram-positive opportunistic pathogen *Enterococcus faecalis* was shown to have the same function regarding the secretion of the major enterococcal peptidoglycan hydrolase, AtlA, which contains a glucosaminidase catalytic domain followed by 6 LysM modules. In *L. lactis* this phenotype was shown to depend on the LysM domain of AcmA. Using a bacterial two-hybrid assay we demonstrated that LcpI interacts with LcpA and LcpB, two proteins that belong to the LytR-CpsA-Psr (LCP)-family of proteins, responsible for attachment of secondary cell wall polymers to peptidoglycan. Our results suggest that LcpI may be involved in the localization of cell wall polysaccharides in the septal region of the lactococcal cell wall. We hypothesize that cell wall polysaccharides could possibly shield peptidoglycan from premature LysM binding during the secretion process and thus ensure efficient secretion of LysM-containing proteins.

B007

Transcript fate of overexpressed membrane proteins in single *Lactococcus lactis* cells

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The Gram-positive bacterium *Lactococcus lactis* has been proposed as an alternative host for the production of heterologous membrane proteins. Its physiological response to overproduction of various membrane proteins has been elucidated[1,2] in order to identify putative bottlenecks during overexpression of heterologous membrane proteins. However, a clear explanation for the differences between well and poorly expressed membrane proteins is lacking. The aim of this study was to identify possible post-transcriptional anomalies that occur during the lifetime of mRNA encoding poorly expressed membrane proteins. We localized overexpressed mRNA molecules coding for various membrane and cytoplasmic proteins in single living *L. lactis* cells using epifluorescence microscopy and the MS2-mRNA tagging system. These studies were complemented with single-molecule fluorescent in situ hybridization. Both techniques consistently showed that overexpressed transcripts of poorly produced membrane proteins accumulate in mRNA-dense polar aggregates while transcripts of normally expressed proteins do not. Therefore, the biogenesis of recalcitrant membrane proteins seems to be hampered earlier than generally assumed.

1. R.K. Marreddy et al, PLoS One, 2011, 6(8), e24060.
2. J.P. Pinto et al, PLoS One, 2011, 6(7):e21873.

B008

Potential of *Lb. plantarum* IBB3036 and *Lb. salivarius* IBB3154 for use as probiotics in animals

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ECO-FCE aims to manipulate the gut microbiome to promote ‘good’ FCE (feed conversion efficiency) in pigs and broiler chickens. Modulation of intestinal microbiota might be achieved through the use of probiotics and/or synbiotics. Probiotics are non-pathogenic bacteria that are capable of surviving and persisting in the host gastrointestinal tract and exert a beneficial effect on the host. In this work, 50 strains from the IBB collection belonging to the genus of *Lactobacillus* were tested for their carbohydrate catabolic potential and enzymatic profile using API CH50 and API ZYM test strips, respectively. As a result, five strains with the typical or the most original and broad spectrum of carbohydrates metabolized were chosen for further studies including survivability at low pH, bile salt susceptibility, adhesion, bacteriocin production and antibiotic susceptibility. It was found that all five strains tolerated bile salts up to 2% concentration, whereas two of the 5 strains maintained viability after incubation at pH lower than 3. The same two strains exhibited adhesion properties and were able to inhibit *Staphylococcus aureus* growth probably due to bacteriocin production. However, inhibition zones were opaque. These two strains (*Lb. plantarum* IBB3036 and *Lb. salivarius* IBB3154) will be further studied in combination with prebiotics in order to prepare synbiotic formulations. The synbiotics will then be injected *in ovo* to determine their effect on broiler chicken gut microbiota post hatch.

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B009

The cooperative behavior of *Streptococcus thermophilus* urease in the yogurt consortium

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Most food fermentation processes involve mixed cultures where different microbial species interact each other. These interactions may have neutral, positive or negative effect on the fitness of fermenting strains. The microbial interactions determining positive effects on interacting micro-organisms, are classified as mutualism and the best example of mutualism in a food process is represented by the yogurt consortium, consisting of the lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. In the yogurt consortium, the interaction between the two species have been classified as a positive proto-cooperation, which enhances the growth rate and the size of each population, whereas the independent growth of the two species in milk led to a lower growth fitness. In this study, we provide the first evidence that urease activity of *S. thermophilus* is able to interfere with the intracellular pH of *L. delbrueckii* thus affecting its physiology during milk fermentation. We therefore consider urease activity an altruistic cooperative trait, which is costly for urease-positive individuals but provides a local benefit because other individuals can take advantage of a urease-modulated pH. Unlike other described cooperative behaviors, urease provides first a benefit to the individual that harbors the enzymatic activity, and then a local benefit. Beside the known interactions occurring between *S. thermophilus* and *L. delbrueckii* in the yogurt consortium, the modulation of cell bioenergetics efficiency by the alkalization of intracellular pH represents a new type of cooperation. Urea-dependent alkalization of cytoplasm acts directly on the third layer of cellular regulatory mechanisms thereby interfering with the kinetic parameters of enzymes involved in homolactic fermentation of both *S. thermophilus* and *L. delbrueckii*.

B010

Bimodal expression of the *Lactococcus lactis* arginine deiminase operon

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Phenotypic heterogeneity describes the development of a population of isogenic (bacterial) cells into distinct phenotypes despite their environment being identical. Inter-individual differences can occur as a result of stochasticity: differences in cell volume, protein content, mRNA content, etc. These differences may cause vast physiological effects, especially in bistable regulatory networks that can diverge a population into cells that are either ‘ON’ or ‘OFF’ for a particular trait. Cell motility is an example of bimodal phenotype development; cells can be either motile or non-motile.
Using transcriptional promoter-*gfp* fusions, it was discovered that the promoter of the arginine deiminase operon, P*arc*, displays a bimodal expression profile when *Lactococcus lactis* is grown in whey permeate. The arginine deiminase pathway produces i.e. ammonia and, indeed, high P*arc* expressing cells display increased acid tolerance. Studies are conducted in order to identify whether the cells in which P*arc* is highly expressed regulate environmental pH to the benefit of all (kin selection) and how the underlying regulatory network supports this bifurcation.

B011

Acidocin B produced by *Lactobacillus acidophilus* M46 is a circular bacteriocin

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Acidocin B is a plasmid-encoded bacteriocin produced by *Lactobacillus acidophilus* M46. Previously, it was reported that acidocin B is a linear peptide composed of 59 amino acids[1]. However, its high sequence homology to gassericin A, a circular bacteriocin from *Lactobacillus gasseri* LA39, suggested that acidocin B might actually be circular as well. We purified acidocin B and the peptide was analyzed by MS/MS sequencing and MALDI-TOF mass spectrometry. A peptide sequence was deduced, consisting of 58 amino acids with a MW of 5621.4 Da, which is 18 Da less than expected from a linear peptide. Based on these results and the genetic sequence, it can be concluded that acidocin B is indeed a circular bacteriocin. The sequence of the acidocin B gene cluster was determined and showed high similarity to that of gassericin A. The elucidation of its solution structure via NMR spectroscopy is now in progress.

[1] Leer *et al*. (1995) Microbiology 141:1629-1635.

B012

Functional analysis of the N-terminal region of endolysin Lyb5 encoded by *Lactobacillus fermentum* temperate bacteriophage fPYB5

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Lactobacillus fermentum temperate bacteriophage φPYB5 uses a two component cell lysis cassette comprised of endolysin (Lyb5) and holin (Hyb5) to burst the host cell. Previous results showed that the expressed Lyb5 protein caused *Escherichia coli* lysis slowly. Here, a putative signal peptide motif (referred to as SP_{Lyb5}) with 29 amino acids was predicted at the N-terminal of Lyb5. In *E. coli*, we showed that the SP_{Lyb5} motif could guide Lyb5 to reach the cell wall through the host Sec pathway. And the SP_{Lyb5} motif was necessary for Lyb5 delivery, as its deletion mutant (Lyb5ΔSP) could not result in host cell lysis, indicating that the SP_{Lyb5} might serve as functional signal peptide. However, the expressed Lyb5 protein appeared in the cytoplasm, cytoplasmic membrane and periplasm fraction with the same molecular mass, suggesting that SP_{Lyb5} could direct Lyb5 across the cytoplasmic membrane without cleavage as the typical signal peptide. And the similar results were obtained using *Lactococcus lactis* as a host for expression of Lyb5. In addition, SP_{Lyb5} motif was able to drive the fused NucleaseB protein to extracytoplasm in *E. coli* as well as in *L. lactis*. These results strongly indicated that SP_{Lyb5} shared obvious characteristics with signal-anchor-release domain both in Gram-negative and Gram-positive hosts, which was firstly identified by experiment in lactic acid bacteria phages. At last, we showed that 41% of the retrieved endolysins from lactobacilli bacteriophages contained putative N-terminal signal peptides, suggesting probable prevalence of sec-exported endolysin among lactobacilli bacteriophages.

B013

Role of peptidoglycan endopeptidases of the NlpC/P60 family in *Lactobacillus plantarum*

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Lactobacillus plantarum is used as a model species to study peptidoglycan hydrolases (PGHs) due to its low PGH abundance (12 members). In this study, we are investigating the role of 4 endopeptidases of the NlpC/P60 family (LytA-B-C-D). Previous work showed that LytA-deficient cells display a strongly altered morphology and aggregate, suggesting the involvement of LytA in division and/or elongation. Analysis of the *lytA* mutant PG indicates that LytA cleaves the stem peptide between D-Glu and meso-DAP. Another NlpC/P60 endopeptidase, LytC, could not be deleted, suggesting an essential role in the cell cycle.
The objectives of the current work are threefold: (i) an in vitro biochemical characterization of LytA and its three paralogs LytB, LytC and LytD, (ii) a genetic investigation of the functional role of the 4 members of the Lyt family (simple and multiple mutants) and (iii) the identification of cell-cycle players affected by LytA depletion and potentially interacting with LytA.
Since these PGHs are toxic when they are cloned in classical expression systems in *Escherichia coli* or *Lactococcus lactis*, we developed a range of conditional expression systems for their direct study in *Lactobacillus plantarum*. In order to create conditional deletion mutants, we used a single cross-over system where gene deletion is coupled with the conditional expression of a copy of this gene by a nisin-controlled promoter. In order to produce tagged or fluorescent coupled proteins, we set up an inducible system borne by a low copy number plasmid which is based on the ComRS system controlling competence in *Streptococcus thermophilus*. These systems are currently exploited for the construction of multiple Lyt-deficient strains, protein purification, and PGH sub-localization studies.

B014

Identification of sirtuin homolog enzymes of *Lactobacillus paracasei*

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Sirtuin is an NAD^+-dependent protein deacetylase, and functions in controlling gene expression of more than 100 genes in *Homo sapiens* via histone deacetylation. It is said to be a longevity gene, because sirtuin-mediated lifespan extension effect has been reported in mammals, nematode, and yeast. In addition, it is known to be involved in stress response to hydrogen peroxide and heat shock in a prokaryote, *Escherichia coli*. On the other hand, little is known about its existence and roles in lactic acid bacteria. In this study, the existence and roles of sirtuin in *Lactobacillus paracasei*, frequently used as probiotics, were examined. Tested strains were *Lactobacillus paracasei* NRIC 0644, NRIC 1917 and NRIC 1981, isolated from different sources. Draft genome sequences of these three strains were determined by second generation sequencer, confirming the existence of sirtuin homolog in the genomes of the three strains. The amino acid sequences of these sirtuin homolog proteins were highly homologous to those of *Lactobacillus paracasei* sirtuin available in the gene bank date base. The enzyme kinetic studies of the purified recombinant bacterial sirtuin proteins showed that the deacetylase activity was comparable to that of recombinant human SIRT1 protein, but with different optimal temperature. The activity was stimulated by resveratrol, but inhibited by suramin. Cholate, a major component of bile, caused severe growth inhibition of lactic acid bacteria. However, pre-treatment of *Lactobacillus paracasei* strains with resveratrol alleviated growth inhibition by cholate. On the contrary, pre-treatment of the bacteria with suramin led to exacerbation of it. Taken together, these data suggested that sirtuin homologof *Lactobacillus paracasei* may play a role in cholate stress response.

B015

Identification of bifidobacterial promoters that are suitable for transposon mutagenesis in bifidobacteria

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Transposon mutagenesis is a powerful tool for functional genomics. However, it has not been reported in bifidobacteria except for the application of the Tn5-based mutagenesis system in *Bifidobacterium breve* strains (Ruiz et al., 2013). Therefore, we have aimed to develop a new transposon mutagenesis system in bifidobacteria. The system requires strong expression of a transposase gene in bifidobacteria for efficient mutant generation. In contrast, its weak expression in the *Escherichia coli* cloning host is desirable for stable construction and propagation of the transposon vector. Therefore, bifidobacterial promoters that are highly active in bifidobacteria but not in *E. coli* will meet these requirements. Here, we developed a new promoter-reporter system and identified the bifidobacterial promoters that are able to fulfill such requirements.
The promoter-reporter system was constructed using the *Bifidobacterium longum* α-galactosidase gene as a reporter and two α-Gal gene-deletion mutants (*E. coli* JW4080 and *B. longum* 105-A *Δaga*) as host strains. Activities of seven bifidobacterial promoters were evaluated by measuring the α-Gal activity in both *E. coli* and *B. longum*.
Among the tested seven promoters, D-xylulose 5-phosphate/D-fructose 6-phosphate phosphoketolase gene (*xfp*) promoters from two *Bifidobacterium* species and a fructose ABC transporter operon (*fruEKFG*) promoter from *B. longum* showed low activity in *E. coli*. In *B. longum*, the *xfp* promoters showed high activity in the presence of glucose. In contrast, the *fruEKFG* promoter was strongly induced in the presence of carbohydrates other than glucose, including fructose, xylose, and ribose. Thus, the *xfp* and *fruEKFG* promoters seemed suitable for the transposon mutagenesis system in bifidobacteria. Applicability of these promoters for the transposon mutagenesis system is currently under investigation.

B016

The influence of lactic acid bacteria strains on the antifungal resistance mechanisms of *Candida albicans* strains

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Infections caused by *Candida* (C.) species represent an important clinical problem and the current antifungal drugs present a high toxicity. Clinical studies revealed that some lactic acid bacteria (LAB) strains are able to increase the effectiveness of some antifungal drugs.
The aim of our study was to determine the mechanism involved in the ability of LAB strains to modulate fluconazole susceptibility of *C. albicans* strains.
In this study 153 LAB strains isolated from fermented products and gastrointestinal tract and a highly resistant strain of *C. albicans* isolated from a patient with vaginal infection were used.
The screening of LAB strains that induce an increase in fluconazole susceptibility of yeast cells was evaluated using disk diffusion assay. *Lactobacillus* (Lb.) *plantarum* CMGB 1 and *Lb*. *rhamnosus* CMGB 38 strains were selected after the screening in order to evaluate the effect exerted on fluconazole susceptibility of *C. albicans* CMGBy 13. Flow cytometry and fluorescence microscopy based on Nile Red accumulation revealed a decrease of yeast efflux pumps activity in presence of LAB strains. *C. albicans* CMGBy 13 fluconazole resistance genes expression was assessed using qRT-PCR technique. The results of the present study highlighted that combination of *Lb. plantarum* CMGB 1 strain with fluconazole had a synergic effect against *C. albicans* CMGBy 13 susceptibility by decreasing ERG11 and MDR genes expression, which are known to be the main mechanisms involved in fluconazole resistances of *C. albicans* strains.
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B017

Transposon mutagenesis in *Bifidobacterium breve*: construction and characterization of a Tn5 transposon mutant library in *Bifidobacterium breve* UCC2003

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Bifidobacteria are claimed to contribute positively to human health through a range of beneficial or probiotic activities, including amelioration of gastrointestinal and metabolic disorders, and therefore this particular group of gastrointestinal commensals has enjoyed increasing industrial and scientific attention in recent years. However, the molecular mechanisms underlying these probiotic mechanisms are still largely unknown, mainly due to the fact that molecular tools for bifidobacteria are rather poorly developed, with many strains lacking genetic accessibility. Indeed transformation efficiencies in bifidobacteria are generally too low and only targeted mutations based on homologous recombination had been successfully achieved in some strains. In this work, we describe the generation of transposon insertion mutants in two bifidobacterial strains*, B. breve* UCC2003 and *B. breve* NCFB2258. We also report the creation of the first transposon mutant library in a bifidobacterial strain, employing *B. breve* UCC2003 and a Tn5-based transposome strategy. This collection of mutants was found to be composed of clones containing single transposon insertions which appear to be randomly distributed along the genome. The usefulness of the library to perform phenotypic screenings was confirmed through identification and analysis of mutants defective in specific carbohydrate utilization abilities.

B018

Draft genome sequence and annotation of *Lactobacillus plantarum* strain Lp90 isolated from Apulian (Italy) wine

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Lactobacillus plantarum is a facultative heterofermentative lactic acid bacteria (LAB), found in many different ecological niches. *L. plantarum* is widely used as starter and probiotic in many food processes. Some strains are claimed to provide health benefits and are marketed as probiotics. Here we report the draft genome sequence and annotation of *L. plantarum* strain Lp90 previously isolated from Nero di Troia, a typical Apulian (South of Italy) wine. *L. plantarum* strain Lp90 has a noticeable ropy phenotype, which was ascribed to its capacity to over-produce exopolysaccharides (EPS). It is the first *L. plantarum* genome coming from a strain of wine origin sequenced. This strain was already characterized in a previous study describing the phenotypic and genomic diversity of *L. plantarum* strains isolated from various environmental niches and in three studies on Lp90 genes coding for small heat shock proteins. The genome of *L. plantarum* strain Lp90 consists of 3,324,076 bp in 33 contigs with a CG content of 44.32%. The genome size and the CG content are comparable those of published *L. plantarum* genomes (http://www.ncbi.nlm.nih.gov/genome/genomes/1108). Among the 3,273 predicted genes, 3,155 were protein coding genes, 34 were identified to be pseudo-genes, while 84 were RNA coding genes (70 tRNAs and 14 rRNAs). The presence of a signal peptide was predicted for 311 proteins. Transmembrane region analysis revealed 869 proteins containing transmembrane helices. The availability of the draft genome sequence of Lp90 represents the base to investigate some peculiar features of this strain, such as the EPS production and the ability to tolerate stress to the wine environment.

B019

***Lactococcus lactis* metabolism and gene expression during growth on plant tissues**

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Although a variety of lactic acid bacteria (LAB) species have been isolated from living, harvested, and fermented plant tissues, the adaptations these bacteria possess for growth in plant systems are currently largely unknown. In this study, we investigated the habitat-specificity and functional traits of *Lactococcus lactis* during growth in an *Arabidopsis thaliana* leaf tissue lysate (ATL). *L. lactis* KF147, a strain originating from mung bean sprouts, exhibited a higher growth rate and reached 7.9-fold greater cell densities during growth on ATL than the dairy-associated strain *L. lactis* IL1403. RNA-seq transcriptome profiling of KF147 identified 853 induced and 264 repressed genes during growth in ATL as compared to GM17 laboratory culture medium. The majority of down-regulated genes encode functions required for transcription, translation, and nucleotide biosynthesis. Genes induced in ATL included the arginine deiminase pathway and a total of 140 carbohydrate transport and metabolism genes involved in xylose, arabinose, cellobiose, and hemicellulose metabolism. The induction of those genes corresponded with *L. lactis* KF147 nutrient consumption and metabolic end-products in ATL measured by GC-TOF/MS untargeted metabolomic profiling. Genes involved in xylose metabolism were among the most highly-expressed by *L. lactis* KF147 in ATL. A xylose isomerase gene deletion mutant was unable to grow in the presence of xylose in XM17. This mutant was able to grow to wild-type levels in ATL, confirming the overlapping functions of carbohydrate metabolism pathways in *L. lactis*. These findings show that certain strains of *L. lactis* are well-adapted for growth on plant tissues and possess specific traits useful for plant-based food, fuel, and feed fermentations.

B020

Genomics of the genus *Bifidobacterium* reveals species-specific adaptation to the glycan-rich gut environment

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Bifidobacteria have been identified in the gut environment of a diverse set of animals, including those of mammals and insects. However, the genetic traits facilitating their adaptation to this highly competitive and variable ecological niche are still poorly characterized. Here, the genomic decoding of 47 representative (sub)species across the *Bifidobacterium* genus revealed a wide variety of genes predicted to be required for the breakdown and internalization of a range of carbohydrates. Such glycan-degrading abilities are expected to be a reflection of the carbon sources present in the ecological niche of bifidobacteria. Furthermore, transcriptome profiling of bifidobacterial genomes supported the involvement of various chromosomal loci in glycan metabolism. The widespread occurrence of bifidobacterial saccharolytic features was confirmed by interrogating metagenomic datasets, thereby supporting the notion that metabolic accessibility to dietary and/or host derived glycans is a potent evolutionary force that has shaped bifidobacterial genomes. This study also underscores the idea of saccharidic resource sharing among bifidobacteria through species-specific metabolic specialization and cross feeding.

B021

Motility of *Lactobacillus curvatus* NRIC 0822: Novel evidence of flagellum-mediated motility outside the *Lactobacillus salivarius* clade

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Lactobacillus sp. is the largest genus within the Lactic Acid Bacteria, with almost 170 species currently identified. Motility has been described in at least twelve *Lactobacillus* species, all belonging to the *L. salivarius* clade. Motility in lactobacilli is poorly characterized. It might confer competitive advantages such as nutrient acquisition and niche colonization, but could also play an important role in immune system stimulation of the host, via flagellin/TLR5 interaction. Herein, we describe for the first time strong evidence of motility in species outside the *L. salivarius* clade, namely in the motile *L. curvatus* NRIC 0822 strain. *L. curvatus* is a member of the *L. sakei* clade and is phylogenetically related to *L. sakei*, *L. fuchuensis* and *L. graminis* species all of which are associated with meat environments. The motility of *L. curvatus* NRIC 0822 was confirmed by phase-contrast microscopy and standard soft agar motility assays. Strain NRIC 0822 was motile at temperatures between 10°C and 37°C, and when utilizing a range of different carbohydrates and in varying atmospheric conditions. In addition, analysis of a newly sequenced draft genome generated by our group showed that the motility genes present in *L. curvatus* NRIC 0822 are organized in a single operon and are very similar (protein sequences >98.5% similarity) to the draft genome of *L. acidipiscis* KCTC 13900. Moreover, the presence of a high number of mobile genetic elements (transposases, integrases) inside and flanking this motility operon suggests recent horizontal transfer between two distinct *Lactobacillus* clades, namely *L. acidipiscis* in the *L. salivarius* clade and *L. curvatus* in the *L. sakei* clade. This study provides novel phenotypic, genetic and phylogenetic insights into flagellum-mediated motility in lactobacilli.

B022

Expression of prophage-encoded endolysins contributes to autolysis of *Lactococcus lactis*

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Analysis of autolysis of derivatives of *L. lactis* subsp. *cremoris* MG1363 and subsp. *lactis* IL1403 lacking the major autolysin AcmA, showed that *L. lactis* IL1403 still lysed during growth while *L. lactis* MG1363 did not. Zymographic analysis revealed that a peptidoglycan hydrolase activity of around 30 kDa is exclusively present in cell extracts of *L. lactis* IL1403. A comparison of all genes encoding putative peptidoglycan hydrolases of IL1403 and MG1363 led to the assumption that (one of) the 27.9-kDa endolysins encoded by the pro-phages *bIL285*, *bIL286* and *bIL309* could account for the autolysis phenotype of IL1403. Prophage deletion and insertion derivatives of *L.lactis* IL1403 had reduced cell lysis phenotypes. RT-QPCR and zymogram analysis showed that each of these strains still expressed one or more of the three phage lysins. Homologous phage lysin genes and lysin activity were also detected in the natural starter culture strains *L. lactis* subsp. *cremoris* E8, Wg2 and HP. Induced expression in *L.lactis* MG1363 of the endolysin from phage *bIL309* of IL1403 resulted in detectable lysis. These results show that phage endolysins of *L. lactis* are expressed during normal growth and contribute to autolysis without induction of phage. Screening for natural strains expressing such endolysins could help in the selection of strains with enhanced autolysis and thus, cheese ripening properties.

B023

Comparative genome analysis reveals strains specific gene clusters involved in exopolysaccharides biosynthesis in *Lactobacillus plantarum*

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Several lactic acid bacteria (LAB) produce capsular and secreted forms of exopolysaccharides (EPS) (Remus et al., 2012). The EPS production is correlated to the presence of specific gene clusters (*eps/cps*) which exhibit a conserved modular organization and include genes encoding both regulatory factors and enzymes involved in EPS biosynthesis, polymerization and secretion, including glycosyl-transferases, which are responsible for the assembly of the characteristic EPS-repeating unit. The *Lactobacillus plantarum* WCFS1 genome encodes 4 *cps* clusters of genes that are associated with surface polysaccharide production. The *cps2A*-J and *cps4A*-J clusters encode all functions required for capsular polysaccharide formation, while the *cps1A*-I and *cps3A*-J clusters lack genes encoding chain-length control functions and a priming glycosyl-transferase (Remus et al., 2012). *L. plantarum* Lp90 exhibited a ropy phenotype which was associated to its capacity to produce EPS. The genetic basis of EPS production was investigated by identification, sequencing and comparative analysis of genes clusters involved in EPS biosynthesis with other *L. plantarum* genomes. It was found that the cluster 4 (*cps4*) is the most conserved, while the cluster 3 presents high homology with *cps3* of WCFS1, ST-III and ATCC 14917 *L. plantarum* strains. Conversely, cluster 1 (*cps1*) is fully present only in *L. plantarum* JDM1, while the first genes of cluster 2 are homologous to the *cps2A*-E genes of other *L. plantarum*. The remaining genes of cluster 2 are homologous to proteins of *Lactobacillus fabifermentans* T30PCM01, probably involved in the exopolysaccharides biosynthesis. This feature makes unique the organization of *cps* clusters *L. plantarum* Lp90 and may explain the ropy phenotype of this strain.

Reference:
Remus DM. et al., (2012). Microb Cell Fact, 11: 1-10.

B024

Investigation of factors affecting aerobic and respiratory growth in *Lactobacillus casei* N87

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Many lactic acid bacteria are genetically equipped for aerobic or respiratory growth but, to date, reports are present only for *Lactococcus lactis* and *Lactobacillus plantarum*.
We investigated the effect of anaerobic (AN), aerobic (30% and 60% of dissolved O_2; AE30, AE60) and respiratory (30% and 60% of dO_2, heme and menaquinone; RS30, RS60) growth on the O_2 uptake, metabolites, activity of pyruvate oxidase (POX), NADH oxidase (NOX), NADH peroxidase (NPR), catalase, antioxidant capabilities and survival to oxidative stresses (H_2O_2, pyrogallol, menadione) of *L. casei* N87. Transcription of *pox* (aerobic pathway), *cydAB* (cytochrome oxidase subunit I and II; electron transport chain, ETC) and *fba* (fructose biphosphate aldolase; glycolysis) was verified by qRT-PCR, while changes in protein profiles by 1D and 2D electrophoresis.
Respiration increased biomass yield compared to AN and AE growth. The highest O_2 uptake was measured in exponential RS cells, suggesting a boost of O_2 consumption by cytochrome oxidase activity (ETC activation). Catalase, noticeable in exponential cultures, decreased in stationary phases when consistent amounts of H_2O_2 (mainly in AE60) were detected. POX, NOX and NPR were highest in AE30 and RS cells but very low in AE60 ones, probably because of inhibition by O_2 and H_2O_2 accumulation. RS60 stationary cells exhibited the greatest radical scavenging activities and robustness to oxidative stresses. Significant numbers of damaged and VBNC cells were found in AE60 cultures, confirming the noxious effect of O_2 and H_2O_2 accumulation. Proteome differed principally as function of growth phase, although AE and RS resulted in increased number of detectable protein spots compared to AN cultivation.
High levels of d O_2 impair functionality and robustness of *L. casei* if respiratory pathways are not activated.

B025

Systematic phenome analysis of *Lactococcus lactis* deletion mutants in genes coding for hypothetical transcriptional regulators

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Characterization of the regulators, the signal governing their activity, the genes they control and the level of control is essential to understand how the bacteria respond to environmental changes. Understanding of a such regulatory network is crucial for obtaining of microorganisms with desirable and specific biotech traits and improving their industrial properties. There are 2310 open reading frames identified in the *Lactococcus lactis* IL1403 chromosome, of which 122 encode transcriptional regulators, and approximately half of them have no function assigned. These proteins on their N-terminal ends have the HTH (helix-turn-helix) DNA binding domain. In this study we constructed several mutants in hypothetical regulatory genes from the RpiR (*yleF*, *yidA*, *yfeA*, *yugA*), MerR (*rmeA*), GntR (*rgrB*) and Xre (*ysgA*, *tagR*, *ps205*) families and subsequently, tested globally by the use of Phenotype Microarrays the phenotypic changes caused by gene inactivation. To gain a comprehensive overview of pathway functions, we tested in obtained mutants their C metabolism; pH growth range; pH control; sensitivity to NaCl and other ions. The results indicate that most of these putative regulators are linked to genes involved in sugar catabolism (in particular to β-glucosides, galactose, maltose and its derivatives) and also may be involved in cell response to osmotic stresses.

B026

Isolation and characterization of oxidative stress-resistant lactobacilli mutants promoting their survival during low-temperature storage

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Oxidative stress is a major factor lowering survival of probiotics during low-temperature storage. Especially strains, isolated from anaerobic environment such as gut, are often sensitive to oxygen and their probiotic function can be limited due to poor survival. To overcome this problem, we isolated oxidative stress-resistant mutants of *Lactobacillus acidophilus* and *Lactobacillus gasseri*, and characterized their mechanisms promoting survival. Streptonigrin(SN), an iron-dependent reactive oxygen generator, was used to isolate oxidative stress-resistant mutants and the survival of these mutants were evaluated at 4°C. Two *L. acidophilus* and one *L. gasseri* SN-resistant strains were shown to increase the viable cell number after 28 days of storage by around 1,000- and 300-fold, respectively, compared to the corresponding parental strain. Both *L. acidophilus* SN-resistant mutants indicated lower H_2O_2-forming NADH oxidase activity and produced small amount of H_2O_2. The expression of *pyrD*, a candidate of H_2O_2-forming NADH oxidase gene, was decreased by 4-fold. These results suggest that decrease of H_2O_2 production may contribute to higher survival of these mutants. By contrast, H_2O_2 production of *L. gasseri* SN-resistant mutant was not decreased. Genomic analysis of the mutant verified the presence of 95 bp deletion in the promoter region of *ileS* gene that encodes isoleucyl-tRNA synthetase. *ileS* expression of the mutant was shown to decrease by 4-fold compared to wild-type strain. We speculated that lower *ileS* expression may induce “stringent response”, thereby improving survival. Consistent with this hypothesis, treatment of mupirocin, a stringent response inducer, increased the survival of *L. gasseri* up to 200-fold.

B027

Impact of fluorescent labeling and flow cytometric cell sorting on viability and vitality of *Lactococcus lactis*

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The production of starter cultures like *Lactococcus lactis* aims at high levels of viability and acidification activity to achieve the targeted performance. Replication as the main criterion for viability has traditionally been measured by plate counting, while the acidification activity of lactic acid bacteria provides a more differentiated picture of bacterial physiology. Distinguishing physiological states even of individual cells is facilitated by combining flow cytometry with fluorescent labeling. Moreover, multiparameter flow cytometry in combination with cell sorting offers the possibility not only to detect but also to physically separate subpopulations representing different physiological states. In order to link the isolated subpopulations to metabolic functions of the initial cell population and thereby to understand the contribution to the overall performance, re-cultivation of fluorescently labeled and subsequently sorted cells is required. For this purpose, it has to be investigated whether and to what extent the viability and functionality was influenced by the applied staining method and the sorting process itself.
We found culturability on plates, growth behavior in liquid media and acidification activity of *Lactococcus lactis* to be negatively affected by fluorescent labeling with carboxyfluorescein diacetate and its succinimidyl ester, propidium iodide and TOTO-1. Furthermore, we will present data showing the impact of cell sorting using a Piezo-based flow diversion system on the viability and vitality of sorted cells. Our results indicate that both fluorescent labeling and flow cytometric cell sorting negatively affect the physiological state of *Lactococcus lactis* cells.

B028

Biofilm formation by *Lactobacillus plantarum* WCFS1 under static and dynamic conditions

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Spoilage of food products is often linked to the presence of biofilms on processing equipment. In the food industry production lines including pipes for products like juices and dressings can be contaminated with lactobacilli causing spoilage. To mimic the dynamics of industrial conditions, we applied dynamic flow to study biofilm formation by *Lactobacillus plantarum*. Cell envelope characteristics of microbes are thought to be crucial for initial attachment and further biofilm development. The objective of this study is to evaluate the biofilm forming capacity under static and dynamic flowing conditions of *L. plantarum* WCFS1 and mutants lacking one or more gene clusters encoding for CPS production (*cps1*, *cps2*, *cps3*, *cps4*) (Remus, 2012) and a mutant lacking the sortase-encoding gene (*ΔsrtA*)(Remus, 2012) predicted to anchor twenty seven surface proteins covalently to the peptidoglycan layer (Kleerebezem, 2003).
L. plantarum WCFS1 forms biofilms of high cell density (12.3 log cfu/cm^2) under dynamic conditions even when the dilution factor exceeds the growth rate by a factor ten. The capacity to form dense biofilms was affected by the presence of the *cps1* gene cluster. Its deletion resulted in a reduction of biofilm cell numbers by 3 orders of magnitude. This effect was specific for dynamic conditions, as in static conditions the number of biofilm cells was comparable to the wild type but with increased matrix formation. Static biofilm formation was negatively affected with the deletion of *srtA*.
Deletion of *cps1* in *L. plantarum* WCFS1 enhances matrix formation in static conditions while cell numbers in the biofilm decreased in dynamic conditions. The phenotype of *ΔsrtA* suggests a role of sortase dependent proteins in biofilm formation in static conditions.

B029

Identification of Restriction-Modification systems of *Bifidobacterium animalis* subsp. *lactis* CNCM I-2494 by SMRT sequencing and associated Methylome analysis

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Bifidobacterium animalis subsp. *lactis* CNCM I-2494 is a component of a commercialized fermented dairy product for which beneficial effects on health has been studied by clinical and preclinical trials. To date little is known about the molecular mechanisms that could explain the beneficial effects that bifidobacteria impart to the host. Restriction-modification (R-M) systems have been identified as key obstacles in the genetic accessibility of bifidobacteria, and circumventing these is a prerequisite to attaining a fundamental understanding of bifidobacterial attributes, including the genes that are responsible for health-promoting properties of this clinically and industrially important group of bacteria. The complete genome sequence of *B. animalis* subsp. *lactis* CNCM I-2494 is predicted to harbour the genetic determinants for two type II R-M systems, designated BanLI and BanLII. In order to investigate the functionality and specificity of these two putative R-M systems in *B. animalis* subsp. *lactis* CNCM I-2494, we employed PacBio SMRT sequencing with associated methylome analysis. In addition, the contribution of the identified R-M systems to the genetic accessibility of this strain was assessed.

B030

Molecular characterization of inulinase genes of newly isolated Bulgarian *Lactobacillus* strains that metabolize prebiotic carbohydrates

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Prebiotic carbohydrates are selectively metabolized by a limited number of microorganisms belonging to genus *Lactobacillus*. The growth and activity of these bacteria are stimulated by fructooligosaccharides (FOS) - prebiotic fructans that usually derived from inulin. The aim of the present study is the selection of *Lactobacillus* strains that convert inulin and the characterization of the putative responsible genes. Four new strains, belonging to species *Lactobacillus casei*, *L. paracasei* and *L. fermentum* were found to grow in media containing inulin and FOS as sole carbon sources. The genes *levH1and levH1*-para, encoding putative inulinases in *L. casei* and *L. paracasei* strains were amplified as PCR fragments and were cloned in pJET 2.1 blunt under T7 promoter control. The genes encoded similar proteins of 1296 amino-acids (with calculated molecular masses of 138.8 kDa) and their extracellular expression was achieved in two different *E. coli* hosts, DH5α and BL21(DE3). The capabilities of the novel *Lactobacillus* strains to convert high amounts of Jerusalem artichoke inulin into lactic acid were investigated in course of several batch processes. The results suggested that the novel inulin-degrading strains are perspective as probiotic/probiotic food supplements. The future development of a biotechnology for L(+) lactic acid production from the cheap substrate inulin is promising as well.
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B031

Cross-talk mediated by Rgg-peptide cell-cell communication systems from *Streptococcus salivarius*

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The oral cavity is presumably one of the most challenging environments considering the tremendous and diversified bacterial community that struggle to thrive coupled to constant and multiple external changes. Remarkably, the commensal *Streptoccoccus salivarius* species is one of the prime colonizers of oral mucosal surfaces that remains predominant in the buccal cavity throughout the human lifespan. Furthermore, several lines of evidence argue in favour of a dampening effect of *S. salivarius* on its closely-related pathogen and dental caries agent *S. mutans*. In all likelihood, *S. salivarius* cells are able to sense the physiological state of *S. mutans* population in order to provide the ad hoc response and endeavour to restrain the spreading of its competitor. Our preliminary results suggest that S. salivarius is able to recognize a signalling peptide excreted by *S. mutans*, via the orthologous ComRS cell-cell communication system involved in DNA transformation and production of bacteriocins.
We are deciphering *S. salivarius* molecular mechanisms that allow intra- and inter-species cross-talk in the framework of streptococcal population dynamics. A newly discovered family of pheromone-responsive transcriptional regulators called Rgg is of particular interest as it encompasses the ComRS system. We systematically deleted rgg genes and assessed the role of Rgg proteins for various phenotypes such as natural DNA transformation, predation through bacteriocin production, biofilm formation, and global fitness. Currently, we are assessing the impact of cross-talk between Rgg members in a single *S. salivarius* strain. Future work will include the investigation of inter-species cross-talk in mixed-species biofilm of *S. salivarius* and *S. mutans* strains for a given Rgg system, starting with the better characterized ComRS system.

B032

The physiological role of two secondary amino acid transporters, SERP1 and SERP2, in *Lactococcus lactis*

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Lactococcus lactis is able to obtain all essential amino acids from casein, the major protein of milk. Nevertheless, it can grow in media containing free amino acids as the sole source of amino acids, implying that transport systems for essential amino acids have been retained. A collection of different transport systems for essential and nonessential amino acids have been described (Konings et al., Antonie van Leeuwenhoek 2002) but so far, only a handful transporters have been characterized. Two members of the APC superfamily of secondary transporters were shown to transport serine in *L. lactis* JP9000 and designated SERP1 and SERP2 (Trip et al., J. Bacteriol. 2013). Here, growth experiments using mutant strains lacking one or both transporters, and in the presence or absence of serine demonstrate that SERP1 is the main serine transporter. In addition to serine, SERP1 exhibits high affinity for threonine and cysteine. SERP2 has a much lower affinity for serine and can support maximal growth in the absence of SERP1 only in medium lacking alanine and glycine. The substrate specificity of SERP2 is L-alanine/D-alanine/glycine/D-serine, which is the same as for the well-studied CycA transporter of *E. coli* that is involved in cell wall synthesis (Robbins et al., J. Bacteriol. 1973). SERP2 is responsible for D-serine toxicity and in conditions of D-alanine auxotrophy, SERP2 is essential for its uptake which prevents the cells from lysing.

B033

Synthesis of alanine and 2-aminobutyrate by an aminotransferase in *Pediococcus acidilactici*

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Pediococcus acidilactici often is a constituent of the non-starter lactic acid bacteria in raw milk-cheese. We observed that this species can degrade serine and threonine and thereby forms alanine and 2-aminobutyrate. To understand the influence of this metabolism on cheese quality and aroma formation, this metabolic pathway was studied in more detail. Amongst other enzymes, aminotransferases may play an important role in this metabolism. Based on genome data, we identified two putative aminotransferases in *P. acidilactici* FAM18098 that are both predicted to be aspartate aminotransferases (EC. 2.6.1.1). Enzymatic characterization of one of these two aminotransferases showed that the enzyme used leucine, methionine, phenylalanine, alanine and 2-aminobutyrate but not aspartate as amino group donors. Furthermore, the enzyme utilized not only 2-oxoglutarate but also 2-ketobutyrate and pyruvate as amino acceptors forming 2-aminobutyrate and alanine, respectively. Gene expression analysis showed that the gene is upregulated during synthesis of 2-aminobutyrate. In summary, the results indicate that the gene product is probably involved in the synthesis of alanine and 2-aminobutyrate.

B034

Detection of *Lactobacillus parabuchneri*, a potential spoilage organism, in milk and cheese

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Histamine can be formed by microorganisms in fermented products such as cheese. High concentrations of this compound can trigger symptoms in consumers who have a histamine intolerance. Microbial decarboxylation of histidine is probably the major cause of histamine formation in cheese. We isolated histamine-producing bacteria from various raw-milk cheeses containing histamine levels higher than 50 mg kg^{-1}. Partial sequence analysis of the *tuf* gene showed that all these bacteria belong to *Lactobacillus parabuchneri* which is closely related to *Lactobacillus buchneri*, a bacterium associated with spoilage in plant-based fermented food and beverages but which is also used in silage fermentation. We sequenced the genome of *L. parabuchneri* FAM21731 using next-generation sequencing technology. The draft genome sequence was compared to public available genome sequences of *L. buchneri* and other related species. Thereby, a gene sequence was identified which seems to be unique for *L. parabuchneri*. Based on this sequence we developed a real-time PCR method to analyze various strains of *L. parabuchneri* and *L. buchneri*. Furthermore, the method was used to detect and enumerate *L. parabuchneri* in cheese and milk samples. The results show that the PCR system is specific for the detection and quantification of *L. parabuchneri* in dairy products. The use of this method is of interest since it can be employed to locate potential sources of *L. parabuchneri* contamination on farms and in dairy plants.

B035

High efficiency *Lactobacillus casei* electrotransformation

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The ability to genetically manipulate lactic acid bacteria extends the range of studies that can be undertaken to better understand their basic physiological and biochemical properties and allows the development of improved industrial strains for the production of proteins and metabolites. Construction of recombinant bacterial strains is often limited by the inability to introduce DNA constructs into recipient cells or by the inability to generate replacement mutations in chromosomal genes. We investigated whether protocols allowing high efficiency electrotransformation of other lactic acid bacteria were applicable to *Lactobacillus casei*. These included the addition of glycine or sodium chloride during growth of the recipient cells, the growth of the recipient cells to different cell densities, the pretreatment of competent cells with H_2O or a lithium acetate-dithiothreitol solution, and variation in electroporation voltage and resistance settings. The five strains tested (12A, 32G, A2-362, ATCC334 and BL23) varied in their responses to these treatments. Transformation efficiencies up to 10^7 transformants per microgram of pTRKH2 vector DNA were obtained which allowed construction of chromosomal gene replacements.

B036

Construction and characterization of prophage-free derivative of the prototype *Lactococcus lactis* subsp. *lactis* IL1403 strain

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Lactococcus lactis IL1403 is the first sequenced prototype strain of *L. lactis* subsp. *lactis* widely used for fundamental and applied research more recently at the level of “omics” and for systems biology approach. Similar to all other lactococcal strains, its genome contains 6 prophage-related sequences which encompass approximately 6.7% of the chromosome and represent a significant part of genomic differences with other lactococcal strains. Both transcriptomic and proteomic analysis of *L. lactis* IL1403 have shown that these prophage-related genes are expressed in various growth conditions. However, it is generally considered that expression of prophages genes can provide to the bacterial host increased fitness, resistance against infecting phages and modify cellular metabolism. Nevertheless, the impact of resident prophages on the different aspects of *L. lactis* physiology has not been deeply explored. Here, we report construction and preliminary characterization of one prophage-free derivative of *L. lactis* IL1403 denoted IL6288. We examine the impact of prophage contents and the role of the individual prophages on different aspects of cell physiology important for industrial and therapeutic applications. These data clearly show that resident prophages can significantly modify i) growth and survival of host strain at different temperatures under aerobic and respiration conditions; ii) its autolytic properties and iii) its susceptibility to infection with virulent phages. All these phenotypes are not an addition of the effects of the individual prophage genes but the result of complex genetic interactions.

B037

Monod revisited: Bet-hedging during bacterial diauxic shift

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When bacteria grow in a medium with two sugars, they first utilize the preferred sugar and only then start metabolising the second one. After the first exponential growth phase a short lag-phase of non-growth is observed, a period called the diauxie lag-phase. It is commonly seen as a phase in which the bacteria prepare themselves to utilize the second sugar. Here we reveal that, in contrast to the established concept of metabolic adaptation in the lag-phase, two stable cell types with alternative metabolic strategies emerge and coexist in a culture of the bacterium *Lactococcus lactis*. Only one of them continues to grow. The fraction of each metabolic phenotype depends on the level of catabolite repression and the metabolic state-dependent induction of stringent response as well as on epigenetic cues. Furthermore, we show that the production of alternative metabolic phenotypes potentially entails a bet-hedging strategy. This study sheds new light on various lag-phases occurring in microbiology and biotechnology and updates the generally accepted explanation of enzymatic adaptation proposed by Monod and shared by scientists for more than half a century.

B038

A novel type of peptidoglycan-binding domain highly specific for amidated D-Asp cross-bridge, identified in *Lactobacillus casei* bacteriophage endolysins

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Peptidoglycan-hydrolases (PGHs) are responsible for bacterial cell lysis. Most PGHs have a modular structure comprising a catalytic domain and a cell-wall binding domain (CWBD). PGHs of bacteriophage origin, called endolysins, are involved in bacterial lysis at the end of the infection cycle. We have characterized two endolysins, Lc-Lys and Lc-Lys2, identified in prophages present in the genome of *Lactobacillus casei* BL23. These two enzymes have different catalytic domains but similar putative C-terminal CWBDs. By analyzing purified peptidoglycan (PG) degradation products, we showed that Lc-Lys is an N-acetylmuramoyl-L-alanine amidase whereas Lc-Lys2 is a γ-D-glutamyl-L-lysyl-endopeptidase. Remarkably, both lysins were able to lyse only Gram-positive bacterial strains which possess PG with D-Ala^{4}D-Asx-L-Lys^3 in their cross-bridge, such as *L. casei*, *Lactococcus lactis* and *Enterococcus faecium*. By testing a panel of *L. lactis* cell-wall mutants, we observed that Lc-Lys and Lc-Lys2 were not able to lyse mutants with a modified PG cross-bridge, constituted of D-Ala^{4}L-Ala-(L-Ala/L-Ser)-L-Lys^3; moreover they do not lyse *L. lactis* mutant containing only non-amidated D-Asp cross-bridge i.e. D-Ala^{4}D-Asp-L-Lys^3. In contrast, Lc-Lys could lyse ampicillin-resistant *E. faecium* mutant with 33 L-Lys^3-D-Asn-L-Lys^3 bridges replacing the wild-type 43 D-Ala^{4}D-Asn-L-Lys^3 bridges. We showed that the C-terminal CWBD of Lc-Lys binds PG containing mainly D-Asn but not PG with only non-amidated D-Asp-containing cross-bridge, indicating that the CWBD confers to Lc-Lys its narrow specificity. In conclusion, the CWBD characterized in this study is a novel type of PG-binding domain targeting specifically D-Asn interpeptide bridge of PG.

B039

Oxidative stress at high temperatures in *Lactococcus lactis* due to an insufficient supply of riboflavin

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Lactococcus lactis MG1363 was found to be unable to grow at temperatures above 37°C in a defined medium without riboflavin, and the cause was identified to be dissolved oxygen introduced during preparation of the medium. At 30°C growth was unaffected by dissolved oxygen and oxygen was consumed quickly. Raising the temperature to 37°C resulted in severe growth inhibition and only slow removal of dissolved oxygen. Under these conditions, an abnormally low intracellular ratio of [ATP] to [ADP] (1.4) was found (normally around 5), which indicates that the cells are energy limited. By adding riboflavin to the medium it was possible to improve growth and oxygen consumption at 37°C, and this also normalized the [ATP] to [ADP] ratio. A codon-optimized redox sensitive GFP was introduced into L. lactis and revealed a more oxidized cytoplasm at 37°C than at 30°C. These results indicate that *L. lactis* suffers from a heat induced oxidative stress at increased temperatures. A decrease in intracellular FAD, which is derived from riboflavin, was observed with increasing growth temperature, but the presence of riboflavin made the decrease smaller. The drop was accompanied by a decrease in NADH oxidase and pyruvate dehydrogenase activities, both of which depend on FAD as a cofactor. By over-expressing the riboflavin transporter it was possible to improve FAD biosynthesis which resulted in increased NADH oxidase and pyruvate dehydrogenase activities, and improved fitness at high temperatures in the presence of oxygen.

B040

Control of natural transformation in *salivarius* streptococci through specific degradation of ComX by the MecA-ClpCP protease complex

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Competence for natural DNA transformation is a tightly regulated process in streptococci. In *mutans* and *salivarius* species, the abundance of the central competence regulator ComX is controlled both transcriptionally by the ComRS signaling system via the pheromone XIP, and post-translationally, by the adaptor protein MecA and its associated Clp ATPase, ClpC. In this study, we further investigate the mechanism and function of the MecA-ClpC control system in the salivarius species *Streptococcus thermophilus*. Using in vitro approaches, we show that MecA specifically interacts with both ComX and ClpC, suggesting the formation of a ternary ComX-MecA-ClpC complex. Moreover, we demonstrate that MecA ultimately targets ComX for its degradation by the ClpCP protease in an ATP-dependent manner. We also identify a short sequence (18 aa) in the N-terminal domain of ComX as essential for the interaction with MecA and subsequent ComX degradation. Furthermore, we investigate the *in vivo* subcellular localization of ComX and MecA during the competence process. Finally, increased transformability of a MecA-deficient strain in presence of sub-inducing XIP concentrations suggests that the MecA-ClpCP proteolytic complex acts as an additional locking device to prevent competence under inappropriate conditions. A model of the interplay between ComRS and MecA-ClpCP in the control of ComX activity is proposed.

B041

Engineering *Lactococcus lactis* for the production of resveratrol, a nutraceutical plant-derived polyphenol

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Plant-derived polyphenols have been shown to induce responses consistent with the beneficial effects of diets rich in fruits and vegetables. These compounds are produced in small amounts, difficult to purify from natural resources and due to their complexity chemical synthesis is a poor option. Hence, the utilization of microbes as platforms for effective production of plant bioactive metabolites emerges as a feasible alternative.
Lactococcus lactis is an industrially relevant microorganism used worldwide in food fermentations. Considering its economical value, *L. lactis* has been intensively studied and a large body of comprehensive data on their metabolism and genetics was generated. The technological breakthroughs in functional genomics, combined with a GRAS status, a relatively simple metabolism, a small genome, and rapid growth, makes this organism an ideal host for production of valuable food and pharmaceutical compounds [1]. Thus, *L. lactis* tailored for optimal production of nutraceutical plant-derived polyphenols is a promising tool to fortify fermented dairy products without the need for food supplements.
In this work, we have engineered *L. lactis* for the production of the stilbene resveratrol from L-tyrosine. A synthetic pathway harbouring the genes codifying the tyrosine ammonia lyase (TAL), p-coumarate-CoA ligase (4CL) and stilbene synthase (STS) was assembled and functional expressed in a LDH-deficient strain. In medium supplemented with L-tyrosine, the recombinant strain accumulated resveratrol and considerable amounts of the intermediate p-coumaroate, denoting a possible bottleneck at the level of 4CL/STS enzymes. Data on growth, end-product and intermediate metabolite profiles will be presented. In order to improve resveratrol production, expression of synthetic modules consisting of different gene combinations and structural organizations is currently under investigation.
[1] Gaspar et al., 2013. Biotechnol Adv, 31:764-88

B042

Comparative genomic analysis of bacteriocin gene clusters among food and clinical enterococcal strains

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Enterococcus strains have become an increasingly important pathogen worldwide. Besides, they are also common constituents of dairy fermented products around the world, such as cheese and sausages, in which it is believed that they contribute to the development of the organoleptic and safety properties of the final product. This is particularly true since they are very prolific in the production of antimicrobial compounds such as bacteriocins, especially active against gram positive bacteria. In our laboratory, we have been studying strains belonging to the genera *Enterococcus* isolated from fermented food products as model microorganisms for the production of bacteriocins. In previous findings we characterized the bacteriocin clusters present in *E. mundtii* CRL35 and *E. faecium* CRL1879 genomes. In the present work, we performed an in silico screening of bacteriocin clusters in enterococci strains isolated from food and clinical samples and compared to those present in our strains. We analyzed 242 partial or complete *E. faecium* genomes deposited in GenBank, we found that six clinical isolates belonging to the Broad Institute (ENGEN0004; ENGEN 0031; ENGEN 0033; ENGEN 0035, ENGEN 0052 and ENGEN 0263 strains) harbored between two to eight bacteriocin genes. Interestingly, we found genes encoding for bacteriocins described in the *Lactobacillus* genus (sakacin Q and sakacin T genes) besides those previously described in enterococcus (enterocin A, B, P, SE-K4, X, NKR-5-3A, L50A and B, enterolysin A). In summary, it seems that bacteriocin genes are widely distributed among *Enterococcus* independently of the origin of the strain. The newly available genomic data and the ongoing developments in other areas might help us to understand their evolutionary origins and their role in microbial interactions.

B043

Prophages from L-lactic acid producing industrial strain of *Lactobacillus rhamnosus*

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Sequencing and annotation of *Lactobacillus rhamnosus* strain used for highly efficient production of L-lactic acid revealed presence of three regions coding for phage associated genes. Two of them coded for complete prophages and third one, for phage remnant. The first prophage inserted at the tRNA-Leu gene had 43,948 bp and a G+C content of 45.07%. Its genome consisting of 68 open reading frames shared no homology with known phages of closely related bacteria. 46 ORFs shared homology with known bacteriophage genes whereas another 20 were found to be hypothetical one and two were of bacterial origin. The second prophage containing of 62 ORFs had 39,221 bp and a G+C content of 45.53% comparing to 46.79% for the host chromosome. It was homologous in 72.8% and 65.2% to the corresponding sequences of prophages from *L. rhamnosus* ATCC 53103 (GG) and *L. rhamnosus* LOCK 900. Both prophages had similar modular arrangement typical for known prophage Lrm1 from industrial strain M1 of *L. rhamnosus*. The third remnant prophage was inserted into transfer-messenger tmRNA chromosome region located between α-galactosidase and integral membrane protein genes. It consisted of 13,782 nt with G+C content of 45.20% and 17 ORFs. The prophage remnant was homologous in 67.5% and 65.6% to the corresponding regions of *L. rhamnosus* LOCK 908 and *L. rhamnosus* Lc705 respectively. Its structure contains limited number of phage genes (integrase, transcriptional regulator, DNA primase, two terminases, portal, capsid and head-tail joining proteins). Our strain unlike of other known *L. rhamnosus* strains is carrying three prophages. The work was partially financed by the project (POIG 01.01.02-10-123/09) of the European Union within the European Regional Development Fund Grants for Innovation.

B044

Environmental Stress affects S-Layer Production by *Lactobacillus acidophilus* IBB801 and *Lactobacillus helveticus* CRL 1177

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Several lactic acid bacteria have the potential to promote the health of the host or to prevent and treat diseases. Such bacteria are referred to as probiotic which must resist multiple stresses, including the gastrointestinal (GI) conditions and food processing. Once cells have survived the GI tract, they can colonize and grow to adequate numbers to exert their beneficial effect to the host. Several factors protect the bacterial cell from harsh environmental conditions such as the presence of S-layer. S-layer proteins are non-covalently bound to the cell wall and assemble into surface layers with high degrees of positional order often completely covering the cell wall. In this study we aimed to gain insight on S-layer production by the health-promoting strains *Lactobacillus acidophilus* IBB801 and *L. helveticus* CRL 1177 subjected to the following culture (stress) conditions: i) presence of bile salts (0.05, 0.1, and 0.2%); ii) incubation temperature (42 or 45°C); and iii) osmotic stress (0.6, 2.0 and 3.0% NaCl). S-layer from lactobacilli was extracted by using LiCl and analyzed by SDS-PAGE and transmission electron microscopy. Under stress conditions, S-protein bands from *L. acidophilus* IBB801 became visible in the gels being stronger in presence of 2% NaCl (osmotic stress) and 0.2% bile salts. In contrast, *L. helveticus* CRL 1177 showed increased production of S-layer in presence of 0.6% NaCl and against heat stress (45° C). S-layer production by *L. acidophilus* IBB801 and *L. helveticus* CRL 1177 was increased under specific stress conditions and helped the strains to maintain cell viability under the assayed detrimental environment. Further studies are needed to prove the S-layer protective role in these bacteria.

B045

Comparative genomics of bifidobacterial species to evaluate the differences between human-residential and non-human-residential species

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The genus *Bifidobacterium* has been classified into more than 40 species, a few of which are widely used as probiotics in the food industry. However, bifidobacterial species have distinctive residential characteristics that might be the result of adaptations to their residential environments. To reveal the potential characteristics of each species, at first, we performed comparative genomic analyses among a total of 67 strains belong to 11 species. Principal component analysis indicated the genetic difference associated with the difference of habituation of each species. Next, investigation was performed on a total of 49 genome sequences, including 15 strains of *B. longum*, 14 strains of *B. breve* and 20 strains of *B. animalis* to evaluate the differences between human-residential (HRB, i.e., *B. longum* and *B. breve*) and non-human-residential (nHRB, i.e., *B. animalis*) bifidobacterial species. Based on functional gene clustering, there were 584 common clusters among the strains. Moreover, there were 166 common clusters among strains of HRB, whereas there were fewer than ten common clusters between strains of HRB and nHRB. Major differences were observed between the HRB and nHRB clusters in their distributions of genes involved in the metabolism of glycans (e.g., human milk oligosaccharides and mucin) and vitamins, adaptive responses to environmental change and surface-associated/extracellular components. These gene distribution differences may provide clues for understanding the mechanisms underlying the unique residential characteristics of each species and their potential property as probiotics.

B046

Mathematical modelling of the gene regulatory network governing competence for natural transformation in *Streptococcus thermophilus*

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In *salivarius* streptococci, competence for natural transformation is tightly regulated by the ComRS system. In the current model, the peptide pheromone ComS is processed, exported outside the cell, and is next re-imported by the oligopeptide transporter Ami. In the cytoplasm, mature ComS directly interacts with the competence regulator ComR. This interaction stimulates the binding activity of ComR dimers on the promoter sequence of *comX*, encoding the alternative sigma factor that controls the expression of genes involved in extracellular DNA importation and processing. The ComRS complex also activates the transcription of *comS*, resulting in a positive feedback loop, which may be essential for the coordination of competence development inside the bacterial population. A lot of questions about the ComRS regulatory cascade still remain open. For instance, it is not clear how environmental parameters are integrated to fine-tune the activity of the ComRS complex. In addition, the competence shut-off mechanism is quite poorly understood. In this study, we developed a dynamic mathematical model of the complex network regulating competence in the *salivarius* model species *Streptococcus thermophilus*. Based on delay differential equations, the model parameters are set according to prior knowledge and experimental data. Growth parameters were integrated in the model in order to capture their effect on ComS concentration (chemically defined medium growth conditions). The outputs of the model are in accordance with prior experimental data, and suggest that the competence shut-off mechanism of *S. thermophilus* is similar to the one taking place in *Streptococcus pneumoniae*. Ultimately, our model will allow us to test different hypothesis on the ComRS regulatory circuit and to reorient future work consequently.

B047

Development of a recombinase-based in vivo expression technology in *Bifidobacterium longum* for identification of specifically-expressed genes in the gastrointestinal tract of mice

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Bifidobacteria inhabit in the human large intestine and exert health-promoting effects. Molecular mechanisms of their commensalism and health-promoting effects have not been sufficiently clarified. Bifidobacterial genes that are specifically expressed in the gastrointestinal tract may provide us with clues for the clarification. Here, we report a development of a recombinase-based *in vivo* expression technology (R-IVET) in *Bifidobacterium longum* aimed to identify the genes specifically expressed in the gastrointestinal tract of mice.
Cre/*loxP* system was used for construction of the R-IVET. A spectinomycin-resistance gene (Sp^R) that harbors *loxP* sequences at its termini (*loxP*-Sp^R-*loxP*) was integrated into *B. longum* 105-A chromosome by a double-crossover strategy. An integrant named 105-A::*loxP*-Sp^R-*loxP* was successfully isolated and showed no growth defect in *in vitro* culture conditions.
A prototype promoterless Cre-expression vector pBFH65 was constructed using a *B. longum* pTB6 replicon. Ideally, Cre recombinase should be expressed only when a promoter-containing fragment was inserted into an upstream region of the promoterless Cre gene. However, Cre expression was detected upon introduction of the pBFH65 into 105-A:: *loxP*-Sp^R-*loxP* where 82.1% of colonies of the pBFH65-introduced strains showed a Sp-sensitive phenotype due to the site-specific recombination between *loxP* sequences by the expressed Cre recombinase. This result suggested that some sequence(s) in the upstream region of the promoterless Cre gene were recognized as promoter(s) in *B. longum* 105-A. Thus, in order to repress the Cre expression, alteration of the RBS sequence and insertion of a transcriptional terminator were conducted in the upstream region of the promoterless Cre gene. Consequently, a promoterless Cre-expression vector pBFK86 that allowed almost no detectable Cre expression in 105-A::*loxP*-Sp^R-loxP was successfully constructed. Feasibility of the pBFK86 for the R-IVET is currently under evaluation.

B048

Comparative phenotype of bifidobacterial species to evaluate the differences between human-residential and non-human-residential species

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Our comparative genomic analyses implied several differences of species property between human-residential (HRB) and non-human-residential (nHRB) bifidobacteria. In this study, we validated whether the genotypic differences, e.g. the compatibility to human breast milk and the ability to produce folate, were expressed as phenotypes. Firstly, *in vitro* test was performed to evaluate the growth of 36 bifidobacterial strains in the breast milk obtained from healthy breast-feeding women, and the ability to utilize human milk oligosaccharides (HMO). Infant-associated HRB showed high ability in utilizing HMO and growth in breast milk whereas nHRB could not utilize HMO and failed to grow in breast milk. The facts that breast milk contains high concentration of lactose as carbohydrate source in the breast milk, suggest the possible existence of antimicrobial components in the breast milk for nHRB. *In vitro* assay found that nHRB strains failed to grow in the presence of lysozyme at a concentration of 50 μg/mL, an average concentration in human milk, whereas *Bifidobacterium longum* and *Bifidobacterium breve* grew well at the same condition. Next, studies were performed to test the ability for folate production by HRB and nHRB. *In vitro* assay showed that high concentrations of folate were produced by HRB compared to nHRB. In addition, *in vivo* test using germ-free and mono-associated mice confirmed the *in vitro* difference. These results validated the difference of HRB and nHRB at phenotypic level in accordance with their genotypic features and provided clues for understanding the mechanisms underlying the unique residential characteristics and their potential function as probiotics of bifidobacteria.

B049

Linoleic acid hydratases from *Lactobacillus plantarum* and *Lactobacillus reuteri* are FAD-dependent C9 hydratases

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Lactobacilli convert linoleic acid with linoleic acid hydratases to 10-hydroxy C18:1 fatty acid (10-HOE), 13-hydroxy C18:1 fatty acid (13-HOE), or 10,13-dihydroxy stearic acid (10,13-HOA) (1, 2). The 10-HOE rather than 10,13-HOA has antifungal property (3). This study aimed to determine whether the catalytic properties of hydratases of different lactobacilli are responsible for the different patterns of linoleic acid conversion.
A phylogenetic tree of linoleic acid hydratases was constructed based on 25 protein sequences including the enzymes from *Lactobacillus plantarum* TMW1460, *Lactobacillus hammesii* DSM16381, *Lactobacillus reuteri* LTH2584, and 6 hydratases that were characterized at the protein level. Enzymes from *L. plantarum* and *L. hammesii* were closely related while the enzyme from *L. reuteri* belonged to a separate cluster. Cultures of *L. hammesii* and *L. reuteri* converted linoleic acid to the 10-HOE as the sole product while cultures of *L. plantarum* produced 10- and 13-HOE as well as 10,13-HOA. The hydratases from *L. reuteri* and *L. plantarum* were cloned, expressed in *E. coli* BL21 and purified from inclusion bodies by affinity chromatography. Linoleic acid hydratases were FAD-dependent enzymes and inactive without the addition of FAD cofactor. Interestingly, both hydratases merely acted on C9 bonds, converting linoleic acid to 10-hydroxy C18:1 fatty acid. In conclusion, the different patterns of linoleic acid metabolites in lactobacilli were not attributable to different catalytic properties of the linoleic acid hydratases and a second C12 acting linoleic acid hydratases may be present in *L. plantarum.*
(1) *J. Agric. Food Chem.* 61:5338. (2) *J. Biol. Chem.* 285:10353. (3) *Appl. Environ. Microbiol.*79:1866.

B050

Diversity of *Streptococcus thermophilus* phage isolated from industrial dairy plants worldwide

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The implementation of starter rotation schemes remains an effective method to minimize phage attack in dairy plants. In an effort to develop intelligent starter rotation strategies to prevent phage infection, an accurate understanding of phage-host interactions and knowledge on the diversity of phage within dairy plants is essential. Twenty-five problematic *Streptococcus thermophilus* bacteriophage were isolated from dairy plants from different global locations including Poland, India and France. Phages were characterized based upon their host range, DNA restriction profile, and packaging mechanism. DNA restriction analysis was performed using *Cla*I, *Eco*RI, and *Hin*dIII. Multiplex PCR was used to classify each bacteriophage as *cos*-, *pac*-, or 5093-type. The results identified 23 distinct DNA restriction profiles with genomes ranging in size from 19-38.6 Kb. Overall, a limited host range was observed for all phage.Twenty bacteriophages were classified as *cos*-type, while three bacteriophages were classified as *pac*-type phage. Two bacteriophages could not be classified using the multiplex PCR method based on their DNA packaging mechanism and structural proteins. The influence of temperature and pH on phage adsorption was also evaluated in select phages. With the exception of Φ5305, reductions in phage numbers was observed when exposed to pH values ranging between pH 4-6.8; no loss in Φ5305 viability was observed at pH 4.0. One step growth kinetic studies revealed latent periods between 10-20 minutes with burst sizes of approximately 100-1000 phage particles per infected cell. Electron micrographs confirmed that all selected dairy phage belonged to the *Siphoviridae* family, morphotype B1. These results indicate that the *S. thermophilus* phages isolated in this study are diverse and highly progressive in nature.

B051

Minimizing the genome of *Lactococcus lactis*

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The 2.54-Mb genome of the nisin producing *Lactococcus lactis* strain N8 contains 2511 genes. Of those, 1261 form a presumed *L. lactis* core genome, and are regarded as essential genes. The aim of this study is to delete non-essential genes excluding nisin operons (n=1239) from the *L. lactis* N8 chromosome, and to examine the influence of the smaller genome on growth and nisin production. This 4-year project was started in autumn 2013, and currently the first deletions are in progress. The genes are deleted from the chromosome by conventional two-step double cross-over strategy. First, a non-replicating plasmid carrying two genomic fragments is integrated into the chromosome by homologous recombination. The integration vector is then removed by a second cross-over event, leading to a potential deletion in the chromosome. For screening and selecting double cross-over colonies, the integration vector carries the sucrose-6-phosphate hydrolase gene *scrB* in antisense direction under the control of nisin promoter. Consequently, the single cross-over strain harboring the integration vector in the chromosome displays sucrose negative phenotype. When the integration vector is removed due to the second recombination, the strain reverts back to sucrose positive and the double cross-over colonies can be found on M17 agar supplemented with sucrose.

B052

Analysis of a spontaneous lysozyme resistant mutant *guaA* reveals a link between nucleotide metabolism and peptidoglycan structure of *Lactococcus lactis*

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The strong peptidoglycan (PG) mesh of Gram-positive bacteria is needed to maintain cell shape and to counteract high turgor pressure as well as cell wall stress. In contrary, high degree of PG elasticity is needed for the growth and separation of bacterial cells. We studied factors affecting the equilibrium between these two opposite call wall features in *Lactococcus lactis* by examining spontaneous mutants resistant to PG hydrolase lysozyme. We found that the large part of such mutants carry deletion of *guaA* gene, involved in purine metabolism. Transcriptional analysis of *guaA* mutant revealed that expression of *pyrB*, encoding aspartate transcarbamoylase responsible for the use of L-Asp for *de novo* pyrimidine synthesis, was considerably decreased. Also, *pyrB* mutant showed increased resistance to lysozyme. The cell wall fine structure determination of *guaA* and *pyrB* mutants indicated increased amount of Asp (Asn) in PG. Since L-Asp is converted to D-Asp and then used for PG cross-bridge formation, we speculate that utilization of L-Asp for effective nucleotide synthesis during exponential growth allows L-Asp to be highjacked away from PG synthesis, leading to appearance Asp-less stem-peptides. Such stem-peptides do not form PG cross-bridges, consequently utilization of L-Asp for pyrimidine synthesis by PyrB may be part of regulational scheme, ensuring elasticity of PG needed during exponential growth.

B053

Physiological and genetic characterisation of lactococcal bacteriophages isolated from whey samples

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Bacteriophages constitute a serious problem in many biotechnological processes by disturbing fermentation processes in dairy plants. Here presented are the results of our research on virulent bacteriophages infecting dairy lactococcal starter cultures. The main objective of our work was to investigate the type, morphology, growth kinetics and genome sequence of isolated phages from various whey samples in Poland.
Phages have been propagated on the commonly used *Lactococcus lactis* laboratory strains (IL1403 and MG1363). Identified phages were classified into two major genetic groups - 936 and C2 - by multiplex PCR analysis. Several phages showed specificity to more than one genetic group which could indicate either presence of a mosaic phage or a co-isolation of a prophage. This result needs further examination. DNA was extracted from each isolated phage and analyzed by restriction endonucleases. This analysis allowed to approximately estimate the size of phage DNAs and establish the similarity level between phages. Based on restriction patterns, the detected phages were assigned into several types. Overall, 15 different phages were selected and subjected to genome sequencing. The complete nucleotide genome sequences will be analyzed and subjected to whole-genome comparison analyses. Furthermore, we characterized the infection cycle of all examined phages by one-step growth kinetics and investigated their burst size and latent time. Electron microscopy of phage particles was performed using JEM 1400 JEOL Co. (Japan 2008).
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B054

The genome of *Lactobacillus nodensis* reveals the basis for flavour production in this industrial adjunct

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Lactobacillus nodensis was recently shown to influence the flavour profile of Gouda-type cheese when added as an adjunct. However, little is currently known about the metabolic potential present in this strain; especially in relation to flavour development. Here, we present the draft genome sequence of *Lb. nodensis* CSK964. The genome was sequenced using a combined strategy of Illumina and Roche 454 paired-end based sequencing technologies. DNA sequence reads were assembled using the MIRA software package. Initial assemblies resulted in 149 contigs in 16 scaffolds. This resulted in a 2.7-Mbp draft genome in 12 scaffolds with an overall GC content of 37.7%. A total of 2,864 protein-coding regions and 6 rRNA operons were predicted. *Lb. nodensis* CSK964 was found to contain an efficient proteolytic system comprising 23 peptidases and the cell wall associated protease, PrtP. Genomic analysis of the adjunct strain, *Lb. nodensis* CSK964 confirm its flavour-forming capacity. Nine esterase genes, potentially important for the “fruity” flavour notes in cheese, were identified in the genome of *Lb. nodensis* CSK964. Interestingly, genomes of LAB typically contain one esterase. Additionally, *Lb. nodensis* was found to contain the full gene complement required for the production of the flavour compounds hydrogen sulphide, dimethyl disulphide, and dimethyl trisulphide.

B055

Strain-specific prebiotics increase efficacy of probiotics

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This work contributes to the ongoing development of more effective multispecies probiotic products. Up till now synbiotic formulations were composed by randomly combining probiotics and prebiotics. We show that potentially more effective products can be developed by specifically selecting pro-and prebiotic combinations.
In order to determine the synbiotic potential of various probiotic/prebiotic combinations seven commercially available prebiotics were screened for their ability to increase growth of 26 different probiotic strains by measuring optical density (OD, 620 nm) in a spectrophotometer. Growth was measured in MRS broth, where glucose was replaced by a prebiotic compound. Based on the results, mixtures of specifically selected prebiotics were composed for several probiotic formulations. Growth and activity of the probiotic mixtures was again determined by OD and lactic acid production respectively.
The results showed that each probiotic strain favoured utilizing specific prebiotics for optimal growth. When multispecies probiotics contained a mixture of the favoured prebiotics for each individual strain, an increase in growth was observed compared to randomly chosen mixtures of prebiotics.
In conclusion, these results indicate that for optimal growth prebiotics should be specifically selected depending on the probiotic strains in the formulation. In this way, strain-specific prebiotics can optimally increase the effect of multi-species probiotic formulations.

B056

A novel transcriptional regulator of proteolytic gene expression in *Lactobacillus helveticus*

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Proteolytic system of *Lactobacillus helveticus* is important for both supplying amino acids for their growth and the production of antihypertensive peptides in milk. Transcription of proteolytic system reported to be repressed by high concentration of amino acids and peptides in the medium. A 26-kDa protein with N-terminal cystathionine β-synthase domains (CBS domain protein), which seems to be involved in the regulatory system, was purified by using a DNA-sepharose bound the upstream regions of the six proteolytic genes that are down-regulated by amino acids. The CBS domain protein bound to the upstream of the *pepV* gene in response to branched chain amino acids (BCAAs). The expression of the *pepV* gene in *Escherichia coli* grown in BCAA-enriched medium was repressed when the CBS domain protein was co-expressed. These results reveal that the CBS domain protein acts as a novel type of BCAA-responsive transcriptional regulator (BCARR) in *L. helveticus*. Footprint analysis using the *pepV* promotor region and gel shift analyses with the corresponding short DNA fragments strongly suggested that the BCARR protein binds adjacent to the *pepV* promoter region. Homology search analysis of the C-terminal region of the BCARR protein suggested the existence of similar sequence to ACT (aspartate kinase-chorismate mutase-tyrA) domain, which has ability to bind amino acids. These results also suggest that the sensing of BCAAs by the ACT like domain might promote the binding of the BCARR to DNA sequences upstream of proteolysis genes, which affects the gene expression of the proteolytic system in *L. helveticus*.

B057

Non-respiratory *Lactococcus lactis* often possess a specific detrimental point-mutation in the *noxA* gene

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Lactococcus lactis can perform respiratory growth when supplemented with heme and O_2. Respiratory growth of *L. lactis* leads to higher final biomass compared to fermentative growth (aer^+). We compared biomass increase as determined by OD. It was shown that among more than 800 *Lactococcus* strains tested, a majority could obtain increased biomass when grown with heme and vigorous shaking (high O_2), compared to growth with heme and no shaking (low O_2). Some *L. lactis* strains failed to respire when both heme and oxygen were available (aer^-). We hypothesized that one or more proteins of the electron transport chain were hindering the heme-dependent respiration. Comparing genome sequences of strict aer^+ and aer^- strains we found SNPs on several genes involved in respiration, with the notable high abundance in *noxA*. PCR targeting the *noxA* gene and subsequent sequencing showed that gene *noxA*, encoding NADH dehydrogenase, was disrupted in most of the aer^- strains. We have sequenced the *noxA* of 36 of the different $aer^{+/-}$ phenotypes and confirmed their $aer^{+/-}$ genotype. We find it very likely that a typical disruption in the NoxA protein leads to an aer^- phenotype.

B058

***In vitro* tyramine accumulation by different *Enterococcus* strains**

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Tyramine is a biogenic amine produced through the tyrosine decarboxylation by tyrosine decarboxylase (TDC) enzyme derived by Gram-positive bacteria in food, especially enterococci. Bacterial TDC is reported to be also able to decarboxylate phenylalanine to produce another biogenic amine, namely phenylethylamine. The aim of this study was to evaluate the ability to accumulate tyramine in model systems by three enterococcal strains, namely *Enterococcus faecalis* EF37 and EF29212, and *Enterococcus faecium* EF12. At a genotypic level, all these strains possessed the tyrosine decarboxylase (*tdcA*) operon that showed the nucleotide sequences typical for each species examined.
The enterococci were grown in rich broth (BHI) added or not with 0.1% (w/v) of tyrosine and incubated at 37°C for 144 hours. Their growth curve were measured by optical density (OD at 600 nm) while their ability to accumulate tyramine was detected by HPLC analysis. In addition, the *tdcA* gene expression of the three enterococci was analyzed by RT-qPCR under the same conditions.
All the tested strains were able to produce high levels of tyramine when tyrosine was added and amine concentration increased concomitantly with the cell number with a relatively small further rise during the stationary phase. Moreover, the results showed that also 2-phenylethylamine could be accumulated. In general, data on the *tdcA* gene expression were concordant with those obtained by HPLC analysis; indeed, the *tdcA* gene was highly expressed during the exponential growth phase, however some differences in the transcript level were observed in relation to the enterococcal strain.

B059

Potential alternative to the ADI-pathway in the catabolism of arginine in *Enterococcus faecalis* V583

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Current literature describes only one existing pathway for catabolism of arginine for *Enterococcus faecalis* V583 in the arginine deiminase (ADI)-pathway, where the enzyme arginine deiminase (*arcA*) catalyzes the deamination of L-argininine. An *arcA* deletion-mutant was constructed in V583 (V583Δ*arcA*) and cultured in a glucose-limited continuous culture (chemostat). Surprisingly L-arginine was still catabolized, suggesting an alternative arginine deiminase or alternative pathway for arginine catabolism exists in *E. faecalis* V583. A shift in metabolism towards more mixed acid fermentation, as well as a significant reduction of biomass was observed for the Δ*arcA* mutant compared to wild type (wt) in the chemostat. A significant effect on growth for the Δ*arcA* mutant compared to wt was also observed in batch culture without any nutritional limitation. The growth impairment was not restored upon complementation, indicating that providing an intact *arcA* gene *in trans*, is not sufficient to restore ADI-pathway function and hence that the mutation is polar. Furthermore, the role of ADI in the pH homeostasis of *E. faecalis* V583 was assessed. However, no significant difference in survival was observed between wt and V583Δ*arcA* at pH 4 in 1mM $MgCl_2$, 20mM $Na_2HPO_4^-$, in the presence of 25 mM arginine. This observation confirms that the importance of ADI for acidurance vary between species of lactic acid bacteria.

B060

STS molecular marker for rapid screening of *Lactobacillus casei* group as secondary adjunct starter

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Flavour perception is very important in the differentiation of fermented dairy products as ripened cheese. Ripened cheese is a dynamic environment involving microbial and biochemical changes which lead to the formation of products characteristics and flavour. The formation of flavours proceeds a rather slowly through several chemical and biochemical reactions during aging. This complex process involves three major metabolic pathways: (1) metabolism of lactate and citrate, (2) liberation of free fatty acids (FFA) and their subsequent metabolism, (3) degradation of the casein matrix of the curd to a range of peptides, followed by degradation to free amino acids (FAA), and ultimately FAA catabolism. These biochemical reactions may be affected by the entire spectrum of cheese microbiota, which is composed of starter LAB (SLAB) and adventitious species including non-starter LAB (NSLAB). NSLAB dominate during ripening thank to their ability to use the major compounds of cheese, such as organic acid (lactic acid produced by SLAB, citric acid of milk), small peptides and FAA, as nutrient sources for grow. The metabolism of NSLAB can lead to the production of different compounds that contribute to the flavour of cheese. Among NSLAB, *Lactobacillus casei* group participate in cheese aroma formation through reactions which rely on the hydrolytic action of enzymes to lactate, citrate and amino acids. In this research work, *L. casei* group metabolic capabilities to form flavour compounds were investigated developing STS (sequence tagged site) molecular marker for identification of genes encoding flavour-related enzymes. Lactate and citrate catabolic pathways of the group were considered.

B061

The effect of growth rate in *Lactobacillus sakei*; analysis of experimental data in light of external information

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Lactobacillus sakei is a lactic acid bacterium commonly found living on fresh meat and fish. Thriving at low temperatures, this microorganism frequently becomes one of the dominant flora during cold storage. It plays a major role in meat fermentation during the production of dry sausages, and has been proposed to enhance the microbial safety of meat products by outcompeting spoilage-causing or pathogenic bacterial species. Effective growth and high acidification activity during meat fermentation are key characteristics of starter cultures used for sausage fermentation to ensure hygienic and sensory quality of the product.
The study of bacteria at a defined growth rate can be achieved in the chemostat. There the specific growth rate is directly manipulated by changing the dilution rate, which creates a controlled and constant environment (so called "steady-state"). We investigated the effect of growth rate in two genome sequenced starter culture strains of *L. sakei* representing the subspecies *sakei* and *carnosus*. Samples at steady-state from growth in anaerobic glucose limited chemostats at high and low growth rates were analysed. To get a complete overview of the strains physiology under the different conditions, we used genome-scale tools in addition to metabolite measurements. These were transcriptome and proteome analyses, and the open-source software Ondex enabling data from diverse biological data sets to be linked, integrated and visualised through graph analysis techniques along with relevant information stored in databases. The present work investigates the metabolic mechanisms underlying the growth performance of the two *L. sakei* strains, and gives a strategy for integrating results from multivariate data analysis and statistical testing of the experimental data into the same platform.

B062

Transcriptional analysis of metabolic genes involved in the growth of *Lactobacillus rhamnosus* during cheese ripening

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All microbiota involved in cheese manufacturing dynamically evolves during ripening. Particularly, starter lactic acid bacteria (SLAB), present at the early stages of cheese-making process, undergo death and lysis, while non starter lactic acid bacteria (NSLAB) become the dominating population during ageing, playing an important role on flavour formation.
Since NSLAB are able to multiply also of 5 order of magnitude in an environment where lactose is completely depleted, which are the metabolic genes associated with their growth in cheese?
Potential substrates for microbial growth are represented by small peptides or amino acids, citrate, lactate, and free fatty acids. Additionally, sugars and phospholipids, nucleic acids and peptides can be released in the cheese matrix when SLAB autolysis starts to occur. These compounds represent carbon sources that could yield pyruvate (i.e. through metabolism of citrate, lactate, amino acids, and nucleotides) or different metabolites.
Previous works (Bove *et al*., 2011; Lazzi *et al*., 2014) have shown that *Lactobacillus rhamnosus* 1019, a NSLAB isolated from PR cheese, is able to metabolize pyruvate and convert it into acetate, when it grows in a cheese-like medium (CB).
In this study we focused on the expression kinetics of genes involved in the growth of *L. rhamnosus* 1019 in CB at different stages of growth, using RT-qPCR on selected metabolic genes (*spxB*, *ackA*, *pta*) to determine their level of activation. Simultaneously, we evaluated the substrate consumption and product formation of organic acids in the different steps chosen for the transcriptomic analysis.
The transcriptional activity of the metabolic genes was studied also in PR cheese samples to evaluate directly from the food matrix gene expression changes in different stages of ripening.

B063

Comparative genomics of *Weissella*

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Weissella is a genus of lactic acid bacteria (LAB) consisting of species formerly included in the *Leuconostoc paramesenteroides* group. Similar to other LAB, they are commonly found in fermented foods but have also been isolated from environmental and human samples. Currently there are twenty known species. In this study, three *Weissella cibaria* genomes were sequenced using Illumia Mi-Seq and Roche 454 technologies. Annotation was done using the Prokka and JGI IMG pipelines. Comparative genomics of the *Weissella* genus as a whole was done using data form the newly sequenced strains and genomic sequence data available online for other *Weissella* strains (n=ten; four *Weissella cibaria*, one *Weissella ceti*, one *Weissella confusa*, one *Weissella halotolerans*, two *Weissella koreensis* and *oneWeissella paramesenteroides*). The genomes had sizes varying from 1.3 to 2.4 Mb. G+C content ranged from 35% to 45%. BLASTClust was used to analyse the pan- and core-proteomes (95% identity cut-off) of *Weissella*. When all of the genomes of the genus were included in the analysis, the pan proteome consisted of 11,025 proteins while the core proteome consisted of only seven. This indicates low homology and high diversity among *Weissella* species. However, when the four *W. cibaria* genomes were analysed alone the pan proteome consisted of 3,242 proteins, while the core proteome consisted of 1,661. The presence of 51% of the *W. cibaria* pan proteome in all strains of this species indicates a much lower diversity when compared to the extensive diversity in the overall genus.

B064

Heterologous expression of dextransucrase from *Weissella cibaria* MG1 in *Lactococcus lactis* NZ9000

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Weissella is a genus of lactic acid bacteria (LAB) consisting of species formerly included in the *Leuconostoc paramesenteroides* group. Similar to other LAB, they are commonly found in fermented foods but have also been isolated from environmental and human samples. The species *Weissella cibaria* and *Weissella confusa* are notable for their production of dextran exopolysaccharide when grown in a medium containing the disaccharide sucrose. Dextran has been shown to improve the texture and rheology of sourdough breads. In addition, it has been shown that use of a dextran-producing strain as an adjunct, or addition of purified dextran alone, can increase moisture retention in Cheddar cheese. In this study, the dextransucrase gene from an EPS hyper-producer stain, *W. cibaria* MG1, was amplified and cloned into *Lactococcus lactis* NZ9000 using the nisin inducible gene expression (NICE) system. The 4.3-kb dextransucrase gene was ligated into pNZ8048 in two separate fragments due to the presence of an Nco1 restriction site in the centre of the gene. Induction and expression of the gene in *L. lactis* NZ9000 conferred the dextran-producing phenotype on this stain. Significant quantities of EPS were produced by this recombinant strain, albeit at lower amounts than that produced by the native *W. cibaria* strain. This study demonstrates the potential for creating a novel starter strain with ability the produce dextran exopolysaccharide that can enhance and add value to the food product and may be of use in the dairy fermentation industry.

B065

Scalable genome-wide characterization of lactic acid bacteria

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With the advance of sequencing techniques the ability to genetically characterize bacterial strains has been extended from single strains to dozens and now even to hundreds of strains. Here we present the *in silico* analysis of over 100 genome sequences of lactic acid bacteria (LAB) from the DSM collection, including over 50 *Streptococcus thermophilus* species and over 50 *Lactococcus lactis* species. We have analyzed multiple aspects of these genomes, including subspecies identification using 16S based taxonomies, a core SNP profile to identify subgroups, plasmid content and their core and pan orthologous gene groups.
The genomes were sequenced at high quality using Illumina technology. Genome sizes were 1.7 MB and 2.5 MB for *S. thermophilus* and *L. lactis* species respectively. A hidden Markov model was used to retrieve 16S sequences and a Naïve Bayesian Classifier was used to determine the species. Genetic profiles of core SNPs were identified to enable whole genome comparisons, allowing detailed comparisons of both the overall diversity and sequence similar sub-groups with different phenotypes. These genome-wide SNP profiles - as based on conserved regions - were compared to phage profiles and displayed a high but not a 100% exact correlation.
Overall, the genome sequences were successfully generated, and analyzed with a dedicated bioinformatics in-house pipeline, in a high throughput fashion. Full genome sequencing and characterization has become an integral part of strain characterization efforts at DSM. The genome sequences were used to accurately determine taxonomies and the diversity via core SNP profiles. The core and pan genome analysis provides leads towards functional subgroups and further understanding of the DSM lactic acid strain collection.

B066

Analysis of regulatory elements in the genetic switch of temperate bacteriophage TP-J34 induced from a *Streptococcus thermophilus* yoghurt starter strain

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Lysogeny, prophage induction and lytic development of temperate *Streptococcus thermophilus* phage TP-J34 is controlled by the genetic switch in the lysogeny module. The genetic switch region separates four adjacent orfs (*crh*, *orf3*, *ltp* and *int*) from the potential lytic cycle-promoting genes *cro* and *ant*, and contains the divergently oriented promoters P_1 and P_2 and several predicted operator sites. The genes coding for putative repressor (*crh*), putative metalloproteinase (*orf3*) and superinfection exclusion-mediating lipoprotein (*ltp*) [1] are transcribed as one polycistronic mRNA starting from promoter P_2. Northern blot and RT-PCR experiments suggested own promoters for *ltp* and *int* (confirmed with 5`RACE PCR obtaining the 5`end of transcripts). *Crh* (essential for the establishment of lysogenization by suppressing lytic genes) was overproduced by heterologous expression in *E. coli* to perform electrophoretic mobility shift assays. Three operator sites in the intergenic regions between *crh* and *cro* (O_{1A}, O_2, O_3) and one between *cro* and *ant* (O_{1B}), respectively, were confirmed by competition assays with synthetic oligonucleotides. Glutaraldehyde was used as cross-linking reagent for Crh oligomerization (i.e., formation of dimers, tetramers and higher complexes). Knock-out experiments with *orf3* gene revealed a key role in induction of the lytic cycle. Orf3 obviously prevents binding of the Crh repressor to its operator sites as indicated by studies on the interaction between both proteins. Cro, the putative repressor of lysogenic genes, only bound to operator O_3 probably resulting in repression of lysogenic promoter P_2.
[1] Ali, Koberg, Hessner, Sun, Rabe, Back, Neve, Heller (2014) *Front. Microbiol.* #**5** (98), 1-23.

B067

Comparative study of the use of molecular tools to evaluate the resistance to wine stress and malolactic performance of *Oenococcus oeni* strains

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Oenococcus oeni is the lactic acid bacteria most adapted to wine stress conditions and it is responsible for Malolactic Fermentation (MLF); for this reason is the specie more frequently used as malolactic starter. The selection of malolactic strains is a task involving a considerable time and effort. The objective of this work was to characterize genetically and to reveal gene expression profiles of a large number of *O. oeni* isolated at different stages of vinification. The existence of relationships between genetic characteristics and expression profiles and MLF ability was inferred. Partially sequencing of *rpo*B and *rpo*C showed that allelic diversity of these genes is higher than that reported by other authors. No clear relationship between alleles and MLF performance was observed. No relationship between number of molecular markers present in strains and their resistance to wine conditions; these results are contrary to other authors' findings. However, we found a clear relationship between higher expression levels of *hsp18*, *rmlB* and *txrA* genes and resistance to ethanol, pH and SO_2, respectively. From the results obtained, expression studies would be considered a good tool to facilitate the selection of *O. oeni* able to perform MLF.

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B068

Biodiversity of *Lactococcus* and *Lactobacillus* strains isolated from cow, sheep and goat milk samples and artisanal dairy products

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Extensive use of lactic acid bacteria in biotechnology and fermentation technologies has contributed to substantial interest in genetics and biochemistry of these microorganisms. The analysis of samples of milk and dairy products, obtained directly at farms located in different regions of the Poland, allowed to create a collection of natural strains of lactic acid bacteria, with a potential for their diverse applications.
This study encompassed microbiological and molecular characteristics of the isolated natural strains of LAB, determination of their taxonomic position as belonging to the genera of *Lactobacillus* and *Lactococcus*, as well as their metabolic potential in respect to carbohydrates catabolism, biosynthesis of enzymes and production of antimicrobial substances. As a result 200 LAB wild-type strains, classified to the genus *Lactococcus* - 150 strains, and the genus *Lactobacillus* - 50 strains. For all these 200 strains the API 50CH (BioMerieux) carbohydrate profiles and Zym API (BioMerieux) enzymatic profiles were determined. In addition, production of aromatic substances, ability to produce exopolysaccharides, tolerance to various concentrations of bile salts and adhesion capacity were also tested. A global phenotype microarray analysis using the Phenotype Microarray analyser (Biolog, USA) for selected, representative *Lactococcus lactis* strains was performed.
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B069

***Lactococcus lactis* YfiA is necessary and sufficient for ribosome dimerization.**

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Dimerization and inactivation of ribosomes in *Escherichia col* is a two-step process involving the binding of ribosome modulation factor (RMF) and hibernation promotion factor (HPF). *Lactococcus lactis* MG1363 protein YfiA^{LI} associates with ribosomes in the stationary phase of growth and is responsible for dimerization of ribosomes. We show that full-length YfiA^{LI} is necessary and sufficient for ribosome dimerization in *L. lactis* but also functions *in vitro* with *E. coli* ribosomes. Deletion of the *yfiA* gene has no effect on the growth rate but diminishes the survival of *L. lactis* under energy-starving conditions. The N-terminal domain of YfiA^{LI} is homologous to HPF from *E. coli*, whereas the C-terminal domain has no counterpart in *E. coli*. By assembling ribosome dimers *in vitro* we dissected the roles of the N- and C-terminal domains of YfiA^{LI} and show that dimerization and inactivation of ribosomes in *L. lactis* and *E. coli* differ in several cellular and molecular aspects. In addition, two-dimensional maps of dimeric ribosomes from *L. lactis* obtained by single particle electron microscopy reveal a marked structural difference in monomer association in comparison to the ribosome dimers in *E. coli*.

B070

GtfA and GtfB are both required for protein O-glycosylation in *Lactobacillus plantarum*

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Acm2, the major autolysin of *Lactobacillus plantarum* WCFS1, was recently found to be *O*-glycosylated with *N*-acetylhexosamine, likely *N*-acetylglucosamine (Glc*N*Ac). Here, we set out to identify the glycosylation machinery by employing a comparative genomics approach to identify Gtf1 homologues, which are involved in fimbriae-associated protein 1 (Fap1) glycosylation in *Streptococcus parasanguinis*. This *in silico* approach resulted in the identification of 6 candidate *L. plantarum* WCFS1 genes with significant homology to Gtf1, namely *tagE1* to *tagE6*. These candidate genes were targeted by systematic gene deletion, followed by assessment of the consequences on glycosylation of Acm2. We observed a changed mobility of Acm2 on SDS-PAGE in the *tagE5E6* deletion strain, while deletion of other *tagE*s resulted in Acm2 mobility comparable to the wild type. Subsequent mass spectrometry analysis of excised and *in-gel* digested Acm2 confirmed the loss of glycosylation on Acm2 in the *tagE5E6* deletion mutant, whereas a lectin blot using Glc*N*Ac-specific succinylated wheat germ agglutinin (sWGA) revealed that besides Acm2, *tagE5E6* deletion also abolished all-but-one other sWGA-reactive, protease-sensitive signals. Only complementation of both *tagE5* and *tagE6* restored those sWGA-lectin signals, establishing that TagE5 and TagE6 are both required for the glycosylation of Acm2 as well as the vast majority of other sWGA-reactive proteins. Finally, sWGA-lectin blotting experiments using a panel of 8 other *L. plantarum* strains revealed that protein glycosylation is a common feature in *L. plantarum* strains. With the establishment of these enzymes as protein glycosyltransferases, we propose to rename TagE5 and TagE6 to GtfA and GtfB, respectively.

B071

Pan-genome analysis of *Lactobacillus crispatus*

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Lactobacillus crispatus is one of the predominant species in the human vagina and considered important for vaginal health. For instance, its presence in the vagina can reduce the incidence of recurrent urinary tract infection and its absence is associated with bacterial vaginosis. The species can also be recovered from a range of other host-associated niches such as the gastrointestinal tract of many animals. Taking advantage of ten *L. crispatus* genome sequences, we have investigated the pan- and core genomic potential of this central urogenital species. Using BLAST and OrthoMCL, the full complement of *L. crispatus* protein-coding genes was assigned to 3,929 orthologous groups of which 1,224 were present in each of the isolates. Mathematical extrapolations of these data to an infinite number of isolates revealed an open pan-genome structure indicating that the genomic diversity present in *L. crispatus* has not yet been comprehensively captured. The estimated core genome appeared to reach a plateau around 1,116 ortholog groups, being notably close in size to the current core and providing a solid foundation for inferring the essential functions and basic aspects of this species. The common core features included many adaptation factors and genetic systems involved in the interaction with the host, production of antimicrobial agents and competitive exclusion of pathogens. This comparative genomic analysis provides new insights into the intraspecific genome variability and the collective molecular mechanisms of *L. crispatus* and provides a plausible explanation for how *L. crispatus* contributes to the well-being of the host and to the prevention of urogenital infections

B072

From NGS sequence reads to pangenomes and gene-trait matching in lactic acid bacteria - A workflow of 2 hours / bacterial strain

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The challenge: With the advent of Next Generation Sequencing (NGS) technologies, sequencing of whole bacterial genomes in a near routine fashion has become affordable.The challenge of today is not to obtain NGS sequences, but to analyze them in a time-efficient and meaningful way.
Objective: To develop a workflow for building species pangenomes as a resource for assessing the distribution of genes of interest, developing sophisticated typing schemes and last but not the least as input for gene trait matching studies.
Methods: Illumina NGS sequence reads were assembled using Genomic Workbench (CLCbio, Denmark). CDS identification, CDS annotation core- and pangenome calculation was done using IOGMA Metabolic Pathway Builder (Genostar, France). MS Excel was used for the compilation of gene presence/absence, cds length, annotation information, nucleotide and amino acid sequences and contig positions. Relations between phenotypes and genes were assessed using PhenoLink (http://bamics2.cmbi.ru.nl/websoftware/phenolink).
Results: Core- and pangenomes were succesfully created for *Streptococcus thermophilus* (36 strains), *Lactobacillus helveticus* (28 strains), *Lactobacillus plantarum* (13 strains), and *Lactobacillus reuteri* (6 strains). Gene trait matching in *S. thermophilus* succesfully connected the strain phenotype “fast acidification”with the presence of the prtS gene for the extracellular proteinase.
Conclusion: The described workflow can be performed in two hours per bacterial strain, the MS Excel output of the developed workflow gives easy access to both the overall and detailed comparison of the coding sequences of the sequenced genomes. In combination with phenotypic data it serves as input for gene trait matching algorithms in order to link phenotypes to genes. Multifasta files can be generated from a free choice of genes i.e. in order to study relationships and develop typing schemes.

B073

***Lactococcus lactis* gene-trait matching revisited**

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Lactococcus lactis is a well-studied lactic acid bacterium that is used in the production of various fermented dairy products. Our previous study using pan-CGH microarrays based on four reference strains (IL1403, KF147, SK11, MG1363) resulted in the identification of about 3900 chromosomal orthologous groups (chrOGs) across 39 *L. lactis* strains. Of these chrOGs, 1268 were found in at least 35 strains. Nearly 600 and 400 chrOGs were present in subsp. *lactis* and *cremoris*, respectively. We now have genome sequences for 38 *L. lactis* strains. Phenotypes included API tests and their antibiotic/metal resistance. Using the sequence data data we identified over 7000 orthologous groups of genes (OGs) of which 1622 are present in 35 or more strains. Here we correlate presence or absence of these OGs with the phenotypic properties of the different *L. lactis* strains. The new sequence data allowed to uncover new genotype-phenotype associations of which we discuss a few examples.

B074

Regulation of natural transformation by the CovRS two-component regulatory system in *salivarius* streptococci

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In *salivarius* streptococci, competence for natural DNA transformation is regulated by the ComRS signaling system, which consists of the intracellular Rgg-type regulator ComR and its cognate competence pheromone ComS. While the mechanism of competence induction by the ComRS complex has been recently unraveled, little is known about the environmental parameters that modulate its activity and/or abundance. Interestingly, among salivarius species, almost all strains become competent upon addition of exogenous ComS, but very few strains display a spontaneous high level of transformation when grown in competence-permissive conditions (i.e. chemically defined medium; CDM). This phenotypic heterogeneity is frequently observed among transformable species but the underlying mechanisms are generally poorly understood. The objective of this study was to identify the regulatory determinants of this phenotypic diversity in *Streptococcus thermophilus*. Strains LMD-9 and LMG18311 were respectively selected as representative of highly and weakly transformable strains. We found that the levels of *comR* and *comX* expression were respectively ~10 and ~1000-fold lower in LMG18311 vs. LMD-9. Through gene deletion and exchange, we demonstrate that these differences are linked to allelic variations in the *rr01-hk01* locus. The locus encodes the CovRS-like two-component regulatory system, which is widely distributed among streptococci and controls the expression of virulence genes in pathogenic species. Our results indicate that, in CDM conditions, this system negatively controls the expression of competence genes in weakly transformable strains. A single and unique amino acid substitution in CovR from LMD-9 relaxes this repressor activity, leading to high transformation frequencies.

B075

Release of bioactive peptides - characterization of two cell-envelope associated proteinases

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Lactic acid bacteria (LAB) are extensively used as starter strains in the manufacture of various fermented products. They are increasingly marketed as health-promoting bacteria. Certain strains have been shown to produce bioactive peptides from milk protein, which are released by proteolytic enzymes. The proteolysis of milk protein by LAB is initiated by cell-envelope proteinases (CEP), which degrade the protein into oligopeptides [1].
In our study, we screened for proteolytically active lactobacilli and lactococci using casein labelled with FITC fluorescence dye [2] and soluble fluorescence was released through proteolysis of casein by the cell-bound proteases. Bioactive activity such like ACE-inhibitory effect was found in the hydrolysate of casein.
Highest proteolytic activity was found in strain 92202, followed by strain 92059. For species identification purposes, we performed 16S rDNA sequencing and used bioinformatical tools (ARB) for species-tree construction. Nucleotide sequence analyses together with physiological sugar fermentation tests suggested that strain 92059 is a *Lactobacillus delbrueckii* subsp. *bulgaricus* and 92202 is a *Lactobacillus delbrueckii* subsp. *lactis* strain, respectively. Presence of proteinase genes in both strains was confirmed by Southern blot assay. Proteinase genes from both strains were amplified via PCR with primers designed on the basis of published sequences of *Lactobacillus delbrueckii* and cloned into pSMART vector for sequencing. Expression of the genes are being optimized in *Lactococcus lactis* with the nisin controlled expression system (NICE), and purified enzymes will be used to degrade milk protein for release of bioactive peptides.
[1] Savijoki, Ingmer & Varmanen (2006) *Appl. Microbiol. Biotechnol*. 71: 394-406
[2]Twining (1984) *Anal. Biochem.* 143: 30-34

B076

Metagenomic analysis of the cocoa bean fermentation process underlines the importance of the involvement of lactic acid bacteria

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Metagenomic analysis of the community DNA of a sample from a spontaneous cocoa bean fermentation process revealed insight into the community diversity and functionality of the cocoa bean fermentation process. A software-independent taxonomic analysis, using both similarity-based and composition-based phylogenetic methods, revealed that the yeast species *Hanseniaspora uvarum*, *H. opuntiae* and *Saccharomyces cerevisiae*, the lactic acid bacteria (LAB) species *Lactobacillus fermentum* and *L. plantarum*, and the acetic acid bacteria species *Acetobacter pasteurianus* are the key players of the cocoa bean fermentation process. Furthermore, occasional microorganisms were retrieved, such as different *γ-Proteobacteria*. A functional analysis of appropriately assembled metagenomic sequence data of the community DNA of the same sample unraveled meta-pathways of the cocoa bean fermentation ecosystem. These included, for instance, homo- and heterolactic fermentation pathways of LAB and methylglyoxal fermentation and gluconeogenesis of enterobacteria. Finally, the metagenomic contig sequences were compared with genomic sequences of microbial strains that are candidate starter culture strains and showed shared functionalities between these strains and the community members mentioned above. All these data will lead to the selection of an optimal functional starter culture mixture for controlled cocoa bean fermentation processes.

B077

Regulation of the lacticin 3147 biosynthetic promoter P_{bac}

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Lacticin 3147, produced by *Lactococcus lactis*, is a member of the lantibiotic class of bacteriocins. Lantibiotics are post-translationally modified antimicrobial peptides that are active against a broad range of Gram-positive targets. The genes encoding lacticin 3147 production and immunity are found on two divergent operons; *ltnRIFE*, which is regulated by the promoter P_{imm}, and *ltn*$A_1A_2M_1TM_2J$, controlled by the promoter P_{bac}. We used a green fluorescent protein expression reporter system to establish if any of the lacticin 3147-associated gene products influence P_{bac} expression. It was revealed that LtnR plays a role as an activator of P_{bac}, in addition to acting as a negative regulator of P_{imm}. Moreover, P_{bac} was subject to negative regulation by *ltn*A_1 and *ltn*A_2. Thus, fluctuations in the levels of LtnR and *ltn*A_1A_2, may serve to maintain lacticin 3147 expression, modification and export at a homeostatic level within the cell.

B078

***In vitro* study of potentially probiotic *Enterococcus* spp. isolated from water-buffalo mozzarella cheese**

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A strain must fulfill certain safety criteria such as absence of virulence factors and lack of antibiotic resistance to be considered as probiotic. Also, it must survive to the conditions in the gastrointestinal tract (GIT) and tolerate the presence of medications that have been consumed by patients under therapy. In this research, the characterization of virulence potential using genotypic tests targeting various genes, the resistance to antibiotics, the tolerance to simulated GIT conditions, and the survival in the presence of sixty-two commercial medications were investigated in five strains of *Enterococcus* spp., isolated from water-buffalo mozzarella cheese, previously identified as *Enterococcus durans* (SJRP17 and SJRP29) and *Enterococcus faecium* (SJRP20, SJRP28 and SJRP65). The strains did not show presence of any genes encoding virulence factors. The strains SJRP20, SJRP29 and SJRP65 were resistant to oxaciclin, SJRP28 was resistant to fosfomycin, vancomicin and teicoplanin, and SJRP17 did not show resistance to any of the tested antibiotics. Strains SJRP17 and SJRP28 showed the lowest (5 log UFC/mL) and the highest (8 log UFC/mL) population, respectively, at the end of simulated GIT conditions, while the population of other strains was above 7 log UFC/mL. Strains SJRP28 and SJRP29 presented the highest (25.81%) and the lowest (14.52%) inhibition, respectively, to the tested drugs. All the strains were inhibited by medications containing ibuprofen, valsartan, enalapril and loperamide hydrochloride in a MIC lower than 20 mg/mL, 8.25 mg/mL, 0.063 mg/mL and 0.4 mg/mL, respectively. The majority of the strains were also inhibited by ketoprofen, paracetamol, metamizole and ciprofibrate. Considering simultaneously all tests, the strain SJRP29 showed the best results, being a good candidate to further investigation of other probiotic and technological properties.

B079

Antibiotic resistance and probiotics properties of lactic acid bacteria isolated from Andean vegetable and animal products

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In Andean regions of America,many traditional fermented foods and beverages are produced. Vegetable traditional products involve quinoa and amaranth whose widespread consumption as grains and fermented flours (sourdoughs) has grown recently due to their high nutritional value and lack of gluten. Fermented potatoes (*Tocosh*) have also been produced from ancient times in the Central Andean region of Peru, whereas llama meat fermented sausages are traditionally produced and consumed in Northwestern Argentina. At present, there is great concern that commensal bacterial populations from food and the gastrointestinal tract (GIT) of humans and animals, such as lactic acid bacteria (LAB) can serve as reservoir for antibiotic resistance genes, which can be horizontally transferred to other microorganisms. On these bases, the antibiotic resistance profiles (EFSA-2012) of eighty LAB strains isolated from Andean products were investigated. The analyzed exhibited a general sensitive profile against ampicilline (AMP), vancomycin (VAN), gentamycin (GEN), kanamycin (KAN), streptomycin (STR), erythromycin (ERY), clindamycin (CLI), tetracycline (TET) and chloramphenicol (CHL). Several strains were resistant mainly to the aminoglycosides (KAN, STR and GEN) while a multi-resistant pattern was found particularly among strains isolated from quinoa /amaranth grains and sourdoughs. However, sensitive LAB strains were observed among Lactobacillus *sakei*, *Lb. reuteri*, *Lb. plantarum*, *Lb. casei* and *Leuconostoc mesenteroides*. On the other hand, to select LAB strains with probiotic potential the resistance to stress factors (lysozyme, bile salts and low pH) present in the GIT, surface properties (hydrophobicity, autoaggregation and adhesion to caco-2 cells) and production of antimicrobial compounds were evaluated. Results allowed selecting *Lb. plantarum* (2 strains), *Lb. reuteri* (1) and *Lb. casei* (1) as the best candidates to be used as probiotics.

B080

The plasmid complement of *Lactococcus lactis* NCDO712

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The lactic acid bacterium *Lactococcus lactis* NCDO712 was isolated from a dairy starter culture in the 1950s and it is the ancestor of the prophage and plasmid cured model strain MG1363. *L.lactis* NCDO712 was described to contain 5 plasmids (~50 kb, 13.6 kb, 8 kb, 3.8 kb, 2.7 kb) of which so far only two have been sequenced. We here sequenced the complete plasmid complement of strain NCDO712, which resulted in the identification of 6 plasmids (55.4kb, 8.6kb, 3.6kb, 2.1kb, 15.5kb, 51.7kb) - one more than initially described. Next to an extracellular protease gene and the genes necessary for lactose utilization, which are on the already sequenced plasmid pLP712, the other plasmid-encoded genes include those of a variety of IS-located transposases, two restriction modification systems (specificity subunits) and stress related genes. The sequencing of the total DNA of strain NCDO712 also allowed identifying of some chromosomal differences between the strains NCDO712 and MG1363. The characterization of *L. lactis* NCDO172 will help establishing it as a relevant model strain because of its functional proximity to commercially used cultures. In combination with the genetically highly accessible derivative MG1363, it will form a useful couple to study industrially relevant traits of *L. lactis*.

B081

Conjugated linoleic acid and γ-aminobutyric acid production by lactic acid bacteria isolated from an artisanal Azorean cheese

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Lactic acid bacteria (LAB) produce a wide range of biogenic compounds, such as conjugated linoleic acid (CLA) and γ-aminobutyric acid (GABA) which are associated with several health benefits. CLA refers to a mixture of positional and geometric isomers of linoleic acid (C18:2, cis-9, cis-12 octadecadienoic acid). These biogenic isomers have been linked to many health-promoting properties including anticarcinogenic, antiatherogenic and anti-obesity activities. Another biogenic compound is GABA, a non-protein amino acid involved in neurotransmission, which may induce hypotension, diuretic and tranquilizer effects as well as antitumorigenic activity. In this study we screened a range of LAB isolated from an artisanal cheese for their ability to produce these two bioactive compounds. One hundred and twelve LAB, previously isolated from traditional Azorean cheese were tested for ability to produce CLA, using spectrophotometric and gas chromatographic (GC) methods. Strains were also assessed for their ability to generate GABA from monosodium glutamate (MSG), by analysing the free amino acid content using an amino acid analyser. Two microorganisms, identified as *Lactobacillus plantarum* were selected for their ability to produce CLA after incubation for 48h with free linoleic acid (0.5 $mg.ml^{-1}$) as substrate. C18:2 cis-9, trans-11 was the most abundant CLA isomer generated (44%). In addition, the CLA isomer C18:2 trans-9, trans-11, known to be anti-atherogenic, was also produced (34%) . Other CLA isomers were also generated as minor compounds. These two and five new strains identified as *Lactobacillus plantarum*, *Lactobacillus casei/paracasei* and *Lactobacillus otakiensis* were also found to produce GABA with concentrations higher than 6mM. The production of CLA and GABA by these strains may provide novel opportunities for the development of probiotic cultures for health improvement.

B082

Regulation of proteolytic system gene expression of *Lactobacillus delbrueckii* ssp. *lactis* CRL 581 by the peptide content of the growth medium

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Since lactic acid bacteria are unable to synthesize many essential amino acids, they possess a specialized proteolytic system that provides cells with essential amino acids during growth in milk, contributes to the development of the organoleptic properties of hard cheeses and can release bioactive health-beneficial peptides from milk proteins. *Lactobacillus delbrueckii* ssp. *lactis* CRL 581 possess a specialized proteolytic system that consists of a cell envelope-associated proteinase named PrtL, transport systems to allow the uptake of the resulting peptides, and several intracellular peptidases, which degrade peptides into amino acids. In this study, we demonstrated that PrtL activity was remarkably reduced in cells grown in the peptide-rich MRS medium compared to the proteolytic activity found in CDM, a peptide-free medium. Transcriptional analyses were performed to assess the dynamic response of CRL 581 cells grown in MRS and CDM. Reverse transcription quantitative PCR (qRT-PCR) was used to quantify the transcription level of fifteen genes (*prtL, oppB, dppA, dppB, opts, optA, oppA, pepP, pepA, pepC, pepO, pepF, pepM, pepN, pepR*) of the proteolytic system of CRL 581 cells grown in both media, with *groEL, 16S,* and *recA* as normalizing genes. The *prtL* gene was repressed 300-fold when CRL 581 cells were grown in MRS compared to the expression in CDM medium. Furthermore, all the transport systems genes evaluated (*oppB, dppA, dppB, opts, optA, oppA*) presented a significant reduced expression level in MRS (23 to 78 fold). Except *pepP*, which was repressed by the peptide content, the other aminopeptidases genes did not show difference in the expression levels. This is the first study describing the proteolytic system gene regulation of *L. delbrueckii* ssp. *lactis* by the growth media peptide content.

B083

Comparative genomic analysis of novel *Lactobacillus rhamnosus* strains of human and dairy origin

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The proven beneficial effects of widely studied strains like LGG, LC705, and HN001 make this species interesting to study candidate genes that contribute to probiosis and adaptability to different environments. However, the beneficial properties associated to probiotics are strain-dependant and should not be extended to other bacteria from the same genus and/or species. In this study, two *Lactobacillus rhamnosus* strains, AMC143 and AMC010 (Biosidus_90), isolated from infants stools and one strain, AMC004 (NC441), from a fermented dairy product were sequenced using next generation sequencing platforms. Phylogenetic trees generated to visualize the evolutionary relationship between strains showed that the sequenced strains were closely related to LC705. Average nucleotide identity (ANIb) scores between strains showed that AMC004 was closer related to AMC010 (ANIb 99.23%) than AMC143 (ANIb 97.62%). Genomes contained between 2,875 and 3,411 coding regions (CDS) and a comparable GC content ranging from 46.9% (AMC010) to 47.8% (AMC143). The three strains contained prophages, phi LC3 was identified in AMC004 and AMC143, and Lrm1 and Lc Nu were present in AMC010. Unlike AMC010 and AMC143, AMC004 produced exopolysaccharides, was unable to use fucose due to the lack of *fucU* and *fucI* isomerases, and was capable of fermenting L-arabinose and methil α-D-mannopyranose. The three strains were capable of using galacto-oligosaccharides (GOS 90%, lactose 10%) as carbon source. Ongoing experiments are targeted to identify genes that contribute to resistance and survival in the gut as well as genes potentially involved in probiosis.

B084

Adhesive properties of *Lactococcus lactis* subsp. *cremoris* IBB477 and their molecular determinants

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In the gastrointestinal tract, adhesion is a prerequisite for bacterial colonisation. Lactococci can be used in food (probiotics) and health-related applications (mucosal vaccine, therapeutic drug delivery), both potentially involving adhesive properties. Bacteria can adhere to the different components of the intestinal mucosa, in particular mucins and proteins of the extracellular matrix (ECM), such as laminin, collagen and fibronectin. Various cell surface-associated proteins were reported to mediate bacterial adhesion. In the present study, molecular determinants, potentially involved in adhesion of the *Lactococcus lactis* subsp. *cremoris* IBB477 strain to intestinal mucosa, were identified.
To this end, the improved high-quality draft genome sequence (2.864 Mb) of IBB477 was generated by shotgun and paired-end reads using Roche-454 platform and by Illumina sequencing technology. Based on the bioinformatic analysis of genome sequence, twelve knock-out mutants in putative adhesion genes were constructed and tested for their adhesive properties to bare and mucin-coated microtiter plates, in comparison with the wild type strain. Two deletion mutants exhibiting low level of adhesion: Δ*mub* and Δ*prtP* (in genes encoding mucus-binding protein and protease, respectively) were chosen for further analysis with the muco-secreting cell line HT29-MTX. Finally, *in vivo* persistence of the targeted strains was evaluated with conventional C57BL/6 mice.
The "Studies of nucleic acids and proteins - from basic to applied research" project is realised within the International PhD Projects Programme of Foundation for Polish Science. The project is co-financed by the European Union - Regional Development Fund. This work was also supported by European Funds Portal Innovative Economy “Centre of medicinal product biotechnology. Package of innovative biopharmaceuticals for human and animal therapy and prophylactics.”
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B085

Polyphosphate accumulation in native and recombinant *Lactobacillus* spp.

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Polyphosphate (poly-P) is a polymer of many individual phosphate residues, linked by phosphoanhydride bonds. Its accumulation in *Lactobacillus* spp. has only recently been seriously acknowledged (Alcantara *et al*, 2013) with evidence indicating a potential role for poly-P accumulation correlating with the cell's stress response.
The role of PPK1, a bacterial polyphosphate kinase, which reversibly catalyses the polymerization of the terminal phosphate of ATP into a polyphosphate chain, has also been more thoroughly investigated. Its presence is intrinsically linked with the formation of distinct poly-P granulations, or volutin granules, underlying the ability of the bacteria to remove phosphate from its environment.
In parallel, *Lacobacillus reuteri*, a member of the enteric microbiota of many vertebrates, has been demonstrated to produce bacterial microcompartments. These are thin-walled, proteinaceous structures inside bacterial cells. These intracellular compartments are 70-150 nm polyhedral structures containing enzymes, enclosed by a thin pore-containing protein shell.
We present data indicating accumulation of poly-P granulations in *Lactobacillus bulgaricus*, a species typically used in the production of yogurt. We also present findings regarding increased poly-P accumulation in *Lactobacillus reuteri*, with the insertion of a recombinant PPK1 gene. Our data highlights the properties of both the recombinant *Lactobacillus reuteri* PPK1 and *Escherchia coli* PPK1, and we also discuss native and potential novel bioengineered connections between polyphosphate metabolism and the production of structural microcompartments in *Lactobacillus reuteri*.

B086

Lactocin Lac705 stimulates the activity of toxin RelE in *Lactobacillus curvatus* CRL705.

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Type II toxin-antitoxin (TA) modules consist of a pair of genes that encodes two components: a stable toxin and a labile antitoxin. The toxin is co-expressed and neutralized with their cognate antitoxin from a TA operon in normally growing cells. Under stress conditions, the antitoxin is readily degraded allowing the toxin to exert their toxic effect causing the cell death. Plasmid pRC18 (18,6 kb) of *Lactobacillus curvatus* CRL 705 encodes a type II TA system and also the two-component bacteriocin lactocin Lac705. The TA module is a single operon that includes a 92-amino-acids antitoxin and a 118-amino-acids toxin that belong, respectively, to the superfamilies PhdYeFM and RelE. In this work, we describe that sub-inhibitory concentrations of lactocin Lac705 increases the toxic effect of RelE toxin in lactocin Lac705-sensitive cells. Strains AR3 and Sac7, two Lac705$^{-/s}$ mutants derived from strain CRL705, showed different sensitivity to the action of lactocin Lac705, being Sac7 four-times more sensitive than AR3. The difference between both cells is that strain Sac7 still has the plasmid pRC18 while strain AR3 is a plasmid-cured derivative. It was observed that sensitivity to Lac705 increased in AR3 cells transformed with plasmid pRA1ta, a plasmid which contains the pRC18-TA functions, showing that the TA module of pRC18 is responsible for the greater sensitivity of Sac7 to Lac705. Similar results were observed when other bacteriocins and antibiotics that target the cell membrane and cell wall functions, which suggest that the toxic function of the toxin RelE could be induced by stress factors whose mechanism of action is associated to the cell envelope.

B087

LsrS: A lantibiotic receptor-like protein encoded by *Streptococcus pyogenes*

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Bacteriocins are ribosomally synthesized antimicrobials produced by bacteria to inhibit other organisms. Bacteriocins select their targets by interacting with specific receptors present on the target-cell surfaces. Recent studies identified mannose phosphotransferase, maltose phosphotransferase, and zinc metalloprotease as receptors for various non-lantibiotic bacterioicins. No receptors for lantibiotic bacteriocins have been identified yet. We used a transposon mutagenesis screen to identify receptors for Smb, a lantibiotic produced by *Streptococcus mutans*. In this screen, we identifieda novel gene in the target organism *S. pyogenes*. This gene mapped within an uncharacterized operon containing three genes, among which two are membrane proteins. One of the proteins, which we named LsrS, putatively functions as a receptor for Smb. Inactivation of *lsrS* rendered the target organism partially resistant to Smb, confirming that LsrS exhibits a receptor-like function. Heterologous expression of *lsrS* in another Streptococcus further clarified that the second membrane protein of the *lsrS* locus is not needed for Smb sensitivity. Whereas, we found that sensitivity to nisin and tunicamycin relies on LsrS and some unknown *S. pyogenes*-specific proteins. A BLAST-P analysis showed that *lsrS* homologs are widely present in streptococci, including the Smb producer strain, *S. mutans*. An *lsrS* homolog, SMU.662, present in *S. mutans*, exhibits a receptor-like function only for Smb but not for nisin or tunicamycin. This is the first report of identification of a lantibiotic receptor-like protein. Surprisingly, we found that this receptor-like protein is also present in the producer organism. Although, the producer strain is protected from the self-produced lantibiotic by the immunity protein, the reason behind the presence of a functional receptor protein in the producer strain is currently not understood.

B088

Lactic acid bacteria as a surface display platform for *Campylobacter jejuni* antigens

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Campylobacter spp. are generally regarded as the most common bacterial cause of gastroenteritis worldwide. It is generally accepted that effective vaccine strategies should be realistic approach to control *Campylobacter* contamination of birds. However, at the present time there is no commercial veterinary anti-*Campylobacter* vaccine on the market.
The overall long-term goal of our work is to construct an effective chicken vaccine for *Campylobacter* using LAB (Lactic Acid Bacteria) strains as carriers. In this study we evaluated the possibility of using GEM (gram-positive enhancer matrix) particles of *Lactobacillus salivarius* isolated from chickens as a binding platform for two conserved, immunodominant, extracytoplasmic *Campylobacter jejuni* proteins: CjaA (member of the ABC transport system) and CjaD (peptidoglycan-associated protein - PAL, a component of the Pal-Tol system, responsible for maintaining cell wall integrity. *C. jejuni* antigens were fused with the protein anchor (PA) of the *L. lactis* peptidoglycan hydrolase AcmA, which comprises three LysM motifs and determines non-covalent binding to cell wall peptidoglycan. Both fused proteins, 6HisxCjaAx3LysM and 6HisxCjaDx3LysM, were produced by an *E. coli* expression system and purified by affinity chromatography. Their ability to bind to non-treated or TCA-pretreated *Lb. salivarius* cells was tested by immunofluorescence and Western blot analysis using polyclonal anti-rCjaA and anti-rCjaD serum. We found that even though CjaDLysM has a higher binding efficiency than CjaALysM, it does not block CjaALysM binding and a significant amount of the fusion was also bound to the GEM surface.
Our results clearly indicate that GEM particles can be used as delivery vehicles for more than one antigen, and GEM particles may represent a good mucosal delivery tool for future applications in different chicken diseases.

B089

Development of strain-specific quantitative PCR for *Lactococcus lactis* subsp. *cremoris*

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A quantitative PCR (qPCR) approach was developed for strain-specific analysis of the microbial composition (component ratios) of mixed-strain lactococcal cultures, with the aim of monitoring culture components in both research and industry to gain a better understanding of the behaviour of individual strains in multiple-strain systems. The Pfaffl strategy was used for relative quantitative analysis of amplification curves, using PCR primers designed from genomic sequences and tested to be strain-specific. Quantitatively distinguishing strains of the same species required (i) establishment of a cell lysis method giving quantitatively equivalent extraction of high-quality DNA from all strains (ii) design of primers (conserved and different between the strains to be analysed) that give high amplification efficiencies, (iii) optimisation of reaction conditions, and (iv) determining DNA target sequences chosen were not prone to variable dosage due to DNA replication intermediates. A simpler PCR assay, useful for quantitatively distinguishing strains that differ in the presence or absence of a restriction site, was also demonstrated. Strain-specific quantitative examination of lactococcal cultures achieved sensitivity comparable to that of conventional colony counting but with the advantage of more directly estimating bacterial cells (on the basis of gene dosage) rather than colony-forming units. CFU counts can be particularly misleading for strains that exhibit different coccal chain lengths. The genome sequence information and primer optimisation required to achieve reliable results mean that the investment needed to set up for analysis of a given culture can be substantial. However, once established, the assay lends itself to examination of multiple parallel samples, thereby increasing throughput and reducing the cost per assay.

B090

Sucessful production, secretion and isolation of Staphylococcal antigens using *Lactococcus lactis* as a host

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For the development and testing of novel proteinaceous antigens of pathogenic bacteria the isolation of these targets isneeded. Since the cytoplasmic production of several naturally secreted proteins of *Staphylococcus aureus* withconventional *Escherichia coli* or *Lactococcus lactis* expression systems resulted in low yields, a new approach was developed for their production. A set of expression vectors for intracellular protein production in *E. coli* has been made to construct derivative vectors for the expression of N- or C-terminally His-tagged fusion proteins with or without a TEV-cleavage site for the removal of the His-tag after or during protein isolation. Based on this set of vectors, new expression vectors for *L. Lactis* were constructed. Upon nisin-induced expression the His-tagged proteins were secreted using a natural signal peptide for Sec-dependent export from *L. lactis*. The tagged proteins were isolated directly from the culture medium using metal affinity chromatography. Lysis of *L. lactis* cells, resulting in a release of cytoplasmic proteins, and degradation of the expressed fusion proteins were severely reduced by using a lactococcal strain that is mutated in the major autolysin AcmA and the membrane-associated protease HtrA. Selected cell surface-exposed *S. aureus* proteins that remained cell wall-attached upon production in *L. lactis* were released from the cells by treatment with 6M urea or 2M KSCN after which they were purified. After isolation the proteins had or could retain their natural activity. More than twenty-five staphylococcal antigens, which are naturally secreted, or membrane- or cell wall-bound (covalently or non-covalently), have been successfully produced and/or isolated. We believe that this approach will be beneficial for successful overexpression and isolation of functionally active secreted proteins of both Gram-negative and Gram-positive bacteria.

B091

Antibacterial effects of nisin A in ethanol solution and metabolome analysis of intracellular substances in exposed indicator cells

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Nisin, an antimicrobial peptide (lantibiotics) produced by dairy-farming LAB *Lactococcus lactis*, has been applied as a biopreservative to foods in more than 50 countries including Japan. It is well known that the antibacterial effects of nisin drastically decrease at neutral and alkaline pH because of low solubility to water. In this study, we have examined efficacy of nisin in the neutral pH range and tested antibacterial effects and mode of action against *Lactobacillus delbrueckii* subsp. *bulgaricus* JCM1002^T, which is one of the most sensitive indicator strains for LAB bacteriocins in our laboratory. Nisin (nisin A) demonstrated strong synergistic effects with 20% ethanol (pH 8.3) and high heat stability at 95ºC for 1h. Metabolome analysis of the intracellular components remaining in the indicator JCM1002^T cells exposed to nisin clarified that major substances such as ATP and amino acids decreased by general nisin mechanism of pore formation in target cell membranes and then efflux of low molecular weight substances from the membrane resulting in death, especially when combined with 20% ethanol; while nisin caused intracellular accumulation of organic acids such as lactic acid in the bacterial indicator cells, indicating a novel knowledge for bactericidal function of nisin. Metabolome analysis might be an effective method to study action mechanisms of antibacterial substances such as antibiotics and bacteriocins under various environmental conditions.

B092

Improving thermal stability of the metastable bacteriocin Lcn972

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Lactococcin 972 (Lcn972) is a narrow-spectrum non-modified bacteriocin synthesized by *Lactococcus lactis* IPLA972. Mode of action studies have shown that Lcn972 is a non-pore forming bacteriocin that specifically binds to the cell wall precursor lipid II, blocking septum formation in dividing *Lactococci*. Lack of homology to other lipid II binding molecules led us to hypothesize that Lcn972 may bear a putative new lipid II binding domain which could be taken as a lead to improve existing antibiotics or engineer new ones. To fully understand the mechanism behind recognition of lipid II, we have firstly solved the 3D structure of Lcn972 by NMR. Lcn972 has a beta-sandwich fold assembling six antiparallel beta-sheets. Unfortunately, the protein unfolds irreversibly rather quickly at room temperature, preventing its use for mapping the interactions with the cell wall precursor. Therefore, disulfide bridges were introduced by site-specific mutagenesis to increase its thermal stability and the recombinant peptides were produced in *Lactococcus*. Two mutants N30CA59C and S15CA26C retained 6 and 60 % of the specific inhibitory activity of the WT peptide. Unfolding, monitored by tryptophan fluorescence, was partially prevented in N30CA59C and apparently inhibited in S15CA26C. Compared to the WT Lcn972, the half-life of S15CA26C at 37 ºC increased from 22 min to over 120 min. Further structural studies of the thermally stable Lcn972-S15CA26C by circular dichroism as well as affinity studies to lipid II are in progress.

B093

GENOBOX: A genomics toolbox for the prediction of functional properties of bacteria

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Fermented foods and beverages such as cheese, yogurt, bread, beer and wine have been in the human diet for centuries. They are the product of the fermentation of yeast and lactic acid bacteria on substrates such as milk, cereals,and fruits. In the context of the European research programme FP7, the GENOBOX project proposes anovel approach to the prediction of functional properties of microorganisms. GENOBOX will allow fast and cost-effective prediction of strains functionality based only onsome of their genomic features. All strains will be storedin a database to allow retrieval and comparison of their genomic features and traceability. GENOBOX is a web application developed using Python Django (version 1.4) on an Apache web server installation and deployed as a virtual appliance. GENOBOX currently hosts the genomes and phenotypic data of *Lactococcus lactis* strains, a few new genomes and a number of bioinformatics tools (for instance BLAST+) that enable comparisons and association of genomic and phenotypic data. The data are linked to popular databases such as the NCBI Nucleotide Database.Strain comparisons are visualized using tables and/or dendrograms generated on the fly from the data stored in the database and according to the user selection. From these dendrograms one could infer for instance properties of a new strain based on its distance from a reference strain. Also, combining thegenotype and phenotype data of multiple strains in a gene-trait matching approach could allow pinpointing gene cassettes that are predictive of a phenotypic property. GENOBOX also allows to query a set of known functional genes against the genomes in the database. The resulting information can be used to predict specific phenotypes and for instance antibiotic resistance and probiotic features.

B094

Study on action mechanisms of a class IIb bacteriocin gassericin T produced by *Lactobacillus gasseri* LA158

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In *Lactobacillus gasseri* isolated from human intestinal tracts and feces, a lot of strains producing a two-component bacteriocin (class IIb), gassericin T, comprised by GatA/GatX has been found. Gassericin T (GatAX) might form pores in the target cell membranes and cause the outflow of the material components from the cells, however the action mechanism remains unclear. In this study, we examined the mode of action using GatA, GatX, and GatAX in each MRS broth culture supernatant of *L. gasseri* LA158 and the destructives for the structural genes of *gatAX*, and tried to estimate the diameter of the pores that gassericin T formed in the indicator cell membranes. After incubation of *L. delbrueckii* subsp. *bulgaricus* JCM1002^T as an indicator strain in each culture supernatant including GatAX (LA158), GatA (LA158*ΔgatX*) or GatX (LA158*ΔgatA*) for 3h, 4h or 8h, the viable bacterial counts were measured, and the materials that flowed out from the indicator cells were collected. Then, each collected sample was subjected to first atom bombardment mass spectrometry (FAB-MS). As a result, three signal peaks of 115 Da (estimated proline), 246 Da (estimated tetramer glycine), and 268 Da (estimated inosine) were detected by GatAX, which were not found alone by GatA and GatX. These data indicate that gassericin T forms the pore of 0.98 -1.2 nm in diameter to the indicator cell membranes as a hexamer.

B095

Cloning and characterization of Sufi: a new Laccase from *Lactobacillus plantarum* able to degrade biogenic amines

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Cell division protein SufI from *Lactobacillus plantarum* J16 with accession number UniProt ID: C6VK53, was described as laccase by Callejón *et al* (2014). SufI is responsible for biogenic amine degradation (BAs) as described in the cited work. The gene *sufI* coding for this enzyme was cloned in a pET-28a plasmid and expressed in *Escherichia coli*. The biochemical characteristics and capacity of SufI to eliminate amines were investigated. The recombinant protein was purified by Ni-NTA purification system, and biochemically characterized with canonical laccase substrates 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), and 2,6-dimethoxyphenol (2,6-DMP). Mass, kinetic constants, thermal profile and pH activity range were determined. Furthermore spectroscopic properties, and oxidation of amines were investigated.
Results shown that SufI showed spectroscopic properties typical of blue laccases, has a molecular weight of ~ 66.5 kDa and exhibited activity towards canonical laccase substrates ABTS and 2,6-DMP. K_M for ABTS was 0.35 mM and V_{max} = 0.034, and for 2,6-DMP the K_M was 1.67 mM and V_{max} = 0.25. Highest oxidizing activity towards DMP was obtained at 60°C. The enzyme was thermostable until 70 ºC, however after 10 minutes incubation of SufI at 85°C no residual activity was measured. Among the biogenic amines, tyramine was a better substrate for SufI than histamine and putrescine.
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B096

The use of engineered and bioengineered nisin derivatives both alone and in combination with essential oils prove effective in controlling the growth of *Listeria monocytogenes*

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Bacteriocins are antimicrobial peptides that are effective against Gram-positive bacteria including *L. monocytogenes*. Nisin is a 34 amino acid polycyclic bacteriocin produced by certain strains of *Lactococcus lactis* subsp. *lactis*. It is generally recognized as safe (GRAS) and is the only bacteriocin permitted as a food additive in over 50 countries. Carvacrol, thymol and trans-Cinnamaldehyde are essential oils from oregano, thyme and cinnamon bark respectively, that have been shown to have antimicrobial activity. In this study, kill curve and growth curve analysis was used to evaluate the activity of nisin and bioengineered nisin derivatives individually and in combination with essential oils to control the growth of *L. monocytogenes*. The bioengineered nisin derivatives exhibited superior potency to nisin A when used alone or in combination with carvacrol, thymol and trans-cinnamaldehyde. These investigations have shown the potential of natural antimicrobial combinations such as bacteriocins and essential oils to achieve higher potency and thus a greater kill/inhibition of food borne pathogens such as *Listeria*. This technology can have many advantages including reduced expenditure and loss for food manufactures and the production of a food product that has its natural properties preserved to the highest extent while maintaining its extended shelf-life.

B097

Metabolic response to hexanal-based oxidative stress of five different *Lactobacillus* species

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Hexanal is one of the most important oxylipins and is an ubiquitous compound present in plants, plants products and foods. Oxylipins play crucial biological role as signals of intra and intercellular communication in plants, vertebrates, invertebrates and fungi. In particular, hexanal can generate oxidative stress response in microorganisms and can cause several types of cell damage, including: metabolic pathway reroutering and disruption, spontaneous mutations, bacteriostatic and bactericidal effects. These phenomena can result in a stress for the cell due to an imbalance that occurs when the survival mechanisms are unable to deal adequately with the Reactive Oxygen Species. Moreover, hexanal induces significant modification on the composition of cell membrane and the release of volatile compounds during the bacterial growth.
In this study the metabolic responses to different concentration of hexanal (1, 5, 10, 25, 50, 300 ppm), at different exposure times (30, 60, 120 minutes), were evaluated on *Lactobacillus sanfranciscensis*, *L. brevis, L. helveticus, L. rhamnosus* and *L. plantarum* through the analysis of volatile compounds by GC-MS-SPME, in order to elucidate specific responses eventually associated to the different species. All the strains responded to the applied stress producing specific volatile compounds linked to detoxification and cell-to-cell communication mechanisms. In particular, the release of molecules (such as acetophenone, isovaleric acid, 2-butyl-octenal and pyrazines) was associated to hexanal concentration (higher amount of hexanal induced higher release) and also to exposure time (longer time of exposure resulted in higher release).

B098

Bioengineered Nisin A derivatives with enhanced activity against both Gram-positive and Gram-negative pathogens

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Nisin is a bacteriocin widely utilized in more than 50 countries as a safe and natural antibacterial food preservative. It is the most extensively studied bacteriocin, having undergone decades of bioengineering with a view to improving function and physicochemical properties. The discovery of novel nisin variants with enhanced activity against clinical and foodborne pathogens has recently been described. We screened a randomized bank of nisin A producers and identified a variant with a serine to glycine change at position 29 (S29G), with enhanced efficacy against *S. aureus* SA113. Using a site-saturation mutagenesis approach we generated three more derivatives (S29A, S29D and S29E) with enhanced activity against a range of Gram positive drug resistant clinical, veterinary and food pathogens. In addition, a number of the nisin S29 derivatives displayed superior antimicrobial activity to nisin A when assessed against a range of Gram negative food-associated pathogens, including *E. coli, Salmonella enterica* serovar Typhimurium and *Cronobacter sakazakii*. This is the first report of derivatives of nisin, or indeed any lantibiotic, with enhanced antimicrobial activity against both Gram positive and Gram negative bacteria.

B099

Growth and metabolism of *Lactobacillus reuteri* in whole grain barley flour

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Lactobacillus reuteri is a bacterium naturally found in the intestine of mammals and birds but has also been isolated from fermented cereals. The occurrence of these bacteria in fermented cereals has stirred questions regarding the origin of these strains and also why cereals makes such a good substrate for this high demanding and otherwise gut associated bacterium. In this study we have analyzed the growth and activity of different strains of *L. reuteri* in whole-grain flour from different cultivars of barley. The strains include commercially used probiotic strains and newly isolated strains from fermented cereals. Bacterial counts were measured as well as pH-reduction and the metabolic profiles for the different combinations of barley and bacteria were determined by 1H-NMR. The results showed significant differences in the growth and pH-reduction between the different barley cultivars and also significant differences between the different strains. For some *L. reuteri* strains there seem to be no significant difference in growth and pH-reduction when grown on the different barley varieties while other strains show significant differences. Using multivariate statistics (PCA and OPLS-DA) we could group the metabolic profiles according to barley varieties but not bacterial strain. This suggests that in this situation the substrate is more important for the difference in production of metabolites than the bacterial strain. More in depth analysis of the NMR-spectra is however needed since parts of certain spectra remain unidentified.
We will also show preliminary results from an epithelial integrity model (IPEC-J2 cells), where a number of the different combinations of barley and *L. reuteri* have been evaluated for their ability to protect against the disruptive effect of enterotoxic *E. coli* on tight junction proteins.

B100

Genomic characterization of pilus-deficient derivatives of *Lactobacillus rhamnosus* GG

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Lactobacillus rhamnosus GG is one of the best characterized lactic acid bacteria and can be considered as a probiotic paradigm. Comparative and functional genome analysis showed that *L. rhamnosus* GG harbors a genomic island including the *spaCBA-srtC* gene cluster coding for the cell surface decorating host-interacting pili. Here, induced mutagenesis approach was used to study the pilus biogenesis in *L. rhamnosus* GG. While mutation selection systems have been used for decades to characterize biosynthetic pathways, recently established next generation sequencing methods allow for rapid characterization of the mutations within genomes. A combination of these two approaches was applied to *L. rhamnosus* GG for the selection of pilus-deficient mutants that were obtained by screening of enriched populations. The isolated mutants were first screened by dot blot analysis using antiserum against pilin proteins. Relevant mutants were selected and the lack of pili was confirmed by immuno-electron microscopy. The pilosotype of 10 mutant strains was further characterized by analyzing pilin expression using Western, dot blot and immuno-fluorescence methods. Moreover, mucus binding assay showed that the mutants did not adhere to porcine intestinal mucus. Comparative genome sequence analysis using the Illumina MiSeq platform allowed us to determine the nature of the mutations in the obtained strains. Three classes of pilosotype determining mutations were detected: mutations in *srtC* gene (Class I), deletions of the entire genomic island containing the *spaCBA-srtC* gene cluster (Class II) and mutations in the *spaA* gene (Class III). Remarkably, only a limited number of collateral mutations was observed. One of the Class I mutants was found to contain only 25 SNPs and can be considered as a candidate for human trials addressing the impact of the absence of pili.

B101

Respiratory condition induces a catabolic shift that promotes long-term survival in *Lactobacillus paracasei*

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Lactic acid bacteria are conventionally classified as obligate anaerobic microorganisms, however several studies support the hypothesis that certain species, such as *Lactococcus lactis* and *Lactobacillus plantarum,* may shift to a respiratory metabolism when exogenous heme and, if necessary, menaquinone sources are added to the cultural medium. Since this capacity has never been investigated in *Lactobacillus paracasei*, the aim of this study was to understand if the same metabolic change may occur in *L.paracasei* LPC-S01 and DG, two strains used industrially as probiotics.

The addition of heme and vitamin K2 (source of menaquinone) to an aerobic culture (Ae-HM) in MRS did not improve *L. paracasei* growth compared to anaerobic (An) and aerobic (Ae) cultures without co-factors. Nonetheless, Ae-HM condition strongly increased long-term cell survival at 4°C in exhausted MRS broth. Interestingly, drastically enhanced cell viability was also observed when *L. paracasei* Ae-HM cultures were prepared in an industrial culture medium.

To understand the molecular basis of this acquired resistance capacity, we performed cytofluorimetric analyses, which evidenced that Ae-HM cells had reduced membrane damage and oxidative stress levels compared to An and Ae cultures. Moreover, we observed by HPLC the production of a higher amount of acetate by the Ae-HM compared to Ae and An cells. This result was also supported by RT-qPCR analysis, which showed higher expression levels of acetate kinase, an enzyme that converts acetyl-phosphate to acetate with production of ATP.

In conclusion, we propose that *L. paracasei* can be able to activate the electron transport chain upon exogenous administration of heme and menaquinone, shifting the metabolism towards the production of acetic acid; consequently, bacterial cells may benefit of a reduced oxidative stress and an enhanced energy yielding, which may preserve cell viability. The respiratory-like metabolism of *L. paracasei* can represent a method to improve the resistance performances of *L. paracasei* probiotic strains at industrial level.

Section C: Ecosystems and Sustainability

C001 **Comparative study of *Lactobacillus helveticus* CRISPR loci**
M. Zago, E. Scaltriti, L. Rossetti, K.I. Sørensen, D. Carminati, S. Grolli, R. Ramoni, G. Giraffa

C002 **Systematic approach in resolving stability of complex starter culture in the context of bacteriophage predation**
M. Spus, J. Wolkers-Rooijackers, T. Abee, E.J. Smid

C003 **Antibiotic susceptibility of autochthonous lactic acid bacteria**
M. Petrusic, Z.R. Radulovic, I.Z. Zuber Bogdanovic, N.M. Mirkovic, D.P. Paunovic, S.B. Bulajic

C004 **Identification and functionalities of lactic acid bacteria isolated from Andean fermented potatoes (tocosh)**
G. Vignolo, .E. Jimenez, E. Ramos Vásquez, D. Zúñiga Dávila, Y. Yépez, E.M Hebert, P.S. Cocconcelli, R. Aznar

C005 **Optimization of lactic acid production by *Lactobacillus casei* from algae cake using a biorefinery approach**
T.J. Overbeck, J.L. Steele, J.R. Broadbent

C001

Comparative study of *Lactobacillus helveticus* CRISPR loci

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Lactobacillus helveticus is a homofermentative thermophilic lactic acid bacterium that is used in the manufacture of Swiss type and long-ripened Italian hard cheeses, such as Emmental, Grana, Parmigiano Reggiano, and Provolone cheeses. In this study, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and their associated genes, linked to a mechanism of acquired resistance against bacteriophages, were studied in 13 *L. helveticus* strains from Italian dairy origin. The obtained sequences and five *L. helveticus* genomes published in online databases were compared. A total of 34 CRISPR loci were identified and were classified into five main families based on CRISPR repeats: Ldbu1, Lsal1, Lhel1, Lhel2 and a new repeat family named Lhel3. Based on Cas protein classification, all CRISPR loci belonged to Type II, because they had the signature protein Cas3. Spacers had a size between 30 and 40 nucleotides; repeats had a size of about 30 nucleotides, with three longer repeats (36-37 bp). In parallel, 24 bacteriophage-insensitive mutants (BIMs) of the phage sensitive strain *L. helveticus* CNRZ892 were analysed to find active CRISPR arrays, with the aim to link the presence of CRISPR loci to phage defense mechanism even in *L. helveticus*. The results suggested that the presence of CRISPR array does not explain the resistance of these BIMs.

C002

Systematic approach in resolving stability of complex starter culture in the context of bacteriophage predation

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Previous studies have shown that complex cheese starter cultures are extremely stable in its composition. This compositional stability of these cultures results from a combination of factors like genetic repertoire, plasmid content and bacteriophage sensitivity and was achieved by the course of evolution during long term use in dairy industry [1]. The main focus of this study was on the role of bacteriophages in the maintenance of functional stability. Many single colony isolates of a complex starter culture were isolated and characterized regarding bacteriophage sensitivity and plasmid content. This information was used in designing defined blends of those single colony isolates. Blends consisting of different numbers of colony isolates were exposed (or not) to bacteriophage predation and sequentially propagated in milk (back-slopping) for many generations. The functionality and population dynamics of the blends were monitored at specified time points.
A systematic approach (from simple to more complex) in defined blends design allowed control over the applied level of diversity. Three different levels of diversity were gradually applied to defined blends: 1) isolates from two different genetic lineages: sensitive and resistant, 2) different levels of bacteriophage sensitivity: sensitive, moderately resistant and resistant among isolates from one of the two genetic lineages used, 3) many isolates (different levels of sensitivity) of all genetic lineages found in the complex starter. Comparative characterization of isolates from initial and end time point of sequential propagation of defined blends was performed to understand the mechanisms controlling compositional stability and maintaining culture complexity.
1 *Multifactorial diversity sustains microbial community stability* Oylum Erkus et al.; ISME Journal (2013), 1751-7362/13

C003

Antibiotic susceptibility of autochthonous lactic acid bacteria

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At present time point, many researchers assume that commensal bacteria, notably lactic acid bacteria (LAB), may represent a reservoir of resistance to antimicrobial drugs. For this reason, the commensal bacterial population such as autochthonous LAB isolated from food and gastrointestinal tract could represent a reservoir of antibiotic resistance genes. Traditional cheeses and fermented milk products made of raw milk represent a means of transmission of genes that carry resistance to antibiotic. According to this, food chain can be considered as one of the main routes of transmission of resistant bacteria between populations of humans and animals. The aim of this work was to evaluate the antibiotic susceptibility of 85 LAB isolated from traditional Serbian cheeses. Antibiotic susceptibility was determined for ampicillin, vankomycin, oxacillin, neomycin, chloramphenicol, gentamycin, tetracycline, erythromycin, kanamycin, penicillin, streptomycin using disk diffusion test and E-test. All the strains showed resistance to kanamycin, while most of the LAB strains showed resistance to streptomycin (94%) and vancomycin (82%). Slightly less number of strains showed resistance to gentamicin (65%), neomycin (56%) and oxacillin (53%). All strains showed sensitivity to penicillin, while several strains showed resistance to ampicillin, erythromycin, tetracycline and chloramphenicol. Strains showed minimal inhibitory concentracion (MIC) for streptomycin ≥24 μg mL^{-1}, gentamicin ≥48 μg mL^{-1}, oxacillin ≥1.5 μg mL^{-1}, tetracycline ≥3 μg $mL^{-1}$and erythromycin ≥1 μg mL^{-1}. The results obtained in this study indicate that resistance of autochthonous lactic acid bacteria isolated from traditional Serbian cheeses to antibiotics, is very important feature in characterisation and selection of strains for starter cultures or potential probiotic bacteria.

C004

Identification and functionalities of lactic acid bacteria isolated from Andean fermented potatoes (tocosh)

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Tocosh is a traditional fermented potatoes-based food produced in the Central Andean region of Peru, which is produced by placing different potato varieties on a straw bed in a hole practiced near a water spring. Besides being the main staple food for local population, *tocosh* is claimed to exert probiotic and antibiotic effects. Samples were collected from 0 (fresh potatoes, T1), 1 (T2) and 8 (M3) month-holes for lactic acid bacteria (LAB) isolation and identification. LAB counts were observed to increase (from 10^4 to 10^8 CFU/g) whereas pH varied from 5.5 to 4.2 during eight months fermentation period. All 152 presumptive LAB were subjected to identification by ISR-PCR and RAPD-PCR profiles, species specific PCR and 16S rRNA gene sequencing. Results showed that fresh and one-month stored potatoes were dominated by *Leuconostoc mesenteroides* (76%, 57%, respectively) and *Lactobacillus sakei* (24%, 43%, respectively) while LAB from 8-months hole potatoes exhibited the highest diversity involving *L. sakei* (42%), *Lactobacillus curvatus* (27%), *Lactobacillus paracasei* (22%) and to a minor extent *Lactobacillus casei*, *Lactobacillus brevis*, *Lactobacillus fermentum* and *Leuc. mesenteroides*. Functionalities of 43 selected LAB strains were assayed and they showed potential phytate degrading activity (74%), but no amylase; ability to grow in free folate media (70%) and tolerance to 12% alcohol (28%). In addition, most of them (53%) exhibited antifungal activity against *Aspergillus oryzae*. Extra or intracellular folate producers were *Lc. mesenteroides*, *Lb. sakei* and *Lb. paracasei*. *Tocosh* enrichment with lactobacilli species during fermentation would support the beneficial effects attributed to this traditional product. Information on unknown microbial genetic resources can be exploited as new functional cultures to improve nutritional and functional quality of other starchy products.

C005

Optimization of lactic acid production by *Lactobacillus casei* from algae cake using a biorefinery approach

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Growing demand for sustainable bio-based products have generated much interest in cost-competitive biorefinery-based technologies that enable the co-generation of a desired end product as well as valuable co-products from as many biomass components as possible. De-oiled algae biomass (called “algae cake”) is currently generated as waste material during biodiesel production and represents a viable fermentable substrate for implementation of a biorefinery approach for added co-product generation. Our group is applying a biorefinery approach to produce lactic acid, a valuable chemical feedstock, via fermentation of algae cake by the metabolically diverse species, *Lactobacillus casei*. Qualitative and quantitative analysis of the monosaccharide and amino acid composition of algae cake showed it contains ~5-6% carbohydrate and 24% protein. Small batch growth experiments showed *L. casei* is capable of utilizing algae cake as a fermentable substrate, but lactic acid yields were unexpectedly low. To identify potential strategies for increasing lactic production, we examined the nutritional requirements of *L. casei* during growth on algae cake. Significant increases in growth and lactic acid production where achieved with supplementation of glucose, casamino acids, and a mixture of essential and stimulatory vitamins. Glucose supplementation had the greatest stimulatory effect on growth and lactic acid production, and enzymatic pretreatment of algae cake with cellulase and amylase confirmed hydrolysis of complex carbohydrates was needed to release a greater content of fermentable sugar for *L. casei*. Research is now underway to secure heterologous expression of these enzymes by *L. casei* and use these strains in an integrated biorefinery process for production of biodiesel and lactic acid from algae.

Section D: Host-Microbe Interactions

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D031 **Insights into glycogen metabolism in *Lactobacillus acidophilus*: impacts on carbohydrate metabolism, stress tolerance and gut retention**
Y.J. Goh, S.. O'Flaherty, T.R. Klaenhammer

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E. Call, S. O'Flaherty, Y.J. Goh, K. Selle, T.R. Klaenhammer

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D036 **Characterization of water-soluble exopolysaccharides produced by *Leuconostoc citreum* isolated from Pico cheese**
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D037 ***Lactococcus lactis* strains delivering IL-10 protein or cDNA to the intestinal mucosa show anti-inflammatory properties in a TNBS-induced chronic colitis model**
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D038 **Characterization of a cell surface serine protease in *Lactobacillus acidophilus* involved in immunomodulation and intestinal epithelial barrier integrity**
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D042 **Exploring the role of lectin-like proteins in *Lactobacillus rhamnosus* GR-1 - host interaction**
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D043 **Detection of luminescent and fluorescent *Lactobacillus reuteri in vivo* and *in vitro***
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E. Wasilewska, D. Zlotkowska, J. Chudzik-Kozlowska

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S. Tynkkynen, M. Miettinen, T. Salusjärvi, R. Viitala

D046 **Probiotics counteract diet-induced obesity and insulin resistance in mice through remodeling adipose tissue and microbiota composition**
C. Grangette, J. Alard, K. Makki, V. Lehrter, V. Peucelle, I. Mangin, A-J. Waligora-Dupriet, B. Pot, I. Wolowczuk

D047 **Modulation of T cell response by pea albumins (*Pisum sativum)* and probiotics orally delivered to Balb/C mice**
D. Zlotkowska, J. Chodzik-Kozlowska, E. Wasilewska

D048 **The influence of the intestinal microbiota modulating factors on the food allergy development**
A. Ogrodowczk, E. Wasilewska, L. Markiewicz, J. Fotschki, B. Wroblewska

D049 **The effect of soybean and whey protein isolates on gut microbiota**
J. Fotschki, E. Wasilewska, L. Markiewicz, A. Ogrodowczyk, B. Kroplewski, B. Wroblewska

D050 **Development of a molecular well-defined vaginal probiotic**
E. Lievens, M. Petrova, S. Malik, J. Vanderleyden, S. Lebeer

D051 **Antipathogenic activity of lactobacilli against *Moraxella catarrhalis*.**
M. van den Broek, I. Claes, S. Lebeer

D052 **The anti-diabetic activity of *Bifidobacterium lactis* HY8101 *in vitro* and *in vivo***
S.H. Kim, C.S. Huh, I.D. Choi, J.W. Jeong, H.K. Ku, J.H. Ra, T.Y. Kim, G.B. Kim, Y.T. Ahn, J.H. Sim

D053 **Selective modulation of the intestinal barrier integrity by *Lactobacillus fermentum* strain AGR1487**
E. Altermann, R. Sengupta, R.C. Anderson, S. Clerens, L. Schofield, W. McNabb, P.J. Moughan, N.C. Roy

D001

Using gene-trait matching to unravel bacterial factors underlying *Drosophila*-lactobacilli mutualistic interactions

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Metazoans establish with microorganisms complex interactions for their mutual benefits. Despite the ongoing interest on the gut microbiota, a clear view of the physiological benefits and molecular mechanisms associated with host/microbiota relationship remains elusive. Therefore the use of simple animal models may help to unravel evolutionary conserved mechanisms underlying the impact of intestinal bacterial in their host physiology. In this light, *Drosophila* has become a powerful model to dissect the mechanisms behind mutualistic host-microbe interactions since it combines genetic and experimental tractability with a culturable microbiota. *Drosophila* gut microbiota is composed of simple and aerotolerant bacterial communities mostly composed of *Lactobacillaceae* and *Acetobactereaceae*.Host nutrition is the most important factor shaping *Drosophila*'s microbiota. *L. plantarum* is a prevalent commensal of *Drosophila melanogaster* and many strains can colonize germ-free animals and remain associated to their host during its entire life cycle. In order to identify bacterial factors underlying this mutualistic relationship we sequenced the genomes of 42 *L. plantarum* isolates and screened them for their ability upon mono-association to promote *Drosophila* larval growth under several nutritional challenges. Those results were used to perform *in silico* gene-trait matching in which the results of the quantitative phenotypic tests have been correlated with genotypic information of each strain. This approach allows the identification of putative molecular mechanisms at play during the association and also candidate genes to study by classical genetic functional methods. These genetic analyses paves the way to further functional studies aiming at deciphering the precise function and mechanisms of action of particular candidate genes underlying *Drosophila*-lactobacilli mutualistic interaction.

D002

Exploiting human host bacteriophages to control *Streptococcus mutans*

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The lactic acid bacterium, *Streptococcus mutans*, is mostly responsible for the initiation of human enamel caries in 70 to 100% of the studied caries cases. *S. mutans* typically produces a highly adhesive dextran that enables it to colonize tooth surfaces via the formation of biofilm. Bacteriophages (phages), viruses of bacteria, play a significant role in shaping microbial population structures in the human body, including the oral cavity. Little is known about *S. mutans* phages and their role on the establishment, maintenance, and pathogenicity of this cariogenic bacterium in the oral cavity. Phages of *S. mutans* may also be of considerable interest for the control and prevention of oral infections. The aim of this work was to isolate and characterize lytic phages targeting *S. mutans* strains. Diverse samples, such as saliva and faecal samples, were screened for the presence of phages targeting *S. mutans*. Phage host range was tested against more than 20 *S. mutans* strains. Phage morphology was assessed with transmission electron microscopy. Phage adsorption assays, restriction profiles, and genome sequencing for a few isolated phages were also performed. By identifying and characterising these *S. mutans* phages we can gain a better understanding and control of population structure, and we can also use phages as pharmabiotics in medicine, i.e. biological entities ‘mined’ from the human host which can be scientifically validated as impacting positively on health.

D003

***In vivo* transcriptome analysis of *Enterococcus faecalis* OG1RF in the mouse intestinal tract reveals adaptive fitness determinants**

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In this *in vivo* transcriptomic study of *Enterococcus faecalis* OG1RF in the intestine of monoassociated mice novel latent and adaptive fitness determinants of *E. faecalis* were identified. From 2658 genes that are present in *E. faecalis* strain OG1RF 124 genes were identified as significantly differentially expressed within the mouse intestinal tract as compared to exponential growth in BHI broth. The groups of significantly up- or down-regulated genes consisted of 94 and 30 genes, respectively, for which 46 and 18 a clear annotation to a functionally described protein was found. These included genes involved in energy metabolism (e.g. *dhaK* and *glpK* pathway), transport and binding mechanisms (e.g. phosphoenolpyruvate carbohydrate PTS) as well as fatty acid metabolism (*fab* genes). Some of these traits have been attributed to not only serve annotated metabolic functions, particularly if they are located at the cell surface like parts of the PTS systems, but also may express additional “moonlighting” functions such as adhesion, tolerance to oxidative stress, long term persistence in the gut, bacteriocin recognition or global sugar metabolism regulation. The novel putative fitness determinants found in this work may therefore be helpful for future studies of *E. faecalis* adaptation to the intestinal tract, which is also a prerequisite for infection in a compromised or inflamed host.

D004

Sticky signaling: Insights into pilus biogenesis of *Lactobacillus rhamnosus* strain GG

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Lactobacillus rhamnosus is a lactic acid bacterium that is extensively used as a natural preservative in diary industry and also marketed as a probiotic bacterium with respect to its health beneficial properties. Remarkably, the *L. rhamnosus* strain GG shows some strong adhesion abilities to human intestinal mucosa due to the presence of cell surface nanostructures called pili. In Gram-positive bacteria, sortase-dependent pili typically mediate the adhesion of bacteria to host epithelial cells and are associated with colonization, host signaling and biofilm formation. The objective of our work is to unravel the molecular mechanisms of the pilus biogenesis required for *L. rhamnosus* GG colonization and persistence in the human intestine tract. Using bioinformatic, biochemical and molecular methods, we identified and characterized novels signals at the N- and C-terminus of pilus-associated proteins that play a pivotal role in the assembly of the pilus structures in *L. rhamnosus* GG. Our findings allowed us to elaborate a regulatory model of the *L. rhamnosus* GG pilus biogenesis and also suggesting that similar signaling mechanisms occur in piliated lactic acid bacteria and other Gram-positive bacterial species.

D005

Species-level metagenomic analysis reveals changes of the gut microbiome following consumption of a fermented milk product

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The gut microbiota (GM) is thought to be involved in the pathophysiology of irritable bowel syndrome (IBS) although its exact role remains unclear. While some probiotics, including the fermented milk product containing *Bifidobacterium lactis* CNCM I-2494 (FMP), have shown promise in ameliorating IBS, their impact on GM remains to be elucidated. The recent development of Next-Generation Sequencing based quantitative metagenomics allows unprecedented species-level resolution of GM structure and potential functions. We aimed to identify the impact of this FMP on the GM structure and function in IBS patients. Stools from IBS patients with predominant constipation (IBS-C) (n=28) enrolled in a 4-week intervention were collected before and after the consumption of the FMP or a control acidified milk product. Fecal microbiota composition and functions were analyzed using species-level metagenomics. *In silico* analyses were used to reconstruct genomes of unknown species and the gut butyrogenic community. The FMP stimulated 3 endogenous *Bifidobacterium* and 2 unknown *Clostridiales* (MGS126, MGS203) and decreased the levels of the opportunist *Bilophila wadsworthia, Parabacteroides distasonis* and *Clostridium* sp. HGF_2. *In silico* analyses revealed that MGS126 and MGS203 are equipped to produce butyrate. Changes in endogenous *Bifidobacterium* were shown to be associated with changes in abdominal pain and feeling of bloating. For the first time, we have used species-level metagenomics to analyze fecal microbiota of IBS patients and identified specific changes of the GM that point towards a potential increase in SCFA, and especially butyrate, production. This model will serve as a frame for future studies required to confirm these observations.

D006

***Lactococcus lactis* CNCM I-1631 targets oxidative stress to ameliorate inflammation in experimental colitis**

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Our previous work showed that a 5 strain-fermented milk product (FMP) containing *Bifidobacterium animalis* sp. *lactis* (CNCM I-2494) and *Lactococcus lactis* (CNCM I-1631) (*LL*1631) improved colitis in *T-bet*$^{-/-}$ *x Rag2* $^{-/-}$(TRUC) mice (1). We aimed to identify the contribution of these 2 strains to the FMP's beneficial effect. By varying the strains used in the FMP, we found that *B. lactis* and *LL*1631 contributed to ameliorating colitis. We posited that *LL*1631 may deliver activities absent from the other FMP species. Using comparative genomic analyses of the FMP strains, we identified genes unique to *LL*1631 that were involved in the oxygen respiration chain. As respiration of oxygen results in reactive oxygen species (ROS) generation, *LL*1631 also possesses genes encoding enzymes that detoxify ROS, such as, superoxide dismutase (SodA). ROS are produced at high levels during intestinal inflammation and cause tissue damage. Thus, we hypothesized that lactococcal SodA played a role in attenuating colitis. Inactivation of the *sodA* gene abolished *LL*1631's beneficial effect in the TRUC model compared to the *wt* strain as demonstrated by histologic colitis scores. Similar effects were obtained in two additional colonic inflammation models, *Il-10*$^{-/-}$ mice and dextran sulfate sodium (DSS)-treated mice. Our efforts to understand how a lipophobic superoxide anion (O_2^-) can be detoxified by cytoplasmic lactoccocal SodA led us to the finding that host anti-microbial mediated lysis was a pre-requisite for SodA release and SodA's extracytoplasmic O_2^- scavenging. *LL*1631 may represent a promising vehicle to deliver antioxidant, colitis-attenuating SodA to the inflamed intestinal mucosa, and host anti-microbial products may play a critical role in mediating SodA's bioacessibility.
Reference1. Veiga *et al*., PNAS, 107:18132-18137.

D007

***Enterococcus faecalis* and *Enterococcus faecium*- healthy, harmless or pathogenic?**

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Enterococci are Gram-positive, homofermentative lactic acid bacteria that occur in a remarkable variety of different ecosystems. *Enterococcus (E.) faecalis* and *E. faecium* belong to the commensal intestinal microbiota of humans and animals, are found in fermented food but are also considered as the primary cause of nosocomial infections in humans.
The aim of this study was to investigate a potential correlation of the origin of isolates with their antimicrobial susceptibilities and/or their reducing metabolic capacity.
A total of 144 *Enterococcus* strains were studied. 52 were isolated from clinical samples, 40 from food fermentations and 47 as commensal isolates. The origin of another 5 strains could not be traced back. Agar disk diffusion technique was employed to determine the phenotypic resistence profiles against 12 antibiotics. Reducing metabolic activity was determined with a resazurin assay.
Linear discriminant analyses (LDA) showed a clear separation of *E. faecium* clinical isolates from the other two groups (food and environmental) due to their low prevalence of teicoplanin resistance and higher resistance against norfloxacin. The origin of *E. faecium* isolates was not discriminated by the reducing activity. Interestingly, in *E. faecalis* the most discriminant variables were reduction activity and norfloxacin resistence, separating clinical isolates from food and environmental isolates.
This study suggests measurement of the reducing metabolic capacity as virulence associated trait at least for *E. faecalis.*

D008

Mucosal immunogenicity of recombinant *Lactobacillus acidophilus* exposing HIV epitope on the S-layer protein

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Lactic acid bacteria are being evaluated as mucosal vaccine-delivery agents. The major surface layer (S-layer) protein of *Lactobacillus acidophilus*, SlpA, is potentially an efficient antigen/epitope-displaying scaffold due to its abundance over the microbial cell surface of beneficial Gram-positive microbes. In this study, a genetically modified *L. acidophilus* strain expressing a 16-mer polypeptide from the membrane proximal external region (MPER) of human immunodeficiency virus type I (HIV-1) on SlpA was established by replacement of *slpA* gene with homologous recombination. The intact cells of the recombinant and their purified S-layer protein were recognized by 2F5, a broadly neutralizing antibody against HIV-1 MPER. Intragastric immunization of mice induced moderate levels of MPER-specific and SlpA-specific antibodies in serum and mucosa secretions. Isotype determination of anti-MPER antibodies in sera revealed IgG2b almost exclusively. Profiling of cytokine production in SlpA-restimulated splenocytes suggested that the specific immune responses were Th1 and Th17 dominant. These findings demonstrate that the *Lactobacillus* S-layer protein can be exploited for the immunogenic display of specific peptides in oral vaccine strategies.

D009

A novel immune stimulatory lactic acid bacteria which enhances anti-viral immunity by activating plasmacytoid dendritic cells

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Plasmacytoid dendritic cells (pDCs) play a crucial role in anti-viral immunity through production of large amounts of type I interferons (IFNs). Although synthetic Toll-like receptor ligands, pathogenic bacteria and viruses activate pDC, there is limited investigation of non-pathogenic microbiota that are in wide industrial dietary use, such as lactic acid bacteria (LAB). We screened for LAB strains, which induce pDC activation and IFN-α production using murine bone marrow-derived dendritic cell culture. Microbial strains with such activity on pDC were absent in a diversity of bacillary strains, but were observed in certain spherical species. Amoung them, *Lactococcus lactis* JCM5805 induced the most prominent activation of pDC. Mechanistic studies revealed that IFN-α induction was TLR9 and MyD88-dependent; a slight impairment was also observed in TLR4-/- cells. Next, we confirmed the effects of *L. lactis* JCM5805 on human pDC *in vitro*. Finally, our randomized, placebo-controlled, double blind study showed that yogurt fermented with *L. lactis* JCM5805 activated pDC activity *in vivo*. This effect was especially greater in subjects whose pDC activity were relatively low. Furthermore, the risk of morbidity from the common cold was suppressed in the *L. lactis* JCM5805 group compared with the placebo group. We have established a large-scale cultivation method of *L. lactis* JCM5805 and several products such as yogurt, soft drinks and tablet that contain *L. lactis* JCM5805 are in the market in Japan.

D010

Comparative genomics of pig intestinal lactobacilli.

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The porcine gastrointestinal tract harbors a diverse and dense commensal microbiota. Lactobacilli are common members of porcine intestinal microbiota and assumed to be important for supporting intestinal health and maintaining the stability of the GIT microbiota e.g. by increasing colonization resistance against intestinal pathogens**.** Recently, a new abundant *Lactobacillus* spp. was identified in the intestine of piglets that was responding to specific diet rich in fructo-oligosaccharides and found to stimulate the pig immune system (Konstantinov et al. 2004 & 2006, Roselli et al. 2007). While first classified as *Lactobacillus sobrius*, subsequent taxonomic studies revealed it to belong to the *Lactobacillus amylovorus* group that includes dominant *Lactobacillus* strains originating from porcine intestine (Jakava-Viljanen et al. 2008). To identify their potential functional properties, including the surface structures interacting with host cells, we have taken a comparative genome sequencing approach of a series of surface layer (S-layer) protein-carrying isolates. The genome of *L.amylovorous* strains with genome size between ~1.8 to ~2 Mb were determined by pyrosequencing, further assembled, and prepared for gap closure. A first comparison showed A high similarity to *L.acidophilus/L.helveticus* genome, The presence of several S-layer protein genes, and the clustering of CRISPR's and CRISPR associated gene cluster. Presently, the genomes are being compared with the aim of providing a deeper insight into the genetics and metabolic capabilities of this *Lactobacillus* group. In addition, this and other comparative genomics studies will allow understanding the high diversity in the presently known *Lactobacillus* genomes. Finally, the genomic characterization of these S-layer positive *L.amylovorus* strains of pig origin will support to the physiological, biochemical and genetic studies that are being performed using these strains.

D011

Moonlighting proteins confer laminin adhesiveness of *Lactobacillus rhamnosus* isolated from fermented Sumbawa mare milk

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Potential probiotic *L. rhamnosus* strains were isolated from fermented Sumbawa mare milk (FSMM) with high binding ability to extracellular matrix proteins including laminin. Adhesion of pathogenic bacteria on laminin is an important process to promote infection, and hence laminin-adhesive probiotic strains may compete with pathogens' infection. The main objective of this study is to identify cell surface proteins (CSPs) of the isolated strains, especially focusing on laminin binding proteins (LPBs). The CSPs of *L. rhamnosus* strains were extracted with six extraction solutions. Each extract was examined by inhibition ELISA with immobilized laminin. The bound CSPs to the immobilized laminin were extracted by addition of 1% SDS, separated by SDS-PAGE, and visualized by CBB and silver staining. Each band was excised from the gel, tripsinized, and subjected to peptide mass fingerprinting. Further, the LBPs were fractionated by gel filtration and followed by inhibition ELISA to determine the major LBP. Under our experiment conditions, we found that CSPs extracted by 1 M LiCl showed the highest inhibition activity without significant effect on cell viability. Several glycolytic enzymes, DNA binding protein, and ribosomal protein were identified in the extracts mass spectrometrically, implying that those proteins are moonlighting on the cell surface of isolated *L. rhamnosus* strains. Judging from the band densities of the bound CSPs on laminin and inhibition ELISA of fractionated CSPs, the ribosomal protein was determined to be the major LBP. Our results suggest that *L. rhamnosus* isolated from FSMM may prevent infection of pathogenic bacteria by competing their binding to laminin.

D012

Short- and long-term changes in the intestinal environment following ingestion of *Bifidobacterium animalis* subsp. *lactis* GCL2505

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Introduction
Bifidobacterium animalis subsp. *lactis* GCL2505 survives and proliferates in the intestine. We evaluated short- and long-term changes in the intestinal environment following ingestion of *B. lactis* GCL2505.
Methods
In test 1, healthy female volunteers (N=53) were divided into 3 groups, and each group ingested *B. lactis* GCL2505, *B. bifidum* which survives but does not proliferate in the intestine, or yogurt fermented by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* for 2 weeks. Fecal samples were collected at days 0 (before ingestion), 1-4, 7, and 14. In test 2, constipated volunteers (N=38) were divided into 2 groups and ingested *B. lactis* GCL2505 or a placebo drink for 8 weeks. Fecal samples were collected at weeks 0 (before ingestion), 2, 4, and 8, and defecation frequency was recorded daily. The number of fecal bifidobacteria was quantified by species-specific real-time PCR.
Results and Discussion
In test 1, the number of fecal bifidobacteria was significantly higher on day 2 compared with day 0, which was maintained to day 14 in the *B. lactis* GCL2505 group. However, no significant change was observed in the other groups throughout the ingestion period. This suggested that proliferation of ingested bifidobacteria in the intestine contributes to a rapid increase of intestinal bifidobacteria. In test 2, the number of fecal bifidobacteria and defecation frequency significantly increased throughout the ingestion period in the *B. lactis* GCL2505 group. However, no significant change was observed in the placebo group from week 4 onward.
Conclusion
B. lactis GCL2505 increases intestinal bifidobacteria levels rapidly and improves defecation frequency throughout a long ingestion period. Compared with non-proliferating bifidobacteria and yogurt, *B. lactis* GCL2505 appears to improve the intestinal environment more effectively.

D013

Strain specific lactobacilli-mediated promotion of juvenile growth: New evidence from strains belonging to several lactobacilli species

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There is growing evidence that intestinal bacteria are important beneficial partners of their metazoan hosts. Recent observations suggest a strong link between commensal bacteria, host energy metabolism, and metabolic diseases such as diabetes and obesity. The *Drosophila* microbiota has already been shown to promote juvenile growth upon nutrient scarcity. More precisely, selected strains of *Lactobacillus plantarum*, a commensal bacterium of the *Drosophila* intestine, have been shown to be sufficient on their own to recapitulate the natural microbiota growth-promoting effect. Intestinal microbiota is therefore starting to be considered as a factor that influences the systemic growth of its host. *L. plantarum*, and the genus *Lactobacillus* more generally, belong to the Lactic Acid Bacteria (LAB), which represent a vast majority of probiotic microorganisms used in food fermentation and in fortified food. In order to assess the role of other LABs on juvenile growth, we have studied the impact of different lactobacilli species on juvenile growth upon nutrient scarcity, using *Drosophila* as an *in vivo* animal model and *L. plantarum* strains as a control. We show that different lactobacilli species have very diverse impacts on juvenile growth, and that this influence can vary within the same species in a strain dependent manner. These analyses pave the way to further functional studies aiming at determining the probiotic potential of a given strain, as well as deciphering its physiological role and metabolic implications, using the powerful *Drosophila* / lactobacilli interaction model.

D014

Bioluminescent *Lactobacillus plantarum* and *Lactococcus lactis* strains for dual color *in vivo* imaging

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Lactic acid bacteria, especially lactobacilli, are common inhabitants of the gastrointestinal tract of mammals, for which they have received great attention due to their health-promoting properties. We describe the development and application of luciferase-expressing lactic acid bacteria (LAB) for noninvasive *in vivo* dual color imaging in the digestive tract of mice. We report the functional *in vitro* expression in *Lactobacillus plantarum* NCIMB8826 and *Lactococcus lactis* MG1363 of the red (CBRluc) and green click beetle luciferase mutants (CBGluc) from *Pyrophorus plagiophtalamus* using a combination of vectors, promoters and codon-optimized genes. We demonstrate that the CBRluc construction is a better performing luciferase system in comparison to the CBGluc construction for the *in vivo* detection of both LAB after oral administration. We explored the possibility to simultaneously detect red and green emitting LAB by dual wavelength bioluminescence imaging in combination with spectral unmixing. The difference in spectra of light emission of the red and green click beetle luciferase mutants and dual bioluminescence detection allowed direct *in vitro* and *in vivo* quantification of two bacterial populations by measuring the red and green emitted signals and thus monitoring the dynamics and faith of the two populations simultaneously. Persistence and viability of both strains given simultaneously to mice in different ratios was studied in anesthetized mice and in mouse feces. The application of dual-luciferase-labeled bacteria has significant potential to allow the *in vivo* study of the interactions of targeted LAB and their mammalian host, e.g. in healthy or diseased status, at different ages or using different modes of administration. This tool also has potential in safety studies and in testing competing interactions between different probiotics.

D015

***Lactobacillus rhamnosus* CNCM I-3690 and the commensal bacterium *Faecalibacterium prausnitzii* A2-165 exhibit similar protective effects to induced barrier hyper-permeability in mice**

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Impaired gut barrier function has been reported in a wide range of diseases and in metabolic and functional gastrointestinal disorders including some of the most emergent pathogenesis concerning developed countries such as obesity and irritable bowel syndrome. In addition, there is increasing evidence suggesting that the gut microbiota tightly regulates gut barrier function and that probiotic bacteria can enhance barrier integrity. Here, we aimed to investigate the effects on the intestinal barrier integrity of *Lactobacillus rhamnosus* CNCM I-3690, a candidate probiotic strain previously known because of its anti-inflammatory properties (1). *In vitro* results using a Caco-2 monolayer cells stimulated with TNF-α pointed out a putative role for the protection of the barrier integrity. We then tested the protective effects of *L. rhamnosus* CNCM I-3690 in a mouse model of low-grade inflammation induced by intra-rectal administration of DNBS which led to colon barrier hyper-permeability. We compared its effects to that of the well-known commensal bacterium *Faecalibacterium prauznitzii* A2-165 previously shown to act protectively on the intestinal barrier in the same mouse model (2). Interestingly, increase of barrier permeability was similarly counterbalanced in mice treated with both strains. Modulation of apical tight junction proteins expression was then analyzed. We showed that *L. rhamnosus* CNCM I-3690 and *F. prausnitzii* A2-165 tended to increase the levels of tight junction proteins Occludin, E-cadherin, Claudin-4, ZO-1 and F11r.
In conclusion, the comparison of *L. rhamnosus* CNCM I-3690 with the commensal anti-inflammatory bacterium *F. prausnitzii* in our colon barrier hyper-permeability model confirms the potential of the *L. rhamnosus* strain for its use in human pathologies involving an increase of intestinal permeability.
1/Grompone *et al*, PLoS One. 2012;7(12):e52493.
2/Martin *et al*., 2014. Submitted

D016

How *Bifidobacterium adolescentis* 22L is genetically adapted to colonize the human intestine through the metabolism of starch

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The human gut is populated by a microbial consortium whose metabolic activities are influenced by, among others, bifidobacteria. Nevertheless, the genetic basis for bifidobacterial adaptation to the human gut is poorly understood. Analysis of the 2,203,222 bp genome of *Bifidobacterium adolescentis* 22L revealed a nutrient-acquisition strategy that targets diet/plant-derived glycans, in particular starch and starch-like carbohydrates. Transcriptome profiling of 22L cultures grown under *in vitro* conditions or during colonization of the murine gut by RNAseq and qRT-PCR assays revealed a set of chromosomal loci responsible for starch metabolism. Conservation of the latter gene clusters in various *B. adolescentis* strains enforces the notion that starch catabolism is an important colonization factor for *B. adolescentis* with concomitant impact on intestinal microbiota ecology.

D017

Evaluation of the bifidobacterial microbe-host crosstalk

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The human intestine is home of an almost inconceivable large number of microorganisms called the human gut microbiota, which can be pictured as an organ placed within a host organism. Within the human gut microbiota, bifidobacteria represent the first most numerous microbial groups encountered in the colon of infants, considerably outnumbering other bacterial groups. Bifidobacteria are anaerobic, Gram-positive, irregular or branched rod-shaped bacteria that are commonly found in the gastro-intestinal tracts (GIT) of humans and most animal and insects. Despite the generally accepted importance of bifidobacteria as probiotic components of human GIT microflora and their use in health-promoting foods, there is a paucity of data about the molecular background covering their probiotic features (e.g., interaction with the host and with the components of the gut microbiota).
Thus, we investigated the genetics underlying bifidobacteria-host interactions and specifically the molecular mechanisms underlying host-microbe responses following colonization of a model murine host with infant bifidobacterial species (e.g., *Bifidobacterium breve*, *Bifidobacterium bifidum* and *Bifidobacterium longum* subsp. *infantis*) or adult bifidobacterial taxa (e.g., *Bifidobacterium adolescentis*), either on their own or in various combinations.
Specifically, functional genomic analyses, by expression microarray, highlighted several genes involved in the interaction with the ecological niche, such as genes encoding proteins involved in the acquisition and breakdown of polysaccharides derived from the diet or from the host. The activities of these enzymes are crucially important for accessing carbon- and energy sources that will shape the intestinal microbiota.
Results revealed that there is an interactive relationship between host and components of the intestinal microbiota and such knowledge increased our understanding of the mechanisms of action of the perceived positive health benefits exploited by bifidobacteria.

D018

Probiotic attributes of riboflavin over-producing lactic acid bacteria strains

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Riboflavin plays a crucial role in human cell metabolism as precursor of coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) (Capozzi et al., 2012). In this study, the riboflavin production of *Lactobacillus plantarum* and *Lactobacillus fermentum* strains was investigated in chemically defined medium (CDM) (Russo et al., 2014) and in a co-cultures system of lactobacilli and human intestinal epithelial cells (Russo et al., 2012). Both strains showed the ability to over-produce riboflavin respect to the control strains. The strains displayed a significant ability to survive to an *in vitro* gastrointestinal transit, an aptitude to form biofilm on abiotic surface, a strong capability to adhere on intestinal cells and to contrast the adhesion of *Escherichia coli* O157:H7. Furthermore, both strains showed antimicrobial activity on agar plate against three human pathogenic bacteria, i.e. *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella enterica.* Resistance to major antibiotics and potential hemolytic activity confirmed the safety assessment. This study aims to demonstrate the combination of probiotic features and riboflavin over-production by the investigated *Lactobacillus plantarum* and *Lactobacillus fermentum* strains and their potential application as endogenousriboflavin producers that couldenrichedthe riboflavin content both in novel fermented food and *in situ* in the host intestine.
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D019

A gut microbial metabolite of linoleic acid, 10-hydroxy-cis-12-octadecenoic acid, recovers the intestinal epithelial barrier impairment in Caco-2 cells partially via GPR40 signaling

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Gut microbial metabolites of linoleic acid have attracted much attention because of their various physiological properties. Dysfunction of tight junction (TJ) in the intestine contributes to the pathogenesis of several intestinal disorders. We evaluated the effects of five novel gut microbial metabolites of linoleic acid on tumor necrosis factor (TNF)-α-induced barrier impairment in human epithelial cell line, Caco-2 cells. Among five gut microbial metabolites of linoleic acid tested, 10-hydroxy- *cis*-12-octadecenoic acid (HYA) suppressed TNF-α-induced changes in TJ permeability and interleukin-8 secretion most potently. HYA recovered the expression of TJ-associated factors, such as occuldin, zonula occluden-1 and myosin light chain kinase, and inhibited the activation of nuclear factor (NF)-κB pathway. HYA also suppressed the protein and mRNA expressions of TNF receptor (TNFR) 2. Furthermore, HYA significantly up-regulated G protein coupled receptor (GPR) 40 expression. However, 10-hydroxy octadecenoic acid, which is an oleic acid metabolite and lacks a carbon-carbon double bond at Δ12 position, did not show these barrier-recovering activities, and down-regulated GPR40 expression. Thus, it was suggested that C=C double bond at Δ12 position in HYA might be crucial for its activity. Collectively, HYA, a gut microbial metabolite of linoleic acid, modulates TNFR2 expression partially via GPR40 signaling to recover the intestinal barrier impairment, and might provide better management of intestinal disorders.

D020

***In vitro* and in silico analysis of probiotic adhesion and inhibition abilities of putative probiotic strains isolated from pig feces**

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Adhesion to the intestinal mucosa is speculated to be an integral probiotic ability as it affects the probiotic's ability to confer health benefits such as immunomodulation and gut protection in the host. However, lactobacilli mechanisms of attachment are not yet well understood so aside from assessment of our sequenced lactobacilli, we also aim to understand the mechanisms involved. In this study, we have used lactobacilli isolates *L. mucosae* LM1 and *L. johnsonii* PF01 along with *L. rhamnosus* GG and pathogens *E. coli* K88 and *S. typhimurium* ATCC 40253, where we have found significant correlations ($p<0.05$) among adhesion, aggregation, and hydrophobicity, as well as between coaggregation and displacement of *E. coli.*MATSadhesion test of the strains showed that the probiotic strains are hydrophobic, and highly basic or electron-donating, where an increased monopolarity of the cell-surface shows better aggregation and adhesion, despite varying hydrophobicity values. Various results were seen in pathogen inhibition, but significant displacement of *E. coli* and *S. typhimurium* were observed, suggesting that higher adhesion ability is important in this inhibition mechanism. Likewise, new bioinformatic approaches in genome sequence analysis were applied to predict and characterize putative cell surface adhesins that are present in these probiotic strains.

D021

Aggregation of *Lactobacillus brevis* mediated by xylan and its relationship with the surface properties and mucin-mediated aggregation of the bacteria

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Although lactic acid bacteria are widely found in nature, the interactions of these bacteria with plants have not been studied well. Thus, screening for *Lactobacillus* strains that interact with plant carbohydrates was conducted. As the result, some *L. brevis* strains in NITE Biological Resource Center (NBRC) were observed to aggregate upon the addition of xylan. The cell surface proteins corresponding to the S-layer proteins varied among the strains. However, the strains that displayed xylan-mediated aggregation retained the ability to aggregate even after the removal of S-layer proteins via LiCl treatment. Therefore, it was seemed that S-layer proteins did not participate in the aggregation. *L. brevis* strains had negative zeta potentials. A correlation between the strength of aggregation and zeta potential was not observed. However, the removal of half amount of S-layer proteins resulted in remarkable decreases in the electric potential and aggregation ability of some of strains. From these observations, it was considered that xylan-mediated aggregation of *L. brevis* was caused by an electrostatic effect between the cells and xylan, which is known to have a negative charge, and that the charge of the cells was influenced by such factors as the condition of S-layer proteins. As the aggregation of the bacteria appeared to be related to an electrostatic action, it was determined whether *L. brevis* aggregated by another substrate. As the result, the bacteria aggregated in the presence of gastrointestinal mucin, which is also known to have a negative charge, and the strength of aggregation among the strains was similar to that induced by xylan. Thus, it was supposed that xylan- and mucin-mediated *L.. brevis* aggregation was caused by a similar mechanism.

D022

Effect of a multispecies *Lactobacillus* formulation as a feeding supplement on the performance and immune function of piglets

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Reduced abundance of intestinal lactobacilli around weaning has been considered to predispose piglets to gut disturbances, e.g. diarrhea. Therefore, dietary supplementation with lactobacilli may help in maintaining better host health around weaning. The aim of this study was to assess the effect of a bacterial supplement containing six strains of the genus *Lactobacillus* in a 3-week feeding trial conducted in recently weaned pigs. Piglets in the supplementation group (n=10) were fed the lactobacilli mixture (total cell count $1x10^{10}$) daily, while those in the control group (n=10) were provided with a probiotic-free placebo. The intestinal survivability of the strains fed to the piglets, as well as the expression of selected cytokines in the gut mucosa was analyzed. The main effect of the lactobacilli supplementation observed was immunomodulation in the piglet intestine, especially in the large intestine. Upregulated expressions of IL-4 and IFN-α were detected in the cecum, with downregulated expressions of IL-8 and TNF in the colon of the supplementation group. In addition, supplementation downregulated the expression of TGF-β1 in the jejunum, ileum and colon. An increased bacterial number was detected in the jejunum of the supplementation group, but no change in the intestinal digesta pH or in the numbers of lactobacilli was induced by the supplementation. Furthermore, the strains which had been supplied could not be isolated from feces, indicating that they had been unable to colonize the piglet intestine in significant numbers. While the lactobacilli supplement used failed to achieve a growth-enhancing effect, and the supplemented strains appeared to have a limited ability to compete with the indigenous intestinal microbiota of piglets, the supplement, however, evoked significant immunomodulatory properties in the piglet intestine.

D023

Inhibitory activity of probiotic lactobacilli against oral Candida under different fermentation conditions - an *in vitro* study

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Objectives: Various clinical studies have shown that probiotics positively affect oral health by decreasing gum bleeding and/or reducing salivary counts of *Streptococcus mutans*. Our aim was to investigate the inhibitory effect of six probiotic lactobacilli against opportunistic oral *Candida* species. Sugar utilization by both lactobacilli and *Candida* was also assessed.
Methods: Agar overlay assay was utilized to study growth inhibition of *C. albicans*,*C. glabrata* and *C. kruse* by *Lactobacillus rhamnosus* GG, *L. casei* Shirota, *L. reuteri* SD2112, *L. brevis* CD2, *L. bulgaricus* LB86 and *L. bulgaricus* LB Lact. The inhibitory effect was measured at pH 5.5, 6.4, and 7.2, respectively, and in the presence of five different sugars in growth medium (glucose, fructose, lactose, sucrose, and sorbitol). Growth and final pH values were measured spectrophotometrically at two-hour time points to 24h.
Results: *L.rhamnosus* GG showed the strongest inhibitory activity against *C. albicans*, followed by *L. case*i Shirota, *L.reuteri* SD2112 and *L.brevis* CD2, in fructose and glucose, respectively . None of the lactobacilli tested affected the growth of *C. krusei.* Only *L. rhamnosus* GG produced slight inhibitory effect on *C. glabrata.*The lower pH values led to larger inhibition zones. Sugar fermentation profile varied between strains. *L.casei* Shirota grew in the presence of all sugars tested, whereas, *L. brevis* CD2 could utilize only glucose and fructose. All Candida species metabolized the sugars tested with the most rapid growth observed with *C. glabrata*.
Conclusion: Commercially available probiotics differ in their inhibitory activity and carbohydrate utilization. The above properties are modified by different pH values and sugars with more pronounced inhibition at lower pH.

D024

Phenotype characterization of a lactobacillar fimbrial operon through recombinant expression in *Lactococcus lactis*

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Gram-positive *Lactobacillus ruminis* is among the more dominant *Lactobacillus* species in the mammalian intestine, and as well, it is recognized as an indigenous (autochthonous) member of the gut microbiota. Despite its remarkable mix ofphenotypic traits and characteristics (e.g., obligate anaerobiosis, flagellar motility, immunogenic responsiveness, and broad host adaptability), *L. ruminis* has surprisingly been less explored functionally, and so then becoming a much-overlooked gut commensal. Recently, we established that a human strain of *L. ruminis* (ATCC 25644) is in fact surface piliated, and thus consistent with genomic predictions that it harbors a sortase-dependent fimbrial gene cluster (from hereafter called the LrpCBA pilus operon). Moreover, we had also demonstrated appreciable binding between this bacterium and two extracellular matrix (ECM) proteins (i.e., collagen and fibronectin), which we presume is partly linked to the adhesive capacity of its piliation. To test this hypothesis, we cloned the so-called LrpCBA pilus operon into *Lactococcus lactis* for the recombinant production of surface-anchored wild-type and tip pilin (LrpC)-deleted pili, with both forms then being examined for binding properties, and as well, for the induction of host immune-cell responses. Here, our data suggests the LrpC pilin constituent of *L. ruminis* piliation plays a pivotal role during bacteria-host(immune) cell interactions as evidenced by its ability to bind to ECM proteins and gut epithelial cells, as well as by its capacity to modulate innate immunity.

D025

Influence to intestinal microbiota and metabolites by feeding anti-obesity probiotics *Lactobacillus gasseri* SBT2055 in diet-induced obese mice.

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The probiotic Lactobacillus gasseri SBT2055 (LG2055) has an anti-obesity effect, but the mechanism is still unclear. In this study, we analyzed intestinal microbiota and metabolites of diet-induced obese mice fed the LG2055 in order to clarify the mechanism of anti-obesity effects of the LG2055. C57BL/6 mice were fed a 10 %-fat diet with/without LG2055 cells for 24 weeks. Consumption of LG2055 resulted in a reduction of body weight gain, fat tissue mass, triglyceride levels in the liver, and pro-inflammatory gene expression in the adipose tissue. Metabolome analysis of feces using gas chromatography/mass spectrometry revealed that feeding of LG2055 significantly increased lactate, nicotinate, glycerol and some free fatty acids ($p < 0.05$). Microbiome analysis of cecum using 16S rRNA pyrosequencing showed that abundance ratios of 6 family, 5 genus and 20 species were significantly different among feeding groups, but the rate of *Bacteroides*/ *Firmicutes*, which had been reported as a factor associated with obesity, was not different. These results suggested that inhibition of fat uptake in intestine and followed improvement in the inflammatory state of the adipose tissue might be a possible mechanism underlying the anti-obesity effect of LG2055.

D026

Development of a non-GMO tuberculosis vaccine, using *Lactobacillus* as a delivery vehicle

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Lactobacillus species have a long record of safe oral consumption and are natural inhabitants of the human gastrointestinal tract. Currently, these bacteria are extensively studied as potential vectors for *in situ* delivery of heterologous proteins, including vaccines. *Lactobacillus* spp. are interesting candidates for these purposes due to their ability to modulate the immune system and to survive the passage through the gastrointestinal tract. However, using bacteria as delivery vectors may be controversial, because they are genetically modified organisms. In the present study we have translationally fused a peptidoglucan binding domain (LysM) to Ag85B-ESAT6, a fusion protein consisting of two potent antigens from *Mycobacterium tuberculosis*.We also made constructs where an additional dendritic cell targeting sequence was fused to the hybrid protein. These hybrid proteins were expressed and purified from *E. coli* and then mixed with living of *Lactobacillus* cells. In this way vaccine-displaying lactobacilli were generated, without these bacteria being genetically modified. The binding capacities of the hybrid proteins to the selected *Lactobacillus* strains wereanalyzed by western blotting, flow cytometry and fluorescence microscopy. Internalization studies with dendritic cells and studies of the immunogenic properties of lactobacillibearing the hybrid proteins are currently in progress.

D027

S-layer as functional surface protein of probiotic strain *Lactobacillus brevis* D6

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The autochthonous strain D6, which was identified as *Lactobacillus brevis,* carries a 45-kDa S-layer protein. This strain displayed the highest percentage of autoaggregation (70.37% ± 5.79 in 5h) among lactic acid bacteria (LAB) strains tested, and the autoaggregation was affected by the removal of S-layer proteins by 5M guanidine hydrochloride (GHCl) (54.87±7.13 in 5h). Since S-layer proteins have been identified within lactic acid bacteria (LAB), only among representatives of *Lactobacillus* species, it was of special interest to study its functional role in Lb. brevis D6. Subsequently, the capacities of the *Lb. brevis* D6 to adhere to extracellular matrix proteins (ECM) and intestinal epithelial cell lines *in vitro* were assayed. The removal of the S-layer proteins reduced the adhesive ability of the strain D6 to Caco-2 cell lines and ECM components, while its adhesiveness to the IPEC-1 cell line was low. Pathogen exclusion through coaggregation ability and survival in simulated conditions of the gastrointestinal tract (GIT) and during freeze drying of *Lb. brevis* D6, before and after GHCl extraction of S-layer proteins from the cell surface, were followed. Results implied on potential S-layer protective functions on *Lb. brevis* D6 cells under aforementioned stress conditions. Additionally, cytokine production in monocyte-derived dendritic cells (moDC) and moDC maturation in response to *Lb. brevis* D6 bacterial cells and its purified S-layer proteins were determined by ELISA and FACS, respectively. However, no immunodulatory effects were observed, besides a slight induction of TNF-a, IL-12 and IP-10 production. The results suggest that the S-layer protein enhances certain probiotic activities of *Lb. brevis* D6, particularly protection in rigorous GIT conditions and adhesion to intestinal cell lines.

D028

Detection of *Salmonella sp*p from fermented diary products

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The growth of *Salmonella enteritidis* was significantly suppressed in some fermented diary products. The objectives of this study was to describe problem in standard methods of detection of *Salmonella enteritidis* from fermented foods. Different kinds of fermented diary products were inoculated with cell suspension of *Salmonella enteritidis.*These samples had final concentration of 30 cfu/mL. After 48 hours the salmonella were found only in one sample when traditional method with peptone water (24 hours) and Rappaport Vassiliadis broth(24 hours) was used. When this method was shortened and used only Rappaport Vassiliadis broth 3 samples was positive.

D029

Unraveling the role of pili and mucus-binding protein in adhesion of a natural isolate of *Lactococcus lactis* to intestinal epithelial cells and to mucins

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Adhesion of probiotic bacteria to intestinal mucosa is important to promote their persistence in the gastro-intestinal tract and their beneficial effects to the host, such as pathogen exclusion or immunomodulatory effects. In this study, we have characterized the molecular determinants involved in adhesion of a selected natural *Lactococcus lactis* strain to both intestinal epithelial cells and mucins.
L. lactis TIL448 strain of vegetal origin was selected among seven tested strains with different surface physico-chemical properties, for its high adhesion to Caco-2 intestinal epithelial cells. In addition, this strain was shown to adhere specifically to mucins, as measured on pig gastric mucin at the single-cell scale and under static conditions, using atomic force microscopy. This was further confirmed at the bacterial population level and under shear flow. Surface proteome analysis, performed through shaving of the bacterial surface with trypsin and LC-MS-MS analysis, allowed to identify two plasmid-encoded proteins potentially involved in adhesion. The first protein displayed pilin characteristics and was encoded in a cluster of four genes typically encoding sortase-dependent pili. The second protein contained two mucus-binding domains. Synthesis of pili at *L. lactis* TIL448 surface was confirmed by immunoblotting detection of high molecular weight forms of pilins associated to the cell wall, as well as by microscopy observations. Adhesion experiments performed with mutants defective either in pili or in mucus-binding protein synthesis revealed that (i) pili but not mucus-binding protein are involved in adhesion to intestinal epithelial cells; (ii) pili and mucus-binding protein play comparable role in adhesion to mucin under static conditions; (iii) mucus-binding protein exhibits a higher contribution than pili in adhesion to mucin under shear flow.

D030

Inhibitory effect of bacteriocinogenic lactic acid bacteria on *Listeria innocua* inoculated in simulated medium of vegetable or fruit juice

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Lactic acid bacteria (LAB) produce a variety of antimicrobial substances such as organic acids, alcohols, diacetyl, H_2O_2 and bacteriocins. Bacteriocinogenic LAB have attracted great interests due to their GRAS status and bacteriocins have potential to be used as safe biopreservatives.
Lactococcus lactis (7.17), *Enterococcus faecium* (13.2) and *Streptococcus thermophilus* (ASR-1) isolated and identified as bacteriocinogenic LAB strains were selected for a series of challenge tests against Listeria innocua inoculated at 10^4 CFU/mL in simulated medium of lettuce or cantaloupe juice. The inhibitory effect of LAB applied at 10^4, 10^6 and 10^7 CFU/mL on *L. innocua* was investigated during tested periods. The LAB and *L. innocua* counts in lettuce or cantaloupe juice were determined at various removal days for up to 28, 21 or 3 days at 4, 10 or 25 °C, respectively. The medium inoculated with *L. innocua* alone was served as the control. Our results indicated that LAB applied at 10^6 or 10^7 CFU/mL to both simulated media significantly inhibited the growth of *L. innocua* at 25 °C for 3 days or at 10 °C for 21 days. LAB inhibited *L. innocua* growth by 3 or 4-5 logs when inoculated at 10^6 or 10^7 CFU/mL, respectively at 25 °C for 3 days. The LAB and *L. innocua* counts had no significant change at 4 °C for 28 days regardless LAB inoculation levels. Comparing with the LAB strains, *Lact. lactis* and *Ent. faecium* had stronger anti-listerial effect than that of *Strep. thermophilus.*These results suggest that *Lact. lactis* and *Ent. faecium* would be used for food protection when applied as protective cultures in food system such as fresh-cut fruit and vegetable products.

D031

Insights into glycogen metabolism in *Lactobacillus acidophilus*: Impacts on carbohydrate metabolism, stress tolerance and gut retention

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In prokaryotic species equipped with the machinery for glycogen metabolism, the co-regulation of glycogen biosynthesis and degradation has been associated with the synthesis of energy storage compounds and crucial physiological functions. These include regulation of central carbon flow, energy sensing and production, stress response and cell-to-cell communication. In some microbes, the ability to synthesize intracellular glycogen has also been implicated in host persistence. Among lactobacilli, complete glycogen metabolic pathway genes are present only in certain species predominantly associated with mammalian hosts or natural environments. This observation sheds light on the potential involvement of glycogen metabolism in the probiotic activity and persistence of lactobacilli in the human gut. *Lactobacillus acidophilus* NCFM possesses a glycogen metabolism (*glg)* operon consisting of *glgBCDAP-amy-pgm* genes. Expression of the *glg* operon and glycogen accumulation were carbon source responsive, growth phase-dependent, and highly induced by raffinose. Mutational analysis of the *glg* genes revealed the involvement of glycogen metabolism in growth maintenance, metabolism of complex sugars (e.g. raffinose) and bile tolerance. *In vivo* competitive co-colonization experiments with a glycogen-deficient Δ*glgA* mutant (with inactivated glycogen synthase) against the parent *L. acidophilus* strain in 129/SvEv germ-free mice resulted in a 2 log reduction of the mutant population one week post-feeding. In subsequent colonization displacement studies, the introduction of parent strain into gnotobiotic mice, previously mono-colonized with the Δ glgA mutant, resulted in population displacement of the Δ *glgA* mutant. Both *in vivo* studies established the significance of glycogen biosynthesis on the competitive retention of *L. acidophilus* in the mouse intestinal tract, demonstrating for the first time that the ability to synthesize intracellular glycogen contributes to gut fitness among probiotic microorganisms.

D032

Influence of *Lactobacillus acidophilus* sortase-dependant proteins on immunomodulation and gut retention

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Surface proteins of probiotic microbes, including *Lactobacillus acidophilus* and *Lactobacillus gasseri,* are believed to promote retention in the gut and mediate host-bacterial communications. Sortase, an enzyme that covalently couples a subset of extracellular proteins containing an LPXTG motif to the cell surface, is of particular interest in delineating bacterial adherence and communication with the mucosal immune system. A sortase gene, *srtA,* was identified in *L. acidophilus* NCFM (LBA1244) and *L. gasseri* ATCC 33323 (LGAS_0825). Additionally, twelve sortase-dependant proteins (SDPs) (i.e. cell surface proteins, adhesion exoproteins and mucus-binding proteins) containing a C-terminal LPXTG motif were predicted in both microbes using the lactic acid bacteria secretome database. Due to the role of sortase in coupling these proteins to the cell wall, *ΔsrtA* deletion mutants of *L. acidophilus* and *L. gasseri* were created using the *upp*-based counter-selective gene replacement system. Inactivation of sortase did not cause significant alteration in growth or survival in simulated gastrointestinal juices. Meanwhile, both *ΔsrtA* mutants showed decreased adhesion to porcine mucin *in vitro*. Murine dendritic cells exposed to either the *ΔsrtA* mutant of *L. acidophilus* or *L. gasseri* induced lower levels of proinflammatory cytokines (IL-6, IL-12 and TNF-α) compared to the parent strains. These data demonstrates the importance of SDPs in the modulation of host immune responses. *In vivo* co-colonization of the *L. acidophilus ΔsrtA* mutant and the parent strain in germ free 129/SvEv mice resulted in a significant one log reduction of the *ΔsrtA* mutant population. Additionally, a similar reduction of the *ΔsrtA* mutant was observed in the cecum. This study shows for the first time that SDPs contribute to gut retention of probiotic microbes in the gastrointestinal tract.

D033

Reuteran from *Lactobacillus reuteri* protect against enterotoxigenic *Escherichia coli* in young pigs

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Enterotoxigenic *Escherichia coli* (ETEC) cause diarrhea in piglets. ETEC K88 fimbriae bind to glycoprotein receptors containing galactose, glucose, and mannose (1) on the intestinal mucosa and deliver heat-labile (LT) and heat stable toxins, causing fluid loss. Reuteran and levan from *Lactobacillus reuteri* prevented ETEC K88 adhering to porcine erythrocytes *in vitro* (2). We determined anti-adhesive properties of bacterial (reuteran and levan) and commercial (dextran and inulin) glycans *in vivo* using small intestine segment perfusion (SISP).
The SISP model used 10 jejunum segments each from 6 weaned pigs (10 kg BW). Five segments were infected with ETEC K88 and five were flushed with saline. Paired segments (ETEC and non-ETEC infected) were infused with 10 g/L of one of 4 glycans or saline for 8 h. High resolution melt-qPCR indicated that *E. coli* were the dominant bacteria in infected segments. Gene copy numbers of K88 and LT in mucosal scrapings and outflow fluid of infected segments were greater ($P < 0.05$) than of non-infected segments. LT amplicons from infected and non-infected segments were only 98% identical over 432 bp, indicating that autochthonous ETEC K88 colonized the pig intestine in non-infected segments. All glycans reduced ($P < 0.05$) ETEC-induced fluid loss. Reuteran tended ($P = 0.06$) to decrease abundance of ETEC K88 genes in mucosa. Fluorescent in situ hybridization indicated reuteran decreased ($P = 0.012$) adhesion of ETEC K88. In conclusion, reuteran reduced ETEC K88 adherence to the small intestinal mucosa by acting as a receptor decoy and may thereby reduce diarrhea in young pigs.
(1) Grange et al. 1998. Infect Immun. 66:1613. (2) Wang et al. 2010. Appl. Environ. Microbiol. 76:4863.

D034

Unravelling the fate of dietary fibers in the murine cecum

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The microbiota of the gastrointestinal tract plays a key role in the digestion of our food. Complex metabolic networks of interacting microbes in the gastrointestinal tract of humans and other mammals yield a wide range of metabolites of which the short chain fatty acids, in particular butyrate, acetate, and propionate are the most abundant products of carbohydrate fermentation. So far metabolic networks were documented in *in vitro* models. In this project interactions between diet, microbiota and host will be quantitatively studied and subsequently modeled using a Systems Biology approach.
In initial experiments the cecum of conventionally raised mice on different fiber diets are analyzed. Determinations of the microbiota composition using phylogenetic microarray technology were complemented with metatranscriptome, metabolome and host derived transcriptome data. This revealed distinct activities of bacterial families in the fermentation of fibres into short chain fatty acids. The *Bifidobacteriaceae,* Lachnospiraceae and *Clostridiaceae* families are active in glycoside hydrolysis and saccharide transport, while *Bacterodetes* and the *Erysipelotrichaceae* families express mainly glycosidases and the *Ruminococcaceae* family is mainly active in the sugar transport. All families express in different ratios enzymes involved in the production of short chain fatty acids. Moreover the butyrate producing bacteria correlate with a set of host genes involved in processes such as energy metabolism, transcriptional regulation and immune system. The metadata obtained is expected to result in refinement of our understanding of the interactions between diet, microbiota and host.

D035

Impact of lactococcal bacteriophages in dairy starters

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Bacteriophage infection of lactococcal starter cultures used in dairy fermentations is recognised as the one of the main causes of disturbance in the manufacture of fermented dairy products. Lactococcal phages are classified into ten taxonomic groups of which the most commonly isolated phagesare members of the c2, 936 and P335 species. Members of the 936 and c2 species are strictly virulent while P335 phages may be either lytic or temperate. In this study, a classification of lactococcal phages isolated from whey samples obtained from a wide variety of geographical locations was performed. Using these whey samples, individual phages were isolated and propagated in order to characterise the genotype using multiplexPCRand restriction profile analysis and also their host range. Of 124 lactococcal phages isolated in this study, the 936 species isolates dominate the population (60%), which is consistent with previous studies, followed by c2 (22%) and P335 (16%) phages. Using restriction profiling of 124 phages, we identified 40 distinct 936 phages, 10 c2 phages and 14 P335 phages. Genome sequencing of a number of these isolates was performed to aid in understanding the genetic diversity within this group of phages.

D036

Characterization of water-soluble exopolysaccharides produced by *Leuconostoc citreum* isolated from Pico cheese

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Exopolysaccharides (EPS) have received increasing attention in recent years due to their contribution for the consistency, viscosity and rheology of dairy products, as well as health promoting benefits such as immunological, antioxidant and prebiotic effects. A total of 106 lactic acid bacteria (LAB) isolates obtained from an artisanal cheese (Pico cheese) were screened for EPS production phenotypes. Only one strain identified as *Leuconostoc citreum* (L3C1E7) produced EPS from sucrose on solid and liquid media and expressed a mucoid phenotype. The water-soluble EPS was isolated, purified, freeze-dried and submitted to analyses for molecular weight, protein and sugar content. The average molecular weight of EPS was found to be 2479 KDa, with a content of 0,52 g/g glucose equivalent and 10% of protein. For the characterization of the EPS producer, a combination of molecular techniques such as polymerase chain reaction (PCR), pulse field gel electrophoresis (PFGE) and plasmid profiling were used for both wild type EPS-producing (EPS^+ and mutant non-EPS-producing (EPS^-). The wild type EPS^+ was stable and sustained sub-culturing for 21 days. EPS tested negative for endotoxin using EndoLISA® test. To investigate the ability of the EPS producer to modulate inflammatory reaction in human intestinal cell line (HT-29), the *in vitro* secretion of pro-inflammatory cytokine interleukin-8 (IL-8) was determined by ELISA Kit. The stimulatory effect of the pro-inflammatory lipopolysaccharide (LPS) on IL-8 secretion was reduced significantly ($P<0.05$) during treatment with live bacteria. In conclusion, this study presents an EPS-producing strain that offers many exploitable opportunities for industrial dairy applications such as novel product development with health promoting potential.

D037

***Lactococcus lactis* strains delivering IL-10 protein or cDNA to the intestinal mucosa show anti-inflammatory properties in a TNBS-induced chronic colitis model**

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Interleukin-10 (IL-10) is the most important anti-inflammatory cytokine at intestinal level. Oral treatments with IL-10 are inefficient because of its sensitivity to the harsh conditions of the GI tract, and systemic treatments cause undesirable side effects. Our aim was to compare the protective effects of IL-10, delivered by recombinant lactoccoci using two novel expression systems, in a murine colitis model mimicking the relapsing nature of inflammatory bowel diseases. The first system is based on a stress-inducible promoter (pGroES/L) for the production and delivery of heterologous proteins *in situ* at mucosal surfaces, and the second allows the delivery to the host cells of an *il-10* cDNA, harbored in a eukaryotic DNA expression vector (pValac). Colitis was induced in female BALB/c mice by intrarectal injection of TNBS. Mice that survived the challenge and recovered their body weight received one of the bacterial treatments or saline solution orally during 14 days. Colitis was reactivated 25 days after the first TNBS challenge with a second injection. Three days after colitis reactivation, cytokine profiles and inflammation in colon samples were evaluated. Animals receiving *L. lactis* delivering pGroES/L: *il-10* or pValac: *il-10* plasmids showed lower body weight loss and damage scores in their large intestines compared to inflamed non-treated mice. Both treatments also increased IL-10 concentration in the intestine, compared to the controls without treatment and maintained an increased ratio of IL-10/ pro-inflammatory cytokines. Our results confirm the protective effect of IL-10 delivered by *L. lactis* either as a protein or as a cDNA in a TNBS-induced chronic colitis model, and provides a step further in the use of genetically engineered bacteria as a valid system to deliver therapeutic molecules at mucosal level.

D038

Characterization of a cell surface serine protease in *Lactobacillus acidophilus* involved in immunomodulation and intestinal epithelial barrier integrity

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Health-promoting aspects attributed to probiotic microorganisms, including adhesion to intestinal epithelia and modulation of the host mucosal immune system, are mediated by proteins found on the bacterial cell surface. Notably, certain probiotic and commensal bacteria contain a surface (S-) layer as the outermost stratum of the cell wall. S-layers are semi-porous, crystalline arrays of self-assembling, proteinaceous subunits called S-layer proteins (Slps). In *Lactobacillus acidophilus* NCFM, the S-layer protein, SlpA, has been implicated in both mucosal immunomodulation and adhesion to host intestinal epithelium. As such, it is critical to explore the properties of S-layers and secreted cell surface proteins as a functional interface for probiotic activity. Here, we describe a gene (*lba1578)* encoding an extracellular, S-layer associated serine protease. To functionally characterize this protein, *lba1578* was deleted from the chromosome of *L. acidophilus*. Following co-incubation with dendritic cells, NCK2282 (*Δlba1578)* demonstrated increased immunomodulation of TNF-α, IL-6, and IL-10 compared to wild-type. Furthermore, there was a significant increase in tight-junction permeability for Caco-2 monolayers exposed to NCK2282 (*Δlba1578)* compared to NCFM parental strain. These data suggest that the S-layer associated serine protease LBA1578 exhibits immunmodulatory properties and may contribute to maintenance and integrity of the intestinal epithelium.

D039

Interaction of *Lactobacillus acidophilus* NCFM grown on different carbohydrates with human intestinal epithelial cells: Adhesion properties and roles of S-layer proteins

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Adhesion of probiotics to the gastrointestinal tract is considered to be an important criterion for colonization. *Lactobacillus acidophilus* NCFM (NCFM) is one of the well-defined probiotic strains isolated from humans and used in dietary supplements and yogurts. Although the adhesion process is complex and involves a variety of proteins, Surface layer (S-layer) proteins have been found to have roles in this adhesion. Combination of probiotics with emerging prebiotics has been shown to alter protein expression in a way that might change the functional properties of the probiotic.
The present study aimed at investigating effects of emerging prebiotics on S-layer protein expression and adhesion properties of NCFM. Adhesion differences by growth on the carbohydrates raffinose, cellobiose, and as control glucose were examined by using the human intestinal HT-29 cell line. 2-D gel electrophoresis, image analysis, and protein identification by mass spectrometry were performed to identify differentially expressed S-layer proteins under these growth conditions.
The results showed that NCFM grown on raffinose and cellobiose significantly increased adhesion to HT-29 cells 2.8 and 2.4 fold ($p<0.001$), respectively, compared to glucose. Protein identification revealed that expression of NCFM SlpX and SlpA proteins was significantly increased in raffinose and cellobiose cultures ($p<0.05$). In conclusion, increased level of S-layer proteins due to utilization of raffinose and cellobiose leads to increased adhesion of NCFM to human intestinal HT-29 cells.
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D040

Characterization of immunomodulatory effects of probiotic bacteria in zebrafish model

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The zebrafish (*Danio rerio)*, already used for the study of the human immune system has an innate response similar to that of humans, representing a relevant model for studying the immunomodulatory properties of probiotic bacteria. The objective of this work is to develop a sensitive vertebrate *in vivo* screening system to assess the ability of probiotic bacteria to colonize the intestine and may also be used to rank the strength of their immunomodulatory effects.
A fish feeding protocol with probiotic bacteria and/or with inflammatory agent (TNBS) was established. The epifluorescence microscopy and transmission electron microscopy were used to visualize the location of GFP-expressing bacteria in the gut. The results show that *Lactobacillus casei* ATCC334 attaches to and colonizes the zebrafish intestine Moreover, inflammation response was assessed in the zebrafish by quantitative RT-PCR and the results show a decrease in the expression of genes encoding pro-inflammatory cytokines TNF-α and IL-1β following the probiotic treatment, suggesting that this bacterium is able to limit inflammation caused by TNBS. A decreased number of recruited macrophages is also demonstrated in the same conditions.
This study highlights the importance of this animal model to characterize the immunomodulatory effects of probiotic bacteria.

D041

The effect of *Lactobacillus casei* 32G on the mouse cecum microbiata and innate immune response is dose and time dependent

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Lactobacilli have been associated with a variety of beneficial health effects and some of these effects have been related to changes in gastrointestinal microbiota. However, the relationship between probiotic dose, changes in the microbiota, and health effects requires further investigation. The objective of this study is to determine if the effect of *Lactobacillus casei* 32G on murine gastrointestinal microbiota and immune responses are dose and time dependent. Eight week-old C57BL/6 mice were fed *L. casei* 32G by oral gavage at doses of 10^6 (low), 10^7 (medium), and 10^8 (high) CFU/mouse for seven days. Mice were sacrificed 0.5h, 3.5h, 12h, 24h, and 72h after the last administration. The time required for *L. casei* 32G to reach the cecum was measured by qPCR. The cecum content and the ileum tissue were collected for microbiata analysis and immune response, respectively. The results demonstrate that 32G reaches the cecum 3.5h after administration. The composition of the cecum microbiota at 3.5h and 12h time point at both medium and high dose differed significantly from 0.5h samples from these treatments. 32G administration at the medium and high doses resulted in increased abundance of *Lactobacillus* in the cecum digesta at all-time points, other than 12h. Additionally, 32G administration decreased *Lachnospiraceae* prevalence at high dose at 3.5h and by all doses at 24h. Gene expression results at 3.5h revealed that 32G administration resulted in up-regulation of Clec2h and IL-10r and down-regulation of Lyz2, TLR-2 and TNF-α. This study demonstrates that *L. casei* 32G's is capable of modulating the murine immune system and cecum microbiata and that these changes are dose and time specific.

D042

Exploring the role of lectin-like proteins in *Lactobacillus rhamnosus* GR-1 - host interaction

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Studies of probiotic lactobacilli indicate key roles for surface molecules in host interactions. Interesting classes of surface molecules are lectins, i.e. proteins that bind carbohydrates with high specificity. Lectins are well characterized in animals and plants, while the information on bacterial lectins is relatively poor. In this study, the role of lectins of the clinically well-documented strain *Lactobacillus rhamnosus* GR-1 is studied in relation with the documented adhesion capacity of the strain.
First, the genome sequence of LGR-1 was screened for gene encoding putative lectins. Corresponding knock-out mutant was constructed using our in house developed genetic methods and phenotypically characterized in comparison with LGR-1 wild type.
One gene encoding a putative lectin-like proteins, renamed here as *llp1,* was identified in the genome of LGR-1. Llp1 contains a highly conserved N-terminal L-type lectin domain and show similarity to the mannose-specific adhesin Msa from *L. plantarum* WCFS1. Mutational analysis indicates that Llp1 mediates specific mannose-dependent adherence to vaginal epithelial cells. The adhesion to the gastro-intestinal epithelial cell line and the cervical cell line was not affected by the mutation, therefore suggesting a possible role for Llp1 in tissue tropism.

D043

Detection of luminescent and fluorescent *Lactobacillus reuteri in vivo* and *in vitro*

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Lactobacillus reuteri is a symbiont that inhabits the gastrointestinal tract (GIT) of mammals and birds. Probiotic strains of *L. reuteri* have shown good efficacy both in animal models and in human clinical trials, and our research aims to increase the understanding of interactions between this bacterial species, the gut microbiota and the host. In this study, we describe for the first time cloning of codon optimized reporter genes encoding click beetle red luciferase (CBRluc) and red fluorescent protein mCherry in *L. reuteri* strains ATCC PTA 6475 of human origin and R2LC of rat origin using the expression vector pSIP411.
In vitro observations of strains with mCherry and CBRluc showed that the genes were stable and that functional proteins were produced. The mCherry signal was pH dependent and the intensity was higher at neutral pH. Furthermore, *in situ* confocal microscopy showed that mCherry-producing bacteria could be detected on a mucosal surface. The CBRluc producing strains generated a stable luminescence *in vitro.*Besides, *in vivo* detection of the CBRluc producing bacteria was performed by whole body imaging in mice after intra gastric inoculation. Additionally, persistency and dosage dependent signal intensity of the recombinant bacteria in the GIT of mice were studied.
In conclusion, we have demonstrated that the two reporter genes are suitable markers for studying *L. reuter* in the GIT. However, the applications of mCherry and luciferase-labeled lactobacilli are not limited to study of bacterial transit and persistency in GIT. We can see significant potential of *in vivo* and *in vitro* studies of the interactions between *Lactobacilli* and structures, cells and microbes present in the GIT using these new tools.

D044

Evaluation of the effect of probiotic preparation in suppressing inflammation in mice with dextran sodium sulfate-induced colitis

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There is an increasing interest in protective and therapeutic effect of probiotics. The study compared the effect three commercial probiotic preparations on dextran sulfate sodium (DSS)-induced colitis. BALB/c mice were gavaged 200 µl of the probiotic preparation ($5x10^9$ CFU/mL) or physiological saline, for 14 days. The probiotic preparations contained: *Lactobacillus rhamnosus, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus helveticus, Lactobacillus lactis, Bifidobacterium longum, Bifidobacterium bifidum, Bifidobacterium breve, Streptococcus thermophilus* and oligofructose (probiotic A); *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* (probiotic B) and *Saccharomyces boulardi* and *Lactobacillus casei* (probiotic C). Colitis was induced for 7 days via administration of 2.5% DSS in drinking water. Disease activity index (DAI) was monitored daily until mice were killed at day 21. Then, colons, mesenteric lymph nodes (MLN) and spleens were taken and examined. It was found that all preparations tested decreased DAI, and myeloperoxidase (MPO) activity when compared to the control group. Similarly, colon length in probiotic receiving groups increased significantly. Probiotic preparations differed, however, in their immunomodulative effects. Feeding mice with probiotic A induced higher amount of $CD4^+$ T cells, whereas feeding with probiotic B increased $CD4^+$ cell in splenocytes but decreased in MLNs. Simultaneously higher amount of $CD4^+/CD8^+$and $CD8^+$T cells was observed in MLNs of mice fed with probiotic containing only *Lactobacillus* strains. Probiotic containing *S. boulardi* and *L. casei* decreased amount of $CD4^+$T cells in MLNs but significantly increased in spleen. Where significant decrease in $CD8^+$ and increased $CD4^+/CD8^+$ cells were also observed.
The present experiments showed that the tested probiotics preparations alleviated DSS-induced biochemical and histological changes and can be used to overcome gut disturbances.
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D045

The effect of killing method on immune response of *Lactobacillus rhamnosus* GG in human macrophages

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The health promoting effects of *Lactobacillus rhamnosus* GG are widely studied and well recognized. The best documented effects are its proven clinical benefits in relieving certain types of diarrhea, reducing the incidence of respiratory infections in children and risk of atopic diseases. Recently comparative genomics of *L. rhamnosus* GG revealed new cell surface structures which may act as immune modulators in a host. Exopolysaccharides, lipoteichoic acids, and pili are reported to affect immune modulating ability of *L. rhamnosus* GG.
We investigated here whether dead *L. rhamnosus* GG cells can produce similar immune response in macrophages as live cells do, and compared the effect of killing methods. Cells cultivated in industrial process and medium were killed using different heat treatments, including spray-drying, UHT treatment and autoclaving. The effect of heat treatments on the amount of live cells was analysed by plating and the effect on cell surface structures as presence of pilus by immunoblotting with pilus antibody anti-SpaC. Human primary macrophages were stimulated with live and dead *L. rhamnosus* GG cells in 1:10 ratio, and the amount of TNF-a, IL-1b and IL-10 produced by macrophages was analysed at 6 and 24 h. The study showed that spray-dried cells gave the most similar immune response compared to live cells, and that the killing method has impact on the immune response and should be considered when dead cell preparations are to be used.

D046

Probiotics counteract diet-induced obesity and insulin resistance in mice through remodeling adipose tissue and microbiota composition

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Alterations in the gut microbiota composition were suggested to play a role in the development of obesity; which is associated with a chronic, low-grade inflammatory state, crucial in the development of insulin resistance. We therefore evaluated whether daily oral consumption of probiotics - selected for their potent anti-inflammatory capacities - could improve metabolism and inflammation in high-fat diet-induced C57BL/6 obese male mice.
We found a strain-specific effect; some strains had no beneficial impact while a mixture led to a significant limitation of body weight gain and to significant improvements of metabolic and immune parameters, including insulin resistance and inflammation. The mixture caused adipose tissue cell-remodeling with reduced inflammatory macrophage accumulation and enhanced levels of regulatory T-cell marker expression, associated with an increase of PPARg. The mixture also impacted on the gut expression levels of fatty acid-binding receptors: decrease of FABP1, CD36 and ApoCII, restoration of GPR41 and GPR43. Importantly, beneficial effects were associated with changes in microbiota composition, notably an increase of *Akkermansia muciniphila*, recently reported to be protective against obesity. Finally, we also demonstrated using the *in vitro* SHIME model that the probiotic mixture favors the production of SCFA, notably butyrate and propionate.
This study provides substantial insight into how the host-microbial mutualism may govern protective effects, notably by positively affecting the gut microbiota composition, the uptake of fatty acids, the adipose tissue cell-remodeling that finally promote metabolic and immune homeostasis. These findings provide crucial clues to the development of more efficient therapeutic approaches and open the potential for future therapeutic treatments in the management of obesity.

D047

Modulation of T cell response by pea albumins (*Pisum sativum)* and probiotics orally delivered to Balb/C mice

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Intestinal mucosa provides a defensive barrier against potential food antigens. Probiotics has been shown as a natural “tool”able to influence the immune response. The aim of the study was determination of immunomodulatory effect of commercial probiotic Multilac® during delivering allergenic fraction of PA- albumin fraction from pea (*Pisum sativum*) to mice.Three groups of Balb/C mice (4-6weeks old), kept in IVC conditions, were used for the study. Mice were immunized orally with: GR I - PBS (control), GR II - probiotic dosed about $5x10^7$jtk/mouse and GR III - probiotic together with PA in dose of $5x10^7$jtk/mouse and 200µg PA/mouse by 21 consequence days. Blood and fecal samples were taken on 0 and 21day of experiment. Specific antibodies level was determinate by ELISA. After termination spleen were taken and lymphocytes were isolated and cultured with/without stimulation agents (PA, probiotics culture medium, PA+probiotics culture medium). It was find that mice gavage with PA with Multilac® gave about two yield lower level specific IgG in blood serum compare to group immunized with PA only. Specific IgA level was not detectable in experimental group. It was found that feeding mice with PA and probiotics induce higher amount of CD4+ T cells during cells stimulation with PA+P then group immunized with probiotics only. In splenocytes culture cytokine level was determinate by CBA test (BD). Splenocytes from GR III produce significantly more IL-10, Il-17A, TNF-b, IL-6 during PA-P or P stimulation then other group.
Presented experiments show that Multilac® together with PA is able to modify immune system response. Multilac® has immunomodulatory in presented route of immunization.
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D048

The influence of the intestinal microbiota modulating factors on the food allergy development

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The composition of the intestinal microbiota has a significant impact on the health of the patient.
The intestinal homeostasis ensures the balance between the defence immune function of the mucosa and systemic tolerance. The state of food allergy indicates an imbalance. Many factors e.g. mother's diet during pregnancy, probiotic supplementation, birth method and the new-born’s diet have a significant impact on the microbiota structure.
The main aim of this study was the analysis of the factors influencing the formation of the intestinal microbiota and the allergy development.
The study included 44 patients of both sex aged 1 to 9 years with and without (control) the symptoms of food allergy. The material for analyses included blood, faeces samples and the questionnaire. The secretion of immunoglobulines and cytokines in patients’ serum as well as secretory immunoglobulins and pro- inflammatory factors in faeces has been determined by means of the ELISA method (BD Bioscience, USA) and the total IgE level was measured with ImmunoCAP (Phadia, Sweden). Food specific serum IgE has been determined using the EUROLINE Pediatric test for allergy diagnosis (EUROIMMUN-AG, Germany). All procedures have been approve by the local ethical committee (Case No. 2/2010) and followed in accordance to the standards of Helsinki Declaration. To optimally distinguish the groups, the statistical analysis was performed.
A positive correlation between the age and the structure of microbiota was observed. In the allergy group reverse correlation between the probiotics supplementation and the level of pro-inflammatory factors was observed. The most significant impact on the structure of microbiota and the state of food allergy in analyzed group revealed the birth method and the newborn diet.

D049

The effect of soybean and whey protein isolates on gut microbiota

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More and more people are reaching for dietary additions in a form of protein supplements. Most of them are based on the whey or soy proteins. The protein supplements are usually used to build the largest possible muscle mass, which undoubtedly has an impact on improving athletic performance, however it can also entail changes in the gut microbiota balance. The aim of the study was to determine the effect of soy and whey proteins on gut microbiota composition.
The experiment was carried out on an animal model - Wistar rats (n=8/group), which were provided free access to water and experimental diet for 28 days. The animals were divided into 3 groups: A - control animals fed with a diet based on casein as the main protein source (supplemented with 0.15% DL - methionine); B - animals fed with a diet containing soybean; C- animals fed with a diet containing a whey protein isolate. The caecal digesta were collected after the experiment and the total number of anaerobic bacteria and a number of lactobacilli, *Clostridium perfringens,* coliform bacteria, fungi and yeast, intestinal enteroccoci, sulfite-reducing clostridia, and *Bifidobacterium* were determined. The experiment was approved by the Local Institutional Animal Care and Use Committee (2010/63/UE). Significant changes in the total number of anaerobic bacteria and sulfite-reducing clostridia content were indicated in the digesta obtained from animals, which were fed with soybean and whey proteins ($p<0.05$). No differences in lactic acid bacteria and other analyzed groups were indicated. The yeast and *Clostridium perfringens* were not detectable in the research material. The supplementation of diet with different protein isolates (plant or protein origin) was associated with changes in bacterial community.

D050

Development of a molecular well-defined vaginal probiotic

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The research field of the human microbiota, studying its role in the balance between health and disease, has never been stronger. In healthy premenopausal women, the vaginal microbiota is mostly dominated by *Lactobacillus* species, with known health benefits. Therefore, this unique niche provides great potential of using probiotic bacteria. However, the underlying molecular mechanisms by which vaginal lactobacilli or applied probiotics contribute to women's health are poorly described. A better understanding of the vaginal microbiota will contribute to a better understanding of properties needed for probiotics which target the vaginal niche and therefore, a better and more tailored selection of probiotic strains.
Several vaginally isolated *Lactobacillus* strains and some well-known urogenital probiotic strains were included in this study. First, the adhesion capacity of the strains to vaginal (VK2/E6E7) and cervical (HeLa) epithelial cells was investigated. Adhesion capacity showed to be strain-specific and dependent on the human epithelial cell line used. Interestingly, a high adhesion capacity was correlated with a high auto-aggregation capacity for *L. crispatus* ATCC 33820 and *L. plantarum* CMPG5300. Furthermore, capacity to form biofilm like-communities on a variety of substrates was investigated. Strong adhesion capacity showed to be related with a strong biofilm formation capacity. To study the immunomodulatory capacity of vaginal lactobacilli, the induction of specific cytokines (IL-8, TNF and IFNb) in VK2/E6E7 cells was determined. A significant induction of pro-inflammatory cytokines IL-8 and TNF in VK2/E6E7 cells was observed for the model probiotic strain *L. reuteri* RC-14. Finally, hydrogen peroxide production by vaginal Lactobacillus was studied. Almost all vaginal strains tested were able to produce hydrogen peroxide, with highest production detected for *L. jensenii* ATCC 25258 and *L. crispatus* ATCC 11440.

D051

Antipathogenic activity of lactobacilli against *Moraxella catarrhalis*.

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Thanks to major advances in next generation sequencing technologies that allow detection of uncultured bacteria and collection of high throughput data in so called 'metagenomic studies', the interest in the beneficial functions of the human microbiota has boomed within the last ten years. However, these studies are mainly descriptive with less attention to **focused functional studies** on selected members. Yet, in addition to the unraveling of the beneficial functions of the microbiota, the application of probiotics has increased significantly during the last decades. Although probiotic *Lactobacillus* spp. are especially known for gastrointestinal applications, they are also present in other mucosal niches of the human body including the skin, the oronasopharynx and the urogenital tract, albeit in different numbers. Therefore, it is interesting to investigate whether the same **molecular framework to identify important characteristics** of gastro-intestinal probiotics can be translated to other niches. In agreement with the main modes of action of beneficial gut probiotics, lactobacilli could also promote health in other niches by (1) having antimicrobial actions against important pathogens, (2) strengthening the mucosal barrier function; and (3) modulating host immunity.
Our results show **antipathogenic activity** of certain lactobacilli against *Moraxella catarrhalis,* a pathogen which is resistant to multiple antibiotics and is involved in various nasopharyngeal diseases like otitis media, chronic obstructive pulmonary disease (COPD) and pneumonia. The exact working mechanism of this activity is now further investigated.

D052

The anti-diabetic activity of *Bifidobacterium lactis* HY8101 *in vitro* and *in vivo*

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The aim of this study was to evaluate the effects of *Bifidobacterium lactis* HY8101 on insulin resistance induced using tumor necrosis factor-α (TNF-α) in rat L6 skeletal muscle cells and on the KK-A^Y mouse non-insulin-dependent diabetes mellitus (NIDDM) model.The treatment using HY8101 improved the insulin-stimulated glucose uptake and translocation of GLUT4 via the insulin signaling pathways AKT and IRS-1(Tyr) in TNF-α-treated L6 cells. HY8101 increased the mRNA levels of GLUT4 and several insulin-sensitivity-related genes (PPAR-g and PGC-1α) in TNF-α-treated L6 cells. In KK-A^Y mice, HY8101 decreased fasting insulin, blood glucose and food intake and significantly improved insulin tolerance. HY8101 improved diabetes-induced plasma total-cholesterol and triglyceride (TG) levels and increased the muscle glycogen content. We observed concurrent transcriptional changes in the skeletal muscle tissue and the liver. In the skeletal muscle tissue, the glycogen synthesis-related gene pp-1 was up-regulated in mice receiving HY8101 treatment. In the liver, the hepatic gluconeogenesis-regulated genes (PCK1, G6PC) were down-regulated in mice receiving HY8101 treatment. *B. lactis* HY8101 can be used to moderate glucose metabolism, lipid metabolism and insulin sensitivity in mice and in cells.

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Selective modulation of the intestinal barrier integrity by *Lactobacillus fermentum* strain AGR1487

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Many *Lactobacillus* species are recognised to promote health benefits such as pathogen inhibition, immunomodulation and enhancement of the barrier function. More recently, the universal claim of beneficial interactions with respective hosts has come under scrutiny.
Tight junctions control the passage of ions and certain metabolites between the intestinal epithelial cells from the lumen to the underlying lamina propria. This flow can be measured by various assays. While trans-epithelial electrical resistance (TEER) measures the paracellular flow of ions across the cell layer, the mannitol flux assay investigates the paracellular movement of small, non-ionic molecules. Both assays were used to ascertain the effect of *Lactobacillus fermentum* strain AGR1487 on intestinal barrier integrity using the human colonic adenocarcinoma Caco-2 cell line.
L. fermentum AGR1487, isolated from the human oral cavity, was shown to decrease TEER of Caco-2 cells by 60 to 80%. This effect was independent of the bacterial growth phase and was mediated by live and UV-inactivated cells (but not by culture supernatant), implying a direct interaction between the microbial cell surface and Caco-2 cells. Analogous to TEER assays, live AGR1487 cells increased mannitol permeability between Caco-2 cells by 3-fold, indicating a destabilisation of the tight junction integrity. In contrast, UV-inactivated AGR1487 did not affect mannitol permeability. This may imply that AGR1487 employs distinct strategies to modulate cell barrier properties by affecting intestinal cells through both microbial surface structures and metabolites.
To identify proteinaceous candidates likely involved in modulating the ion flux across the Caco-2 cell barrier, isolated cell surface proteins of strain AGR1487 were fractionated using HPLC. A sample with activity in TEER assays was subjected to proteomic analyses and peptide fragments were compared to the non-redundant NCBI protein database and to a custom AGR1487 ORFeome database.

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E001

Dietary administration of probiotic *Lactobacillus paraplantarum* L34b-2 enhanced growth, disease resistance and humoral immune response in *Pangasius bocourti*

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Probiotic bacteria have gained increasing interest as a growing concern of high antibiotics employed in aquaculture. Improved resistance against infectious diseases can be achieved by the use of probiotics. The potential probiotic *Lactobacillus paraplantarum* strain L34b-2 was isolated from Thai traditional fermented beef called “Som Nau”. This bacterial strain was selected based on its ability to produce antibacterial activity against bacterial fish pathogens, together with its tolerance to gastrointestinal conditions and adhesion to mucin. In feeding experiments, the strain L34b-2 was administered to “Pla Mong” fish (*Pangasius bocouti)*as dietary supplement at ~10^7 $CFUg^{-1}$ feed for 2 weeks before challenged with the fish pathogen, *Aeromonas hydrophila*. It was found that the probiotic-fed fish grew and survived much better than the controls. The innate immunity of the fish was also examined. The serum lysozyme, complement and bactericidal activities were significantly higher in probiotic-fed fish compared with those in the control fish. These results strongly support the potential use of *L. paraplantarum* strain L34b-2 as probiotics in *Pangasius* fish aquaculture.

E002

Isolation and screening of lactic acid bacteria to be used as probiotics in catfish

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Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. The objectives of this study were to isolate and select potential probiotic, lactic acid bacteria using common *in vitro* screening assays such as antibacterial activity, tolerance to gastrointestinal conditions and adhesion ability. The selected *Lactobacillus plantarum strain* L42g isolated from Thai traditional fermented food displayed strong antibacterial activity against two fish pathogens tested: *Aeromonas hydrophila* and *Streptococcus* sp. The strain L42g well tolerated simulated gastrointestinal fluids and exhibited high adhesion ability towards mucin. After feeding orally to catfish, *L. plantarum* L42g not only enhanced the fish growth but also conferred a protective effect against *A. hydrophila*, the fish pathogen tested. Therefore, *L. plantarum* L42g is a good candidate as the potential probiotic to be used in catfish rearing.

E003

Isolation and characterisation of lactic acid bacteria with anti-fungal properties and their application in malting and brewing

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Fungi are an important problem in the malting and brewing industry, due to their ability to produce mycotoxins on the field, during storage as well as during the malting process. Mycotoxins can cause immuno-repression in humans and animals as well as negatively influence malt and beer quality. Lactic acid bacteria (LAB) are known to produce different compounds with antimicrobial activity. In this presentation antifungal compounds from malting / brewing LAB have been characterised at a molecular level and their effectiveness proven in pilot scale malting trials. The impact of the biologically acidified malt on beer quality was also investigated. Large scale screening of LAB for antifungal activity identified a number of strains which showed strong inhibition against *Fusarium* species. The antifungal compounds of the strains were isolated and characterised at a molecular level using a wide range of analytical tools (GC-MS, HPLC, NMR etc). The impact of these antifungal strains was evaluated in pilot scale malting trials, and their influence on the malt and beer quality was assessed using a range of EBC/MEBAK based analytical methods. Challenge trials using *Fusarium* species revealed that the strains inhibited the fungal growth during malting, as well as improving the malt quality and reducing the formation of mycotoxins. The interaction between the starter strains and fungi was studied using scanning electron microscopy.
The reduction of fungal growth during malting has a positive impact on the production of malt, as well as on malt and beer quality. In particular, the gushing problem, which has been attributed to high level of fungal contamination in malt, could be solved by using the antifungal strains.

E004

Carnitine-rich yoghurt as a promising functional food

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L-carnitine expressed by *cai* operon was known to play a significant rule in transferring long-chain fatty acids inside the mitochondria. Subsequently, β-oxidation and degradation of such fatty acids can be performed. L-carnitine was reported to perform different vital physiological effects on humans since it helps obese patients to lose body weight significantly, improves body performance, enhances recovery from exercise, improves insulin activity and reduces diabetes type 2 risks, prevents atherosclerosis and helps angina patients to exercise without chest pain, reduces the progression of life-threatening diseases including cancer, AIDS, CHD, and lung diseases, treats the symptoms of hyperthyroidism disorders, slows down the progression of mental deterioration in Alzheimer's disease, prevents osteoporosis through aging, improves male fertility and reduces the oily skin and gives a smooth overall appearance. Furthermore, L-carnitine is a quaternary ammonium compound with a wide antimicrobial activity that inhibits most G (+), G (-) bacteria, different yeasts and molds; whereas, it was found to improve some G (+) beneficial bacteria such as lactic acid bacteria.
The expression of L-carnitine by lactic acid starter is the target of the present study. This could be performed by cloning *cai* operon into *Lactobacillus bulgaricus* DSM20080 and/or *Streptococcus thermophilus* 425. This approach can be accomplished using pLEB590; a multicopy expression vector derived totally from lactococcal DNA. The heterologous expression of L-carnitine by latter lactic acid starters could be a promising strategy to produce carnitine-rich yoghurt that gains all the former mentioned vital physiological benefits and improves the shelf-life of yoghurt itself. Furthermore, L-carnitine over-expression by lactic acid bacterial transformants can be a cheap source for L-carnitine production as a promising food additive used generally in food industries.

E005

Hyaluronic acid-rich yoghurt as an elderly functional food

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Hyaluronan is a complicated mucopolysaccharide (a repetitive sequence of glucuronic acid and N acetyl glucosamine) occurring naturally throughout the human body. It is beneficial in skin-care products as an excellent natural moisturizer. It has many valuable uses as it enhances fertility, moisturizes tissues and joints, smoothes skin, activates white blood cells (induces immune system), decreases bacterial inflammations, reduces the incidence of chronic bronchitis, accelerates wound recovery, makes vision clearer, improves sleeping, improves hair growth, enhances bone density and cures osteoporosis. It is well-known that hyaluronic acid concentration in the human body decreases by about 60% through aging (over 60 years old). Therefore, it is a vital supplement for the elderly. The present study deals with the expression of Hyaluronic acid by lactic acid starter culture. Such aim could be achieved by cloning *hasA* as well as *hasB* genes of *Streptococcus thermophilus* 425 into *Lactococcus lactis* subsp. *lactis* MG1363 via a multi-copy expression vector (pLEB590) derived totally from lactococcal DNA. Such modification could allow the over-production of Hyaluronic acid after the optimization of the transformants fermentation conditions. Latter lactic acid bacterial transformants could be promising cultures used to produce hyaluronic acid-rich yoghurt. Such product could be a vital functional food for both sick and healthy elderly.

E006

Fermentation of 'ogi' with some lactic acid bacteria isolated from various sources and their ability to produce β-galactosidase

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Seventeen lactic acid bacteria (LAB) were isolated from yellow maize gruel, soil samples of three fruit trees (mango, guava and banana), different parts of tilapia fish such as gills and intestine as well as fish pond sediment and water. These LAB isolates were used as starter cultures for the fermentation of maize gruel ('Ogi") made from grains of *Sorghum bicolor* for 48 hours. Their proximate composition was determined using standard methods. Result of the proximate analysis shows that *Lactobacillus plantarum* from moist mango soil had the highest protein and crude fibre contents of 11.48 and 1.95% respectively while the least was observed in *Streptococcus lactis* with protein content of 3.89%. Studies on β- galactosidase production by LAB were performed in an automatic incubator and samples were taken at every 6 hours interval for 3 days for enzyme assay at 28ºC. All the LAB isolates showed enzyme activities but *Lactobacillus fermentum* showed the highest activity of 247.78 U/ml at pH 7.5 after 54 hour of incubation while there was no activity at 60 hour of incubation. The least activity (153.35 U/ml) was observed in *Lactobacillus bulgaricus* from fish intestine at pH 6.6 at 36 hours of incubation. *Lactobacillus fermentum* can be a potential producer of β-galactosidase which may have applications both in industry and biotechnology.

E007

Optimized phage-robust cultures

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Lactic acid bacteria are widely used as starter cultures for the manufacture of cheese and other fermented milk products. However, during the fermentation process the strains can become susceptible to bacteriophage attack, resulting in delay or even failure of the fermentation process and thereby causing significant economic loss.
As a starter culture supplier, DSM takes the phage robustness aspect of its cultures very seriously and takes care to supply its customers with cultures of the highest phage robustness standard. The basis for the development of these phage robust cultures consists of high-throughput characterization of phage-host interactions and rational design of blends based on these data.
In addition, the market performance of our cultures is monitored by extensive whey testing and phage analysis. By using state-of-the-art software analysis this data is visualized to spot trends in phage developments, performance of specific culture product ranges and benchmarking of specific dairy factories against the overall industry. This allows us to be ahead of the game and being able to take corrective action early on.
In this poster, several examples of these methods will be shared.

E008

Aroma formation by lactic acid bacteria at extremely low growth rates

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In some industrial food fermentation processes lactic acid bacteria (LAB) encounter long periods of nutrient limitation, for instance during cheese ripening or dry sausage production, resulting in extremely slow growth. Nevertheless, particular LAB survive these periods and still contribute to flavour formation. This study focuses on the formation of aroma compounds by LAB growing at near-zero growth rates. Recently, different microorganisms, including *Lactococcus lactis* and *Lactobacillus plantarum*, have been grown in retentostat cultures at low growth rates (reaching doubling times of more than a year) reflecting the behaviour in a natural environment with restricted nutrient availability. The cells remained metabolically active and viable and increased robustness was observed. In this study dairy-derived LAB with high aroma formation potential will be grown in retentostat cultures. Aroma formation will be analysed using different protein rich defined media reflecting industrial conditions. By measuring gene expression profiles, information will be obtained on the regulation and activity of genes involved in metabolic processes associated with aroma formation.

E009

Exopolysaccharides come in many shapes and sizes

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Exopolysaccharides (EPS) are secreted by various lactic acid bacteria and play an important role in the rheological properties of dairy products. One of these producers is *Streptococcus thermophilus*. In our research we have examined 14 different strains of *S. thermophilus* for their EPS production and the characteristics of this EPS. Clustering the data on quantity, molecular weight and gyration radius of the EPS resulted in three main clusters. One cluster containing strains with a high EPS production and molecular weight, another cluster containing EPS with a very high gyration radius and the last cluster contains strains with both a low amount and a low gyration radius of EPS. These clusters can also be linked to their rheological features during growth (high and low viscosity).
Within the clusters more diversity is being found based on the sugar composition of the EPS. Of the 14 examined strains 11 different EPS compositions were found. This high diversity can be used to customize the texture of cheeses and yogurts.

E010

Determination of lactic acid bacteria nutritional requirements using an MTP robotic system

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Lactic Acid Bacteria (LAB) are fastidious micro-organisms that require many complex nutrients to sustain their growth. In order to gain knowledge on LAB nutritional requirements, an in-house robotic system solution was developed to determine rapidly auxotrophies of *Streptococcus thermophilus* and *Lactococcus lactis* strains.
Growth kinetics of the bacteria of interest are monitored in a 96 well microtitre plate (MTP). Maximum Optical Density (OD_{max}) and maximum growth rate (μ_m) are measured on a Chemical Defined Medium without omission (Full CDM) or with one single component omission (Single Omission Media). The impact of each ingredient (i.e. approx. 50 molecules such as amino acids, vitamins, [3DOTS]) on bacterial growth is then ranked as non-essential, stimulatory or essential.
Compared to previous manual methods described in the literature, the use of this robotic system will improve results consistency (eliminate human error) as well as generate results in a much faster manner.

E011

Characterization of some bacteriocins produced by lactic acid bacteria isolated from fermented foods

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Lactic acid bacteria isolated from different sources (dairy products, fruits, fresh and fermented vegetables, fermented cereals) were screened for antimicrobial activity against other bacteria, including potential pathogens and food spoiling bacteria. Six strains have been shown to produce bacteriocins: *Lactococcus lactis* 19.3, *Lactobacillus plantarum* 26.1, *Enterococcus durans* 41.2, isolated from dairy products and *Lactobacillus amylolyticus* P40, *Lactobacillus amylolyticus* P50, and *Lactobacillus oris* P49, isolated from *bors* (fermented wheat bran). The first three bacteriocins were heat stable, low molecular mass polypeptides, probably belonging to class II bacteriocins. They have a broad inhibitory spectrum, including strains of *Listeria monocytogenes* and *Staphylococcus aureus*. The other three bacteriocins were heat labile, high molecular mass proteins, with a very narrow inhibitory spectrum, most probably belonging to class III bacteriocins. The activities of all six bacteriocins were stable in a wide pH range, but were supressed by the treatment with proteinase K and pronase. A strong synergistic effect of some bacteriocins mixtures was observed, especially when bacteriocins produced by *Lactobacillus amylolyticus* and *Lactobacillus oris* were combined, or when these bacteriocins were combined with the ones produced by the strains isolated from dairy products. We can conclude that fermented foods are still important sources of new functional lactic acid bacteria. Among the six characterized bacteriocins, there might be some novel compounds, with interesting features. Moreover, the bacteriocin-producing strains isolated in our study may find applications as protective cultures.
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E012

Potential application of lactic acid bacteria exopolysaccharides in food and beverage industries

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The growing interest of governments and industry in developing healthy and natural alternative foods and beverages that will fulfil the consumer drive towards a healthy lifestyle and natural diet has led to an increase in traditional lactic acid bacteria fermentation research. Lactic acid bacteria exopolysaccharides (LAB-EPS) have long been known as interesting food ingredients since they can have positive physical/functional effects on foods/beverages such as yoghurts, breads, juices and sports drinks by acting as (depending on the food) hydrocolloids, humactants or as contributors to mouth-feel.
In this presentation, the results of (i) *ex-situ* LAB-EPS application as a replacement of low-protein/wholemeal flour functionality for bread-making purposes, and (ii) *in situ* LAB-EPS application for the production of novel barley wort-based beverage will be illustrated, respectively.
Generally, the *ex-situ* LAB-EPS application in wheat bread improves dough and bread quality, in terms of specific volume and hardness, in particular when low-protein and wholemeal flour was tested. Additionally LAB-EPS functionality showed dosage dependence.
In wort-based beverage, the organoleptic modulation of beverage, using *in-situ* produced EPS, was achieved with the improvement of rheological profile of the resulting fermentate. Additionally, small amounts of organic acids were formed, and ethanol remained below 0.5% (v/v), the threshold volume for a potential health claim designation.
The results suggest that *ex-situ* and *in-situ* LAB-EPS application could be potentially used as strategic tool to compensate the low technological performance of the raw material and for the production of a range of novel, nutritious and functional beverages.

E013

Potential use of antimicrobial substances from *Lactobacillus plantarum* KUB-KJ174 and KL-1 against bacterial contaminants from bakery production process

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Bakery products have become to be a convenient food for lifestyles of Thai people. It is generally considered to be microbiologically safe food due to the high temperature up to 170°C used for baking process. However, the conveyors used to transfer at the post-baking process are the most likely source of bacterial contamination. This presentation aimed to study the efficiency of two antimicrobial substances (AMS) PP-174 and PP-KL1 from *Lactobacillus plantarum* KUB-KJ174 and KL-1, respectively against bacterial contaminants by conveyor model system. Two target AMS were preliminarily determined for their inhibition activities against the growth of 75 bacterial contaminants from puff pastry production process. Three of them, *Enterobacter asburiae* TPC-T14B, *E. asburiae* TPC-T23 and *Bacillus cereus* TPC-T15B exhibiting high resistance to both PP-174 and PP-KL1 were used for further inhibition determination by conveyor model system. One hundred microliters of each target culture were dropped on a piece of conveyor and incubated for overnight to perform conveyor contamination. Consequently, either 100 µl of PP-174 or PP-KL-1 at the concentration of 1, 5, 10, 50 and 100% were overlaid on each target contamination area for 18 h and later determined for their inhibition activities by standard plate count method. The results showed that the 1% of PP-174 and PP-KL-1 displayed the inhibition activity against the strain TPC-T23 for 100 and 75% while 5% did against the strain TPC-T14B for 1.44 and 8.56%, respectively. However, no concentration of the PP-KL1 could inhibit the growth of TPC-T15B while 50-100% of the PP-174 displayed 100% inhibition activity. Therefore, the PP-174 would be a potential AMS used as a cleaning solution to avoid bacterial contamination of the tools in production process.

E014

Heading for an economic industrial upgrading of crude glycerol from biodiesel production to 1,3-propanediol by *Lactobacillus diolivorans*

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The production of biodiesel has dramatically increased over the last few years leading to a surplus of crude glycerol as waste product. To create an economically feasible biorefinery concept for biodiesel production the crude glycerol needs to undergo a value-adding step to produce high-value chemicals like 1,3-propanediol.
Lactobacillus diolivorans turned out to be a good natural producer of 1,3-propanediol yielding 85 g/l 1,3-propanediol with a productivity of 0.45 g/l h in a fed-batch cultivation process using crude glycerol from biodiesel production derived from palm oil. Crude glycerol of different origins has been successfully tested showing no inhibitory effects on growth or production. Sugar necessary for the formation of biomass was replaced by hydrolysate from lignocellulosic material and in combination with crude glycerol from palm oil 75 g/l 1,3-propanediol could be obtained with a productivity of 0.36 g/l h.
A toolbox for the genetic engineering of *Lactobacillus diolivorans* including a transformation method as well as the expression vector pSHM for homologous and heterologous protein expression was developed to further improve the production process. The functional expression of genes was proved by the heterologous expression of the green fluorescent protein resulting in 40-60 fold higher fluorescence of the obtained clones compared to the wild-type strain. Homologous overexpression of a putative NADPH-dependent 1,3-propanediol oxidoreductase improved the 1,3-propanediol production by 20% in batch cultures.

E015

Selection of folate producing strains of *Streptococcus thermophilus* for a soft cheese production

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Folates are compounds having a chemical structure and nutritional activity similar to folic acid. In humans and other animals, the requirement of folates is satisfied by dietary sources because the body cannot synthesize them. The natural capacity for folates production by certain microorganisms has a potential to be exploited, either to replace the fortification with folic acid, the chemically synthesized form of folate that can cause adverse effects in some individuals, or to develop fermented foods naturally bio-enriched in folate. In this work, a large number of *Streptococcus thermophilus* strains was tested for the ability to accumulate folates. The first step was the selection of presumptive folate producers, which consisted in the search for the genes involved in folate biosynthesis. Gene expression analysis and microbiological assays for the quantification of total folates were then carried out on strains owning the genes for folate production. Subsequently, different approaches were applied to the best producers strains to maximize the folate production, e.g. the optimization of culture conditions (variation of pH and temperatures of growth) or a selection “for cultivation”, consisting of repeated cultivation of overproducers on synthetic medium in absence of folic acid. Selected strains were also tested, alone or in mixture, to verify the folate overproduction. HPLC analysis allowed the quantification of folate and the identification of the chemical forms of folate produced. The overproducer strains selected *in vitro* were then applied in soft cheese. This study opens new perspectives on the application of functional *S. thermophilus* strains for the design of dairy fermented products containing elevated levels of *in situ* produced form of folate.

E016

Antimicrobial biodegradable food packaging impregnated with bacteriocin 7293, a novel bacteriocin produced by *Weissella hellenica* BCC 7293

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The objective of this study was to develop an antimicrobial biodegradable food packaging by using Bacteriocin 7293 (Bac7293), a novel antimicrobial peptide produced by *Weissella hellenica* BCC 7293. Bac7293 was loaded into poly(lactic acid)/sawdust particle biocomposite film (PLA/SP) using diffusion coating technique. The maximum amount of Bac7293 incorporated into the pre-heated PLA/SP was achieved at 19.54 ± 2.87 μg/cm^2 after 30 min of diffusion coating, whilst PLA film without sawdust particle could not absorb Bac7293. According to the JIS Z 2801:2000 standard testing method, the produced PLA/SP impregnated with Bac7293 (PLA/SP+Bac7293) effectively inhibited both Gram-positive and Gram-negative food-borne pathogens(*Pseudomonas aeruginosa, Aeromonas hydrophila, Listeria monocytogenes, Escherichia coli, Salmonella typhimurium* and *Staphylococcus aureus*). Antimicrobial activity of PLA/SP+Bac7293 film remained unchanged even after storage at 25 °C for 12 months. In a food model study, PLA/SP+Bac7293 film effectively inhibited growths of *P. aeruginosa , A. hydrophila , L. monocytogenes , E. coli , S. typhimurium* and *S. aureus* artificially inoculated on fresh Pangasius fillet during storage under refrigeration. A reduction of 4 to 6 log cycles of all tested pathogens on fish fillet was observed. After 14 days of storage, the overall migration of PLA/SP+Bac7293 film to fish fillet was estimated at 2.46 to 3.01 mg/dm^2, which was much lower than the overall migration limit regulated by the commission of the European communities.

E017

Critical parameters for successful CINAC

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The International Dairy Federation (IDF) is a non-profit organization that represents the dairy sector worldwide. The IDF plays an important role in guarding the quality of the dairy industry by, among other things, setting standard protocols and procedures for analytical methods performed within the dairy branch in collaboration with ISO. One of the keystone protocols is for the measurement of the acidification kinetics of lactic acid bacteria, generally referred to as CINAC. Although the procedure set by the IDF is detailed and a ring test was conducted to ensure that the protocol could be followed and that comparable results could be obtained, the critical parameters governing the technique are neither emphasized nor described as such.
In this presentation we will describe the critical parameters we identified when implementing the IDF-prescribed CINAC protocol at DSM Food Specialties. These are of interest to industrial parties as well as academic institutions because of their effect on the reproducibility and continuity of the acidification kinetics measurements.

E018

Study of biotechnological method for detoxification of feed contaminated with ochratoxin A and pathogenic bacteria

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Safe feed production is one of the factors important in obtaining food free from chemical and microbiological contaminations. Biotechnological methods of feed decontamination are very demanded. Ability of lactic acid bacteria to ochratoxin A detoxification by biological transformation or/and adsorption to the cell walls, is the special feature connected with specified strains.
Lactobacillus plantarum S KKP 880 was isolated from natural environment and is capable to eliminate ochratoxin A and inhibit the growth of undesirable microorganism such as toxins producing moulds and pathogens: *Salmonella* sp., *Escherichia coli* and *Clostridium perfringens*. The efficiency of the preparation consisting of the *Lactobacillus plantarum* S strain was studies of the agricultural farms. The results showed the positive effect of the preparation on the reduction of ochratoxin A content and pathogens presence. Addition of the bacterial preparation to the meadow sward and whole maize plants contaminated with ochratoxin A, caused the lowering of ochratoxin A level by more than 70 %during ensiling process, in relation to the content of mycotoxin in raw plants. In silages treated with bacterial preparation the growth of *Salmonella* sp. and *Escherichia coli* was completely inhibited, the number of coli bacteria decreased by more than 100 times and the number of moulds over 1000 times lower, compared with silages prepared without bacterial preparation. The feed contaminated by ochratoxin A and pathogenic bacteria, after the process of lactic fermentation provided by strain *Lactobacillus plantarum* S may be employed in nutrition of breeding animals as being safe products.

E019

Study of application of lactic acid bacteria strains with special activities for food and feed quality improvement

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The ability of lactic acid bacteria (LAB) to synthesize a wide variety of useful metabolites as well as rapid development of molecular biology a growing interest in industrial practice these microorganisms. Think guiding research was to identify the metabolic activity of LAB isolated from preserved vegetables, raw milk and digestive tract of farm animals as well as to define the environmental conditions to allow maximum utilization of this activity. In studies of this work it was found that the growth of the lactic acid synthesis by assessing the strain is dependent on the concentration of the saccharides (glucose, lactose, sucrose) in the medium the cultivation time and the test LAB strain. In laboratories MRS medium was prepared in a high lactic acid content higher compared to the culture in minimal media selection. In the context of tests it was also found that the proportions of lactic acid synthesized form D (-) and L (+) are dependent primarily on the LAB strain but also the carbon source its concentration and the cultivation time. The dynamics of the acetic acid synthesis was dependent on the concentration and type of carbon source. All the tested LAB strains were characterized by antimicrobial activity against pathogenic bacteria to the test both Gram-positive and Gram-negative the degree of inhibition was varied and dependent on the strain and culture conditions. The level of synthesis of lactic acid and other relevant metabolites in food technology may be adjusted by changing the environmental conditions while the selection of appropriate strains of LAB. Biodiversity LAB and their ability to adapt to changing conditions can be a tool to be used for various industrial applications.

E020

Latin-style fresh cheese enhances probiotic strains survival in the gastrointestinal tract

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For a food to be categorized as probiotic it is crucial that bacteria can live/grow in foods, but also survive during gastric transit. In the present study, fresh cheese produced with potential probiotic bacteria was tested in order to evaluate this cheese-type as a food carrier for the delivery of viable microorganisms into the gut. A total of 25 LAB strains identified as *Lactobacillus casei/ paracasei* (12), *Lb. plantarum* (5), *Lb. otakiensis* (1), *Lactococcus lactis* (3), *Leuconostoc mesenteroides* (3) and *Ln. citreum* (1), were exposed to simulated gastric juice (pH 2.5) and intestinal environment (0.3% of bile salts and 0.1% of pancreatin). Acidic environment drastically reduced LAB counts and no viable cells were detectable after 3 h of exposure. Most LAB counts were also reduced after incubation with bile salts and pancreatin. In a parallel experiment, the protective effect of Latin style fresh cheese on LAB cultures was then investigated. These cheeses are made without addition of any starter culture and are ready for consumption immediately after production. The same LAB strains were then inoculated during cheese manufacture and samples were submitted to the same conditions (pH 2.5 and bile salts/pancreatin). No loss of viability was observed in either environment as initial counts were maintained over a 3 h period. Therefore, fresh cheese greatly enhanced the survival of LAB strains in simulated gastric juice and intestinal environment, which is most likely due to the buffering capacity of this cheese. In conclusion, the use of fresh cheese as a probiotic food carrier presents real advantages in the survival of probiotic bacteria especially during gastric digestion, enhancing delivery within the gut and thereby maximizing health benefits.

E021

Application of potential probiotic bacteria and omega-3 fatty acids in yogurt production and impact on sensory quality

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Application of probiotic bacteria and polyunsaturated omega-3 fatty acids (omega-3 PUFAs) in functional food production can achieve many positive effects on human health.
The objective was to investigate the survival of autochthonous potential probiotic bacteria *Lactobacillus paracasei* 08*, Lactobacillus gasseri* IM 105 and commercial *Lactobacillus acidophilus* LA 05and the influence of omega-3 PUFA, on the sensory quality of yoghurt.
Experimental yogurt variants were produced using starter culture *Lactobacillus delbrueckii* ssp*. bulgaricus*, *Streptococcus thermophilus* (Chr. Hansen) and potential probiotic: *Lb. paracasei* 08 (Collection of the Department for Food Microbiology, Faculty of Agriculture, University of Belgrade), *Lb. gasseri* IM105 (Collection of Institute of Dairy Science and Probiotics, Biotechnical Faculty, University of Ljubljana) and commercial *Lb. acidophilus* LA 05 (Chr.Hansen). For each probiotic strain three variants of yogurt were produced: 1, without omega-3 PUFAs; 2, with 100mg/L omega-3 PUFAs; 3, with 200mg/L omega-3 PUFAs. Viability of probiotics and sensory evaluation were observed for 3 weeks.
The amount of *Lb. paracasei* 08 was 10^{8}-10^{9} cfu/ml during storage. Cell counts of *Lb. gasseri* IM105 and *Lb. acidophilus* LA 05 were maintained at the level 10^{7}-10^{8} cfu/ml. Addition of both concentrations of omega-3 PUFAs did not significantly affect on the sensory quality of yoghurt, especially those which were produced with *Lb. paracasei* 08. Average sensory scores of yogurt produced with probiotic strains were ranged 4.06 - 4.99, which corresponds to 81.20 - 99.80 % of the maximum quality.
Results indicate that autochthonous potential probiotic strains can be successfully used in the production of yoghurt fortified with omega-3 fatty acids as a new functional dairy product.
Key words: potential probiotic bacteria, yoghurt, omega-3 fatty acids, sensory quality

E022

Identification and characterization of potential starter cultures for commercial low salt cucumber fermentation

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Sodium chloride free cucumber fermentation demands the utilization of starter cultures to assure safety and product consistency. Commercial starter cultures developed from and for vegetable products are lacking in most countries. Hence, the objective of this study is to identify and characterize potential starter cultures isolated from commercial cucumber fermentations that can: (1) rapidly ferment cucumbers, (2) spearhead the fermentation or co-ferment with selected lactobacilli, (3) lacks malic acid decarboxylase, (4) aids in preventing secondary cucumber fermentation, and (5) possess acceptable safety profile. A group of 7 *Lactobacillus plantarum/pentosus* isolates out of 342 lactic acid bacteria obtained from two geographically distant commercial cucumber fermentations were selected for their ability to rapidly decrease the pH in cucumber juice media, and co-ferment with *L. brevis*, while potentially preventing secondary fermentation spoilage by *L. buchneri* . This group of isolates were more rigorously identify by sequencing *pheS* (phenylalanine t-RNA synthase), *rpoA* (RNA polymerase) and *dnaK* (heat shock protein 70). All of the tested cultures were classified as *L. pentosus* except for one isolate. The ability of these cultures to produce biogenic amines, and D- and L- lactic acid; decarboxylase malic acid and to utilize xylose was evaluated. The potential of these cultures to rapidly ferment cucumbers using low salt cover brine was confirmed in industrial scale fermentations in 38,000 L open top tanks. Understanding that the majority of the best performing lactic acid bacteria in commercial cucumber fermentation are *L. pentosus* instead of *L. plantarum* does change the traditional picture of the dominant microbes in such environment. Availability of suitable starter cultures for commercial scale salt free cucumber fermentations open ventures for the production of probiotic pickles.

E023

Nisin production under hemin-stimulated respiration at fed-batch fermentation

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In this study, nisin production of *L. lactis* N8 was optimized by independent variables of glucose, hemin and oxygen concentration in fed-batch fermentation which respirated with hemin stimulation. The effects of independent variables were determined and the response surface method was applied for optimization. Different glucose, hemin and oxygen concentrations were found effective on the nisin production of *L. lactis* N8 in relevant fed-batch fermentation. Response surface model was able to explain the nisin production of *L. lactis* N8 in fed-batch fermentation system with high fidelity where this model was given R^2 value above 98 % and insignificant lack of fit. Accordingly, the equation developed indicated the optimum parameters for glucose, hemin and dissolved oxygen were 8 g L^{-1} $^{h-1}$, 3 µg mL^{-1} and 40%, respectively. While 1711 IU mL^{-1} nisin production was produced at *L. lactis* N8 in control fed-batch fermentation, 5410 IU mL^{-1} nisin production was achieved within the relevant optimum parameters where the respiration was stimulated with hemin. Accordingly nisin production was enhanced 3.1-fold in fed-batch fermentation with using hemin. As a conclusion the nisin production of *L. lactis* N8 was developed extensively, by stimulating the respiration by adding hemin in the fed-batch fermentation resulting also in increasing the biomass.

E024

Biodiversity of heterofermentative lactic acid bacteria in traditional cheese DL starters

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Heterofermentive lactic acid bacteria are an important part of traditional mesophilic cheese DL-starters where they grow in close symbioses with the homofermentative *Lactococcus* population. *Leuconostoc* (*Leu*) is believed to be the main group of heterofermentative bacteria in these starters with *Leu. mesenteroides* being the most abundant species. The aim of this study was to explore the biodiversity of heterofermentative bacteria in traditional cheese DL starters. A modified MRS medium with vancomycin was used to isolate heterofermentative bacteria from two traditional Danish DL starters (A and B), and puls field gel electrophoresis (PFGE) where used to differentiate the isolates to strain level. 16S RNA sequencing and specific carbohydrate fermentation was used to identify species and differentiate between the subspecies of *Leu. mesenteroides*. Both starters contained several strains of *Leu. mesenteroides*, with starter A having 12 different strains and starter B had five different strains. Two *Leu. pseudomesenteroides* was found in starter B whereas this species was not found in starter A. Three *Lactobacillus danicus* strains was isolated from starter A. This novel species has earlier been isolated from other Danish dairy starters and cheese made from these starters. The results from this study revealed that traditional cheese starters may contain several strains of *Leuconostoc*, which make them robust against phage attack and a rich source for isolating strains for putative technological applications.

E025

Screening of Lactic Acid Bacteria from giant breed dog faeces for probiotics

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Selection of probiotic bacteria from Saint Bernard dog's faeces, 8 isolates were chosen from 64 isolates bacteria by undergoing all bacteria morphologically and testing biotic capability to examine of which isolates and kind of bacteria are capable being proper to be big dogs supplementary diet. According to the undergone morphological experiment found A1 - A8 are gram positive bacteria forms and lactic acid producing only. A1 - A7 isolates are catalase negative, but A8 is catalase positive. Lastly A1, A2, and A4 could grow up at both low and high temperature circumstances, while A3, A5, A6 and A7 could not grow at low temperature and A8 grew improperly at high temperature. The examination of probiotic capabilities through which bacterial isolates are most resistant to NaCl, bile salt No.3 and acidity. After being tested for 72 hours found that A8 was resistant to 18% NaCl, A7 was resistant to 8% bile salt No.3 and A7 was resistant to pH 2 - 10. Comparing the results to Bergy's Manual found that isolates of A1 - A7 may be *Streptococcus* spp., and A8 may be *Micrococcus* spp.

E026

Antimicrobial activity of *Lactobacillus plantarum* strains and antimicrobial properties of *L. plantarum* small heat shock proteins

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The aim of this study was to investigate *Lactobacillus plantarum* strains possessing antimicrobial features to be proposed as potential bioagents and biopreservant stains that could prevent the growth of pathogenic bacteria in fermented food. A total of 85 *Lactobacillus plantarum* strains, including strains overproducing small heat shock proteins, were investigated for their antimicrobial activity against the pathogenic bacteria *Escherichia co*li O157:H7, *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella enterica*. Firstly, the antimicrobial effect was evaluated using over-night cultures (ONC) of lactobacilli by agar spot test. The antagonistic effects of *L. plantarum* strains against pathogens were detected measuring the growth inhibition halos on plates. All strains showed significant antibacterial activity and we classified *L. plantarum* strains as no, mild, strong and very strong inhibiting strain when inhibitions zone of 1 mm, 2 mm, 2-5 mm and more than 5 mm were produced. Subsequentially, 15 strains showing strong and very strong antimicrobial activity were selected and their cell-free supernatant (CFS) was harvested and used for agar diffusion and microdilution bioassays over an incubation time of 24 h at 30 °C, in order to investigate the substances determining the antimicrobial ability. Overall, the results showed an interesting antagonism depending both on *L. plantarum* strain analysed and pathogens bacteria tested. Moreover, antimicrobial activity was observed by *L. plantarum* strains overproducing small heat shock proteins. The applications of *L. plantarum* strains exhibiting antimicrobial activity need to be considered as safety bio-alternative to traditional antibiotics used against human infection and to chemical substances used in the food processing.

E027

A meta-analysis of host - phage interaction matrices in *Streptococcus thermophilus*

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Streptococcus thermophilus is one of the most important dairy starters. Its interaction with bacteriophages in dairy environments (both cheese and yoghurt production plants) has been described, and the high specificity of bacteriophage-host relationships in this species has been attributed to the CRISPR-CAS defense mechanism. Host - phage interactions are usually presented as rectangular matrices with bacteriophages on columns and hosts on lines. These matrices, if not reordered, are of little assistance in individuating groups of bacteriophages with similar host ranges or in analyzing the structure of the matrix. Recently, Flores et al. (PNAS, 2011, 108, E288-97) described a number of approaches for analyzing the statistical structure of host - phage relationships and pointed out that the significant modularity of matrices for *S. thermophilus* phages (with blocks of hosts attacked by limited number of bacteriophages) contrast with the general trend for nestedness and this was postulated to be dependent on the CRISPR-CAS defense mechanism (Weitz et al., Trends Microbiol. 2013, 21, 82-91). We examined 7 published studies for which phage - host matrices were available and some unpublished results on phages isolated from natural milk cultures and found that, although a modular structure is found in many studies, the parameters describing the matrix seems to be partly dependent on the nature of the study, its size and on the isolation strategy. In addition, matrix cluster analysis proved to be a rapid tool for reordering lines and columns of the matrix to find cluster of phages with similar host ranges, and network analysis using an open source graph visualization software (Gephi, https://gephi.org/) provided additional tools to explore and represent the structure of the networks.

E028

Characterization of natural milk cultures for the production of high-moisture Mozzarella cheese: variability in performance and species composition

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Natural milk starters (NMS) are used in Italy for several cheese types, including high moisture Mozzarella. NMS are produced by thermization (63°C, 15 min) of raw milk followed by incubation at 42°C. The procedure is repeated daily with backslopping. Our objective was to use culture dependent and independent methods to evaluate the dominant bacterial community, to assess the variability in performances over repeated cycles of reproduction and the presence of *Streptococcus thermophilus* bacteriophages.
Raw milk and NMS (CN) were obtained from an artisanal cheese making plant. Two replicates of laboratory NMS (CL) were prepared. From the 2° cycle raw milk was inoculated (0.5% v/v) with the previous day batch, and the procedure was repeated 13 times.
pH, acidity, microbial counts and acid production ability were evaluated. For selected cycles PCR-DGGE of v3 region of 16S rDNA and of *lacSZ* operon were used to evaluate species and strain composition and an electronic nose was used to evaluate the aromatic profile. Multiplex PCR was used to detect bacteriophage DNA. Isolation, typing (RAPD-PCR) and molecular identification was carried out for the final cycle.
Microbial counts and technological parameters of the cultures were variable, but tended to stabilize over time. Both culture dependent and independent methods confirmed that the microbiota was dominated by *S. thermophilus. Lactobacillus helveticus , L. delbrueckii , Enterococcus faecalis* and *Lactococcus lactis* were also present.
Differences were found between CL and CN and even between the two replicate CL. Several *S. thermophilus* strains were present in each culture but only 2 *lacSZ* profiles were found. DNA of *cos*- type phages was detectable since early cycles but an enrichment step was needed to isolate a limited variety of virulent bacteriophages.

E029

Characterization of the microbiota present in quinoa flour fermentation and evaluation of their ability to produce exopolysacharides

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The development of methods to enhance the palatability and organoleptic properties of gluten free foods using quinoa, a nutrient rich grain, presents an opportunity to meet consumer expectations with innovative fermented quinoa bread. Towards this goal, red quinoa, black quinoa, and quinoa real were ground and fermented, as well as two commercial quinoa flours. From these sourdough fermentations, a total of 54 and 16 bacterial and yeast isolates, respectively, were obtained and identified. *Pediococcus pentosaceus* and *Saccharomyces sevazzii* were the dominant microbes found in these fermentations The dominant bacterium and yeast were cultivated on MRS and Yeasts and Molds plates, respectively, supplemented with 2% glucose, lactose, fructose, starch or sucrose to evaluate their ability to produce exopolysaccharides. Exopolysaccharides are expected to enhance the rheological properties of the bread, making the texture similar to that of a wheat bread. Thirteen of the 54 bacterial isolates were identified as exopolysaccharide producers, as well as twelve of the 16 yeast isolates, by looking at the sliminess or ropiness of colonies grown on supplemented media. *Pediococcus pentosaceus* stood out as the predominant EPS producing-bacterium in quinoa fermentation, thus a viable candidate for use as a starter culture. With the use of exopolysaccharide producing bacteria as starter cultures in quinoa bread, gluten free bread will be a cost effective, nutritious and palatable alternative to gluten containing products.

E030

Intracellular and extracellular expression of *Bacillus thuringiensis* crystal protein Cry5B in *Lactococcus lactis* for use as an anthelminthic

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Bacillus thuringiensis crystal protein Cry5B (140 kDal), and a truncated version of the protein (tCry5B, 79 kDal), are lethal to some nematodes. Genes encoding the two crystal proteins were cloned into a high copy number vector in *Lactococcus lactis* to determine whether the protein expressed in this host would have a lethal effect on *Caenorhabditis elegans* and other nematodes. Western blots revealed that constitutively expressed Cry5B and tCry5B were present in both cell lysates and in cell supernatants. The survival of *C. elegans* was negatively affected when the nematodes were exposed to live *L. lactis* cells containing the plasmids. The *cry5B* gene was cloned into pMSP3535H3 in attempts to improve expression with nisin induction. Three hours after nisin induction at concentrations up to 200 ng/ml, intracellular Cry5B was strongly induced without adversely affecting cell viability or cell membrane integrity. The crystal protein genes were also cloned into plasmid pTRK1061, which encodes a promoter and transcriptional activator reported to invoke low level expression of prophage holin and lysin genes in *Lactococcus* lysogens, resulting in a leaky phenotype. Cry5B and tCry5B were actively expressed in the lysogenic strain *L. lactis* KP1, and found primarily in cell supernatants. Log phase cells containing the leaky system grow normally. Lactate dehydrogenase assays indicated no marked increase in cell lysis from the leaky system, and that Cry5B, but not lactate dehydrogenase, was able to leak from the cells. Taken together, these results indicate that the crystal proteins Cry5B and tCry5B can be expressed intracellularly and released extracellularly in *L. lactis*, showing promise that an anthelminthic could be delivered orally in a food-grade microbe.

E031

Antimicrobial activity of lactic acid bacteria on biofilm formed by *Listeria monocytogenes*

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Lactic acid bacteria are proposed as an innovative eco-friendly strategy to control relevant food-borne pathogens including *Listeria monocytogenes* and *L. monocytogenes* represent a safety concern for the food industry due to its ability to survive and grow under several harsh conditions associated with food processing and preservation. In addition, *L. monocytogenes* may persist in food plants and equipment since able to form biofilms. In the present work, we screened 152 lactic acid bacteria for their ability to inhibit the growth of *L. monocytogenes* serovar ½a and 4b from animal and vegetable origin. The antagonistic effect was investigated by analysing the halo of inhibition on agar plates co-inoculated with LAB strains or in presence of the correspondent cell-free supernatant. The same approach was employed to evaluate the reduction of biofilm formation on glass, polystirene, and stainless steel. *L. monocytogenes* biofilms were quantified spectrophotometrically and the viability of the pathogen assessed by qPCR. LAB were clustered in four categories according to their inhibitor effect. The strains with the stronger antagonistic activity suggest a potential employment to control *L. monocytogenes* proliferation and the corresponding biofilm formation in food processing and plants.*"The research leading to these results has received funding from the European Union 's Seventh Framework Programme for research, technological development and demonstration under grant agreement n°289719".*

E032

Biodiversity of autochthonous *Oenococcus oeni* strains isolated from Apulian wines

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Winemaking is a complex process that involves two different fermentation steps: alcoholic fermentation (AF) and malolactic fermentation (MLF). MLF consists in a decarboxylation of L-malic acid to L-lactic acid and leads to increase in wine pH, decrease in wine sourness, increase of microbial stability and bacterial production of various secondary metabolites that contribute to wine quality. Usually MLF is performed by lactic acid bacteria, mainly *Oenococcus oeni* strains, inoculated at high concentrations, enough to ensure survival and malolactic activity. *O. oeni* strains shown a large degree of phenotypic and genotypic heterogeneity. In this work, we studied the genetic and technological diversity of *O. oeni* strains isolated from Apulian wines undergoing spontaneous MLF to select strain suitable for autochthonous malolactic starter cultures design. We studied the genetic diversity of 50 *O. oeni* strains using two molecular methods (Variable Number Tandem Repeat-VNTR and Multi Locus Sequence Typing-MLST), and malolactic performances. VNTR identified 30 profiles, of which 11 unique profiles, while MLST distinguished only 8 different sequence types (STs) among 20 strains, resulting have different VNTR profiles. Nevertheless MLST techniques allowed to find six new STs and two new alleles, respectively, for gene *rpoB* and *purK*. This study confirmed the worldwide presence of two *O. oeni* phylogenetic subpopulations (A and B). All the strains have been screened for their capacity of degrading malic acid, with two different times of bacterial inoculum, either together with yeast (co-inoculum) or after the completion of alcoholic fermentation (sequential inoculum). *O. oeni* strains studied shown different malolactic performances. Experimental scale-up allow us to select two *O. oeni* strains as potential biotypes for starter cultures.

E033

Preliminary assessment of the microbiological quality of raw and fermented milk (Lben) from cow and camel of southern Morocco: Isolation and characterization of antagonistic lactic acid bacteria

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Samples of raw and fermented milk (Lben), of both camel and cow, collected from different zones of southern Morocco were analysed to evaluate their microbiological quality and to isolate BLIS-producing lactic acid bacteria (LAB). The following groups of microorganisms were investigated: total aerobic mesophilic flora (TAMF), fecal coliform (FC), total coliform (TC), caseolytic germs, *Staphylococci* (Staph), lactic acid bacteria (LAB) and yeasts and fungi. The analysed samples were generally highly contaminated and the microbial counts markedly variable among samples. A total of 1000 strains randomly isolated from different samples were screened for the production of bacteriocin-like BLIS active against *Bacillus subtilis, Staphylococcus aureus* and *E. coli*. 55 LAB strains were shown to be active against at least one of the indicator strains. Among them, two strains called Z3.7 and Z3.10 were selected for their inhibitory activity, heat stability and pH acid resistance. They were identified as *Streptococcus thermophilus*, throughout API 20 Strep and API 50 CH tests. In fact, they both lack haemolytic activity, and were susceptible to chloramphenicol. These characteristics insure their safety aspect and their potential application in fermented food as starter and preservative culture.

E034

Characterization and screening of the microflora of lemon coagulant soy-cheese for preliminary starter culture(s).

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Naturally fermented soy- cheese (fermentation time: 12 h at 37^{o}) was produced and its microflora was enumerated, isolated and identified. The dominating fermenting microorganisms (lactic acid bacteria) were screened for possible use as starter cultures based on acidification profile and physico-chemical properties of the soy- cheese produced. The total viable cell count of the naturally fermented soy- cheese was 5.30 x 10^6 cfu/g and that of the lactic acid bacteria count reported 4.15 x 10^5 cfu/g. The microflora of the soy- cheese includes; *Aerococcus viridians, Micrococcus kristinae, Micrococcus varians, Leuconostoc lactis, Lactobacillus fermentum , Lactococcus lactic* spp*. cremoris, Lactobacillus pentosus, Lactobacillus delbrueckii* spp. *bulgaricus, Lactobacillus brevis, Escherichia coli, Aeromonas hydrophila, Saccharomyces cerevisiae* and *Candida sake*. The lactic acid bacteria isolates from the naturally fermented soy- cheese were used as single culture to produce soy- cheese. The pH of the soy- cheese ranges from 5.41 to 5.74 and its protein content ranges from 27.34% to 51.85%. Among the lactic acid bacteria used as starter culture, the results indicated that *Lactobacillus delbrueckii* spp. *bulgaricus, Lactobacillus pentosus* and *Leuconostoc lactis spp. cremoris* yielded the highest protein contents in soy- cheese. Hence, it can be deduced that they hold potential for use as starter cultures and the combination of soy-cheese isolates in mixed culture fermentation trial is recommended.

E035

Cold stress improves the survival of *Lactobacillus plantarum*

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The stress-resistance of bacteria is affected by the physiological status of the bacterial cell and the environmental factors such as pH, salts and temperature. In this study, we report the stress response of *Lactobacillus plantarum* L67 after the freeze-thawing cycles. The response of cold stress to the *csp* genes (*csp* C, *cspL* and *cspP*) and ATPase activities was then evaluated. The cold stress was adjusted to 5ºC when the bacteria were growing at the mid-exponential phase. A comparative proteomic analysis was performed with two-dimensional gel electrophoresis (2D SDS-PAGE), and a Matrix Assisted Laser Desorption/Ionization-Mass Spectrometer. Only 56% of the *L. plantarum* L67 cells without prior exposure to cold stress survived after four consecutive freeze-thawing cycles. However, 78% of *L. plantarum* L67 cells survived after freeze-thawing conditions the incubation temperature was downshifted to 5°C for 6 h under freeze-thawing conditions. After applying cold stress to the culture for 6 h, it was then stored for 60 days at 5°C, 25°C and 35°C separately. The cold-stressed culture *L. plantarum* L67 showed 8% higher viability than the control culture. After applying cold stress for 6 h, transcript levels of two genes (*cspP* and *cspL*) were up-regulated to 0.4 (*cspP*) and 0.2 (*cspL*) when compared to the control. However *cspC* was not up-regulated. Proteomic analyses show that it was increased after reduction of the incubation temperature to 5°C. In conclusion, cold acclimation constitutes a real advantage in bacterial competition against spoilage and pathogenic psychrotrophic bacteria in terms of food preservation.

E036

Comparison of membrane methods for laboratory purification of EPS produced by lactic acid bacteria

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Exopolysaccharides (EPS) synthesized by lactic acid bacteria (LAB) play an important role in stabilizing and enhancing the viscosity and water binding properties of fermented dairy products and also contribute to the mouth-feel, texture and taste perception of those products. Various methods have been reported for isolation of EPS for quantification and characterization purposes. The primary aim of this study was to compare three membrane-based methods for recovering EPS: dialysis, ultrafiltration and centrifugal filtration. Initial evaluation employed a model system, paralleling established procedures for extraction of EPS from milk-based cultures and fermented foods. Simulated product extracts were produced (ethanol-precipitate from a TCA-supernatant of reconstituted skim milk) containing added dextran. These extracts were then subjected to dialysis, ultrafiltration and centrifugal filtration using ~10 kDa cutoff membranes and recoveries were compared. The recovery trend was consistently ultrafiltration>dialysis>centrifugal filtration, with the lowest variability shown in UF.
The methods were also applied to LAB cultures (each possibly producing a chemically different EPS) in reconstituted skim milk. The ultrafiltration method (EPS estimation 159 - 391 mg/L of culture) gave generally higher estimates of EPS compared to dialysis (78 - 380 mg/L) and centrifugal filtration (76 - 332 mg/L). Not all yields were consistent with the trend established using the dextran model. Based on this study, four strains (*Streptococcus thermophilus* ASCC871275 and ASCC870753, *Lactobacillus delbrueckii* ssp*. bulgaricus* ASCC871092 and ASCC87840) have been selected for further analysis of the production and characteristics of their EPS.

E037

Probiotic survival in symbiotic yogurt-like cereal-based beverage

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The scientific literature and market trends testify to the growing emphasis on the development of dairy-like vegetable based products, including food matrices based on fruit, vegetables and cereals, able to meet the needs of consumers allergic/intolerant, on cholesterol-restricted diets, and/or vegans. Together with the formulations based on soy flour, yogurt-like products produced from cereal flours, have been receiving increasing attention due to the good balance of texture, flavor, and nutritional properties in the final product. As substitutes of dairy productions, the corresponding non-dairy foods yogurt represent a novel alternative for the consumption of probiotic bacteria.
In order to produce yogurt-like cereal-based beverages, a mixture of rice, barley, and oat flours and concentrated red grape must were used. In addition to standard formulation, we prepared a β-glucans enriched beverage using a flour of barley selected for its β-glucans content (12 g of β-glucans per 100 g of flour). β-glucans have been reported to be highly fermentable by the intestinal microbiota in the caecum and colon, and can enhance both growth rate and lactic acid production of microbes isolated from the human intestine. Throughout the technological phases, a commercial yogurt starter cultures and a selected strain of *Lactobacillus plantarum* were separately used to perform lactic acid fermentation (30 °C, 8 hours). During the storage at 4 °C, we assessed the survival of 7 probiotic strains (*Lactobacillus acidophilus* LA5, *L. plantarum* CECT 8328, *Lactobacillus johnsonii* CECT 289, *Lactobacillus fermentum* CECT 8448, *Lactobacillus reuteri* CECT 925, *Lactobacillus paracasei* subsp*. paracasei* LC-01 , *L. plantarum* WCFS1) in the standard yogurt-like cereal-based beverage and in β-glucans enriched beverage (symbiotic yogurt-like cereal-based beverage).

E038

NADH oxidase of *Streptococcus thermophilus* is required for the effective yogurt fermentation with *Lactobacillus delbrueckii* subsp. *bulgaricus*

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Yogurt is produced by *Lactobacillus delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*) and *Streptococcus thermophilus* (*S. thermophilus*). It is widely known that proto-cooperation between these bacteria is important for efficient yogurt production and further information is required to understand this phenomenon precisely.
We previously reported that dissolved oxygen (DO) suppresses yogurt fermentation with an industrial starter culture composed of *L. bulgaricus* 2038 and *S. thermophilus* 1131, and also found that reducing the DO in the medium prior to fermentation (deoxygenated fermentation) shortens the fermentation time. We recently found that deoxygenated fermentation primarily increased the cell number of *S. thermophilus* 1131 rather than that of *L. bulgaricus* 2038, resulting in earlier L-lactate and formate accumulation. Our study suggested that DO is mainly removed by *S. thermophilus* 1131. The results using an H_2O-forming NADH oxidase (Nox)-defective mutant of *S. thermophilus* 1131 (*Δnox*) revealed that Nox is the major oxygen-consuming enzyme of the bacterium. Yogurt fermentation with the *S. thermophilus* mutant *Δnox* and *L. bulgaricus* 2038 was significantly slower than that with the wild *S. thermophilus* 1131 and *L. bulgaricus* 2038, and the DO concentrations of the mixed culture of the former combination did not decrease to less than 2 mg/kg within 3 hr.
Therefore we concluded that Nox of *S. thermophilus* 1131 contributes greatly to effective yogurt fermentation, presumably by removing the DO in milk. The results with other combinations with six strains of *S. thermophilus* and two strains of *L. bulgaricus* supported this conclusion.

E039

Use of Design of Experiments to determine Design Space - a robust region of operation assuring product quality in industrial production of starter cultures and probiotics

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Chr. Hansen is a worldwide supplier of lactic acid bacteria for starter cultures and probiotics. The success of our cultures relies on the ability to develop optimized and robust production processes meeting the quality requirements with regards to cell counts, storage stability and of course activity.
In our pilot plant we are continuously improving our process knowledge and understanding. We use a statistical approach to design experiments for optimization of new production processes testing relevant fermentation parameters for their effect on product quality. With the possibility of running 20 pH-controlled fermentations at a time, we have tested up to eight factors (process parameters) in a fractional factorial design with four centerpoints. The effect of the tested factors on product quality (*e.g.,* cell count and storage stability) was evaluated and the best model for each response was developed. Based on these models, the optimal setpoint value for each factor was identified and Monte Carlo simulations were used for making an estimation of the Design Space. The Design Space identifies the largest possible variation for each factor that will still result in a product that meets all the quality criteria. This predicts both the optimal factor settings as well as the region of acceptable variability for each of the factors.
Design of Experiments and Design Space estimation enables us to define the critical process parameters for product quality and their optimal settings. Furthermore, it allows us to understand the robustness of our production process and identify the safe region of operation within which the process complies with all the specifications for the product.

E040

***Lactobacillus pentosus* and yeasts survival to simulated human gastrointestinal transit using fermented olives as a carrier**

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Lactobacillus pentosus 119 and 13B4 strains were used as starter cultures in small-scale olive fermentations. Olives, Gordal variety, were processed according to the traditional method. Two fermentation vessels were inoculated with *L. pentosus* 119 and the two other with *L. pentosus* 13B4. At the end of fermentation, samples of olives were withdrawn and the bone was removed. Each pitted olive sample was then divided into two aliquots: one of them was homogenized in a stomacher apparatus and the other one was treated with a cocktail of enzymes. The simulated human digestion was carried out as follows: homogenized olives or released microorganisms from biofilm were incubated in human saliva, then incubated in gastric juice and finally treated overnight in intestinal juice. To test the survival of lactobacilli and yeasts at each step, samples were withdrawn and then spread in appropriate selective culture media. The presence of the inoculated *L. pentosus* strains was assessed by RAPD-PCR analysis, and the yeast were identified by RFLP of the 5.8S ITS region. In addition, samples at each step were processed for scanning electron microscopy (SEM).
In spite of a reduction in the number of viable cells, both inoculated *L. pentosus* strains were able to survive to the complete simulated digestion in the samples of homogenized olives. On the contrary, they were not able to survive to neither gastric nor to pancreatic digestions in the samples of the extracted biofilms. On the other hand, yeasts were able to survive to the complete digestion in both types.
This study demonstrates that the fermented olives are potentially a suitable product for the delivery of the probiotic microorganisms to consumers.

E041

NMR spectroscopy as a tool to understand metabolic pathways of *Lactobacillus plantarum*

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The efficacy and robustness of lactic acid bacteria is of high commercial value to DuPont. In order to generate basic knowledge about metabolic pathways and to gain better process understanding, NMR spectroscopy is highly useful. NMR spectroscopy is less sensitive than HPLC and GC, but NMR has the advantages of being unbiased, rapid and quantitative, with an inherently high structural information level thereby allowing for detection of small metabolites. Typically 80-100 supernatant samples can be prepared by just adding buffer and the analysis can be finished overnight. In combination with chemometrics, correlations between biological effects, metabolites and pathways can be discovered.
We performed cultivations with *Lactobacillus plantarum* as model strain at different pH set points to evaluate the impact of environmental changes on the metabolism. We will present data giving an insight into the metabolic pathways of *Lactobacillus plantarum* cultivated at different pH set points.

E042

***Leuconostoc mesenteroides* and its added-value in Dutch-type cheese manufacturing**

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The species *Leuconostoc* is recognized for its relevance in the food industry. *Leuconostoc* plays an important role in the manufacturing of various dairy products (like buttermilk, quark and cheese), fermented vegetables such as sauerkraut, and sourdough and the production of the biopolymer dextran. Although *Lactococcus* strains are the main players in the manufacturing of Dutch type cheeses, *Leuconostoc mesenteroides* contributes to several key attributes of the distinctive product. This representative of the lactic acid bacteria, however, has gained considerably less research attention. In an effort to close the gap between experience and knowledge and understanding, we studied the contribution of *Leuconostoc mesenteroides* in the production of Dutch type cheeses. On basis of a strain collection of *Leuconostoc* strains of various origins and a DNA micro array on basis of the ATCC 8293 strain, isolated from an olive fermentation, in combination with a gene knock-out bank of said strain, we obtained various insights with respect the contribution of *Leuconostoc* to various aspects of cheese manufacturing.

E043

Caciotta cheese manufactured with the addition of potentially probiotic non-starter *Lactobacillus* isolated from raw ewes' milk produced in Sardinia: Compositional, physicochemical, microbiological and sensory characteristics

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This work evaluated the effect of potentially probiotic autochthonous *Lactobacillus*strains on compositional, microbiological, physicochemical, and sensory characteristics of Caciotta cheese produced from pasteurized ewes' milk. One control cheese made with the addition of commercial lactic cultures (CC) and three types of experimental Caciotta cheese (C1, C2, C3) made using three different combinations of autochthonous adjunct cultures, each one containing a different *Lactobacillus* strain selected for its probiotic potential, were manufactured in an industrial plant. Conventional culturing, isolation and genotypic characterization by Rep-PCR in addition to a combined PCR-DGGE approach using total cheese DNA extracts and DNA extracts obtained from LAB isolates, were used to monitor the presence and viability of probiotic *Lactobacillus* strains. Cheeses were analyzed at 2, 15, 30 and 60 days of ripening. After 30 days of ripening, the mean values for the compositional parameters of all the probiotic cheeses, including the control, were in the range: 44-46% moisture, 20-22% protein and 26-28% fat. The pH ranged from 4.79 to 5.12. As for lactic acid bacterial counts, the number of lactococci evolved quite similarly in all type of cheeses while significant higher levels of lactobacilli ($p< 0.05$) were detected in cheeses made with the addition of probiotic autochthonous strains, these latter showing a high viability (10^8-10^9 cfu/g of cheese) during the whole ripening period. The use of adjunct probiotic strains did not adversely affect acceptability of the cheeses. Cheese C1 obtained the maximum scores for flavor/taste at 30 days of ripening. This work has been funded by RAS (L.R. 7/2007).

E044

Gene expression profile of proteolytic system of *Lactobacillus casei* Zhang during the late stage of milk fermentation

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Lactobacillus casei Zhang is a probiotic bacterium isolated from koumiss in Inner Mongolia of China, which has been commercially used as starter in the manufacture of dairy products. To study gene expression profile of *L. casei* Zhang during growth in milk, a whole genome microarray was used. Compared to *L. casei* Zhang grown to late logarithmic phase in milk, 61 genes were significantly up-regulated (>5 fold) in stationary phase, whereas 26 genes were down-regulated. Collectively, these data showed that the majority of the identified genes was involved in the carbohydrate metabolism and energy production, followed by genes involved in nucleotide metabolism, inorganic ion transport, amino acid transport and metabolism, chaperone, etc. This study demonstrates the fundamental effects of cultural conditions on the transcriptome of *L. casei* Zhang. Moreover, it improved the understanding of growth and survival mechanism of the bacterium during the late stage of milk fermentation.

E045

Tyramine production of enterococci under different pH, NaCl concentration and incubation temperature

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The genus Enterococcus comprehends of lactic acid bacteria (LAB) present in several fermented food in which they play a controversial role. In fact, they can produce tyramine in high amounts thanks to the most efficient tyrosine decarboxylase system among LAB, which is considered a species characteristic for *Enterococcus faecalis* and *Enterococcus faecium*. The decarboxylation activity depends on several environmental factors and on the efficiency of the cytoplasmic enzymes involved (i.e. tyrosine decarboxylase (TyrDC), tyrosine permease (TyrP) and tyrosyl-tRNA synthetase (TyrS)).
In this work the tyramine production of three enterococci (*Ec. faecalis* EF37, *Ec. faecalis* EF29212 and *Ec. faecium* EF12) was analysed under different conditions. These strains were previously studied for their ability to produce tyramine in rich (BHI) and limiting (Bover-Cid and Holzapfel medium) conditions. Cells at the end of exponential phase were collected and resuspended in a citrate buffer at a concentration of about 8.3 Log cfu/ml and the production of tyramine and cell viability were monitored over a period of 48 h. The conditions tested included pH (from 4 to 7), NaCl concentration (from 0% to 15%) and incubation temperature (from 20°C to 45°C). Under the same conditions, also the tyramine produced by a purified commercial TyrDC was monitored.
The results showed that the tyramine production seems to be regulated by cell capacity to intake tyrosine for the cytoplasmic decarboxylation rather than by the efficiency of TyrDC.
Among the considerate variables, temperature was the most influencing factor on tyramine accumulation for enterococci cells.
This study provides important information about the strategies that could be adopted in order to control tyramine accumulation in fermented foods.

E046

L-threonine catabolism to glycine in *Lactococcus lactis* IL1403

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The catabolism of threonine in lactic acid bacteria (LAB) has brought about interest due to the fact that this amino acid can be degraded to acetaldehyde, an important flavor component in dairy products, and glycine by threonine aldolase (TA) [1]. The model organism *Lactococcus lactis* subsp*. lactis* IL1403 is known to produce glycine from glucose or serine [2] and the existence of a TA in this LAB has not been verified.
We carried out continuous cultivations (D-stats) in 3 parallels at 0.2 h^{-1}, where we changed the concentration of L-threonine in the medium from 1.2-4.1 mM. This led to an increase in L-threonine utilization and glycine production, whereas the consumption of L-serine decreased and the expression of the protein encoded by *glyA* (responsible for degradation of L-serine to glycine) was not significantly increased. These results hinted that when L-threonine is abundant in the environment, *L. lactis* IL1403 prefers the latter over L-serine for glycine biosynthesis and that a TA is probably responsible for the conversion. Irregardless, the production of acetaldehyde was multiple times lower than the production of glycine. Acetaldehyde can be converted to ethanol by alcohol dehydrogenase (AdhE), but the production of ethanol was constant throughout the experiments and the expression of AdhE was decreased.
Herewith we hypothesized that there might be an alternative glycine biosynthesis pathway present in *L. lactis* IL1403, that is initiated by threonine dehydrogenase [3]. Additional analyses to prove this hypothesis are currently in progress.

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E047

Engineering functionality of complex starter cultures

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The use of starter cultures is an essential aspect in cheese manufacturing. Starter cultures may consist of single strains or mixtures of different strains and species of mainly lactic acid bacteria. An undefined mixture of different strains and species can be regarded as a complex ecosystem. Starter culture “Ur”, used for the production of Gouda cheese is an example of a complex starter culture and it contains 7 genetic lineages of *Lactococcus lactis* and 1 of *Leuconostoc mesenteroides*. Complex ecosystems are known for their stability in composition, even during the process of back-slopping, where new milk is inoculated with whey from the previous batch.
We set out to investigate the compositional stability of starter culture Ur during propagation at different temperatures. In addition, Ur was reconstituted from the 8 defined genetic lineages in 1:1 starting ratio. Propagation was carried out in fat free milk and the inoculated milk was incubated at 20˚C, 25˚C and 30˚C. These temperatures were selected to possibly favor strains that have different optimum growth temperatures. After 28 transfers (~200 generations) the composition of Ur and the reconstituted mixtures was determined using qPCR with lineage specific primers. Sequential propagation of Ur in milk at different temperatures induced changes in the relative abundance of different genomic lineages, but none of the lineages were lost.
We also demonstrated that reconstituted Ur, in comparison with original Ur, leads to a similar end-point composition after 28 transfers as Ur. This demonstrates the robustness of the original Ur culture and suggests interactions between the different lineages.

E048

Synthesis of fucosylated oligosaccharides using crude enzyme extracts from *Bifidobacterium longum* subsp*. longum* RD 47

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Human milk oligosaccharides (HMOs) are mostly fucosylated at their non-reducing termini. It has been proposed that HMOs function as a prebiotic for bifidobacteria. New *Bifidobacterium* strain (*B. longum* RD47) has been isolated whose cell extracts can synthesize fucosylated oligosaccharides from lactose and fucose as substrates. The synthesized oligosaccharides have been analyzed by thin layer chromatography (TLC) and mass spectrometry. To improve the production yield of fucosylated oligosaccharides, the effect of the medium composition on the production of enzymes from *B. longum* RD47 was evaluated and the oligosaccharide synthesis reaction conditions were optimized. Furthermore, the related genes have been cloned from the genome of *B. longum* RD47 and overexpressed in *E. coli*. The synthesized fucosylated oligosaccharides may provide a new constituent for the HMOs and novel prebiotic food ingredient.

E049

Physiological characterization of potentially probiotic lactic acid bacteria isolated from olives: Evaluation of short chain fatty acids and analysis of extracellular proteomes

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Probiotic strains may exert positive effects on human health by various mechanisms, among which the production of bio-active metabolites, including short chain fatty acids (SCFA). All the SCFA, mainly acetic, propionic and butyric acid, display beneficial effects on human health; butyric acid is the most interesting for its role in the prevention and treatment of colonic diseases.
In this study the ability to produce SCFA, directly or indirectly through the production of lactic acid, by 17 potentially probiotic lactic acid bacteria was investigated. Among them, 14 strains are *Lactobacillus plantarum*, 1 is *Lactobacillus pentosus* and 2 are *Leuconostoc mesenteroides*. Propionic and butyric acids were quantified by gas chromatography; acetic and lactic acids were quantified by specific enzymatic kits. All the tested strains displayed the ability to produce significant amounts of acetic and lactic acids (in the range of g/L) and just small amounts of propionic and butyric acids (in the range of mg/L).
The extracellular proteomes of the most promising strains, *Lb. pentosus* S3T60C and *Lb. plantarum* S11T3E, were evaluated by coupling 2-DE and MALDI TOF-TOF mass spectrometry. This is an interesting approach to explore a probiotic strain, since secreted proteins represent the first contact between bacteria and the host after ingestion. Six and seven proteins, in different isoforms, were identified from *Lb. pentosus*/S3T60C and *Lb. plantarum* S11T3E, respectively. All of them have a predicted extracellular location, indicating the effectiveness of the used protocol. Interestingly both the strains secrete an extracellular transglycolase, potentially involved in the production of exopolysaccharides, and several proteins involved in cell wall renewal. Moonlighting proteins are not present, except for a small amount of glyceraldehyde 3-P dehydrogenase secreted by *Lb. plantarum* S11T3E.

E050

Matching genome and transcriptome of *Lactococcus lactis* strains with robustness towards industrial stresses

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Lactococcus lactis is industrially employed to manufacture various fermented dairy products. The most cost-effective method for preservation of *L. lactis* starter cultures is spray drying, but during this process cultures encounter heat and oxidative stress, typically resulting in low survival rates. However, viability of starter cultures is essential for their adequate contribution to milk fermentation, supporting the ambition to better understand and improve their robustness phenotypes.
We have measured the ability of 39 *L. lactis* strains to survive these industrially relevant stresses. This set of strains contained different *L. lactis* subspecies and strains from dairy as well as plant origin were included. The observed 4-log variation in heat and oxidative stress survival was compared with genomic content, resulting in the identification of genes associated with robustness.
Besides presence, also activity of genes can play an important role in the observed diversity in robustness. Therefore, four *L. lactis* strains (MG1363, IL1403, KF147 and SK11) with varying robustness phenotypes were fermented under twelve different conditions, varying in temperature, salt concentration, pH and level of oxygen. Cells were harvested at exponential phase of growth for transcriptome analysis and stress survival measurements. The varying growth conditions resulted in up to 4-log differences in robustness towards heat and oxidative stress. Moreover, clear differences in gene expression profiles were observed. Correlation of robustness phenotypes and gene expression levels revealed transcriptome signatures for oxidative and heat stress survival. For strain MG1363 this included the *metC-cysK* operon, involved in methionine and cysteine metabolism, which triggered us to grow this strain in the absence of cysteine, resulting in elevated expression levels of the *metC-cysK* operon and concomitant enhanced robustness towards oxidative stress.

E051

Metabolic modeling of a single *Leuconostoc mesenteroides* and seven *Lactococcus lactis* strains identifies the possible metabolic dependencies within a complex bacterial starter culture.

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Many starter cultures are multistrain blends of different lineages of (lactic acid) bacteria used in a variety of (food) fermentations. These multistrain starters are known for their high robustness of the culture performance during industrial processes, when compared to single or defined cultures. The metabolism of these lactic acid bacteria is well understood, yet there is a lack of understanding the microbial interactions between the strains in a culture and whether these interactions play a role in the increased robustness of the culture performance. In this study, a genome-scale metabolic modeling approach was conducted on a representative collection of strains originating from the Ur cheese starter culture. The goal of the study was to identify metabolic dependencies between the strains (a single *Leuconostoc mesenteroides* and seven different *Lactococcus lactis* strains) in the culture. The models were obtained by reconstructing a genome-scale metabolic model on the basis of the genome annotation, followed by a manual curation step. Flux balance analyses (FBA) were performed on different combinations of these models using PySCeS. The nutrient composition of milk was used as medium composition input. The FBA predicted an increase in overall biomass when the complete repertoire of strains are modeled in the same system. Furthermore, optimizing the ratio of thedifferent lineages of lactic acid bacteria in the model does not result in an exclusion ("washout") of one of the strains, indicating a complex metabolic dependency between the strains.

E052

Fructose induces gene expression of mannitol 2-dehydrogenase of *Lactobacillus reuteri* CRL1101

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Mannitol, a polyol produced by certain heterofermentative lactic acid bacteria, has multiple industrial applications, being mainly used as natural sweetener in the food industry due to its low-caloric, low-glycemic, and anti-cariogenic properties. Under certain culture conditions these microorganisms may use fructose as an alternative electron acceptor to produce mannitol using the mannitol 2-dehydrogenase (MDH) enzyme. We aimed to investigate the effect of fructose on mRNA *mdh* expression, MDH synthesis and activity, as well as mannitol production by *Lactobacillus reuteri* CRL1101. The strain was grown in a chemically defined medium (CDM) with 2% glucose and 5% fructose (CDM_F) as carbon sources at 37 °C for 24 h. CDM with 7% glucose (CDM_G) was used as control. Cell growth (CFU/mL), pH, mannitol production and residual sugar concentration (HPLC), *mdh* gene expression (qPCR), MDH synthesis (2D-PAGE) and activity (spectrophotometrically) were determined. *Lb. reuteri* CRL1101 produced 20 g mannitol/L in CDM_F after 24 h-incubation. Although no mannitol was detected in CDM_G, basal MDH activity values were 0.5 ± 0.1 U/mg prot at all assayed time points were obtained. In presence of fructose, a 6-fold increase in MDH activity (3.0 ± 0.2 U/mg prot) was found after 8 h-incubation compared to the control. Interestingly, *mdh* gene expression was 55-fold higher in CDM_F than in CDM_G at the same incubation time. This observation is consistent with differential MDH expression as a clear proteomic spot at 8 h-incubation in CDM_F but not in CDM_G was detected. Our findings demonstrate that the presence of fructose highly induces *mdh* gene and protein expression in *Lb. reuteri* CRL 1101.

E053

Control of biogenic amine production by *Enterococcus faecalis* and *Streptococcus thermophilus in vitro* through the use of *Lactococcus lactis* bioprotective cultures

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Histamine and tyramine are the biogenic amines (BAs) mostly implied in food poisoning and can be formed through histidine and tyrosine decarboxylation by several bacteria. Among the strategies to contrast BAs accumulation, the use of competitive microbial cultures can be proposed. In dairy environments this role can be played by lactococci, known as producers of antimicrobial substances, which can exert antimicrobial activity against a wide spectrum of bacterial species.
In this study the *Lactococcus lactis* strains LCCG27, LCEG46 and LCN17 were tested for *in vitro* competition against the tyramine producer *Enterococcus faecalis* EF37 and the histamine producer *Streptococcus thermophilus* PRI60. The lactococcal strains were shown to produce bacteriocin-like compounds in solid media. Genetic data determined that LCN17 produced nisin Z, LCEG46 produced lacticin 481, while the third strains did not possess any of the genes coding for the most common lactococcal bacteriocins. This finding suggested that *L. lactis* LCCG27 produces a new bacteriocin.
In particular, the three lactococci were inoculated separately at a level of about 7 Log CFU/ml in medium added with 0.05% (w/v) of tyrosine or histidine with different inoculums of EF37 or PRI60 (from 2 to 6 Log CFU/ml) and incubated at 30°C. The samples were analyzed for BAs concentrations and bacterial growth was modeled. Also the growth and BAs production of decarboxylase-positive strainscultured alone were monitored.
The results showed that, in co-cultured samples, BAs accumulation was reduced with respect to control samples, depending on initial inoculum and strains and, in general, lactococci exerted a greater inhibiting activity against *S. thermophilus* PRI60. These data confirm the great potentiality of bioprotective *L. lactis* strains for controlling the growth and activity of decarboxylase-positive strains.

E054

Development of allergen free total nitrogen sources for biomass production of lactic acid bacteria

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Lactic acid bacteria have very complex nutritional requirements, and have been cultured using components such as dairy and soy ingredients, as these provide the essential nutrients for their growth. Since many of these bacterial cultures are used in food applications, allergenic media components are not desirable as this reduces the number of customers.
The purpose of this study was to develop allergen free medium formulations for *L. acidophilus*, *L. lactis* and *L. bulgaricus*. Various yeast extracts with different degrees of processing were combined with peptones derived from non-allergenic sources such as pea, cotton, rice and corn. Initial medium development was performed utilizing high throughput automated growth curve generation at a microtiter scale in BioScreen C. Acid production was also monitored in a Berthold LB940 plate reader in combination with a pH dependent fluorophore. The individual components as well as combinations of them that showed the best final OD, growth rate and acidification profiles were selected for second round of optimization, where their ratios were systematically changed and assessed for the same parameters. Finally the optimized mixtures were validated in pH controlled bench scale fermentors, and the number of CFU/mL was determined at the end of the fermentation. The results were compared to traditional allergen containing mediums.
Our results show that by optimizing a combination of yeast extract and peptone it is possible to utilize an allergen free media to cultivate these strains of Lactic acid bacteria. The allergen free medium's developed by Kerry gave comparable or superior growth when compared to medium utilizing an allergen containing nitrogen source.

E055

Comparison of lab-scale and industrial spray drying process for *Lactobacillus plantarum*.

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Common used process to produce dry lactic acid bacteria (used as starter cultures or probiotics) is the batch freeze drying process. Freeze drying allows a high survival rate and stability of the bacteria and a good scalability of the process. Due to high energy consumption and the complex design of a freeze drying facility, many efforts were undertaken to find other ways of drying lactic acid bacteria (e.g. fluidized bed, vacuum or spray drying). Especially spray drying is a cost efficient technique to produce heat sensitive products like skim milk or instant coffee powders in large amounts and can be also applied for lactic acid bacteria (LAB) drying. Most of the current available studies on LAB spray drying are based on lab-scale equipment. This type of equipment is very useful to show the feasibility of spray drying a product (physical properties). Under these conditions, high survival rates and good stability of LAB could be achieved. To determine the scalability of small-scale results in industrial scale, data from lab and production runs of a *Lb. plantarum* culture were investigated regarding cell count and survival.
Comparing cell counts (survival rates) from a commercial available spray dried *Lactobacillus plantarum* culture show differences between lab and industrial scale. Two different temperatures (inlet or outlet temperature) were kept similar in a lab-scale spray tower to compare survival rates reached in an industrial tower. In both cases much higher survival rates could be achieved under lab-scale conditions. We will present the impact of both spray drying scales on the survival of *Lb. plantarum*.

E056

Application of single and mixed cultures of *Lactobacillus rhamnosus* and *Enterococcus faecium* for L-lactic acid fermentation process

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Optically pure stereoisomers of lactic acid economically produced by large scale fermentation are required for the production of biodegradable polylactide (PLA). We applied single and mixed cultures of *Lactobacillus rhamnosus* and *Enterococcus faecium* for the fermentative production of L-lactic acid using glucose, fructose and xylose as single substrates or in combination. Fermentation medium contained (150 g×l^{-1} glucose, 125 g×l^{-1} glucose + 25 g×l^{-1} fructose, 50 g×l^{-1} D-xylose, 100 g×l^{-1} glucose + 50 g×l^{-1} D-xylose), yeast extract 5 g×l^{-1}, $MgSO_4 \cdot 7H_2O$ 0.2 g×l^{-1}, $MnSO_4 \cdot H_2O$ 0.05 g×l^{-1}, KH_2PO_4 2.6 g×l^{-1} and $CaCO_3$ (28 or 84 g×l^{-1}) as neutralizing agent. Fermentation process was carried out at 42^{o}C for 72h with gentle shaking (100 rpm). Biomass, reducing sugars, and lactic acid were estimated using HPLC or chemical methods. The optical purity of product was estimated by D and L lactate determination kit “Megazyme”. Biomass concentration in all cultures after 72 h of cultivation reached value from 2.46 to 4.00 g×l^{-1} being highest for *L. rhamnosus* grown in D-xylose and lowest for *E. faecium* in glucose medium. The best production of lactic acid (146.2 g×l^{-1} and 142.3 g×l^{-1}) was observed for *L. rhamnosus* in glucose+fructose and glucose medium, respectively. *E. faecium* produced 44.1 g×l^{-1} of lactic acid in D-xylose medium whereas *L. rhamnosus* only 21.7 g×l^{-1}. Mixed cultures of both strains produced less lactic acid than *L. rhamnosus* alone in all four sugar combinations. Glucose inhibited xylose metabolism in glucose + D-xylose medium. Purity of L-lactic acid isomer varied between 83.3 and 100% depending on sugar and strain combination. The work was partially financed by the project (POIG 01.01.02-10-123/09) of the EU within the European Regional Development Fund.

E057

Applicability of a *Lactobacillus amylovorus* strain as co-culture for obtaining a novel naturally bio-enriched in folate fermented milk

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The ability of 55 strains from different *Lactobacillus* species to produce folate was investigated. In order to evaluate folic acid productivity, lactobacilli were cultivated in the folate-free culture medium (FACM). Most of the tested strains needed folate for growth. The production and the extent of vitamin accumulation were distinctive features of individual strains. *L. amylovorus* CRL887 was selected for further studies because of its ability to produce significantly higher concentrations of vitamin (81.2 ± 5.4 µg/L). The safety of this newly identified folate producing strain was evaluated in healthy mice. No bacterial translocation was detected in liver and spleen after consumption of CRL887 during 7 d and no undesirable side effects were observed in the animals that received this strain. This strain in co-culture with previously selected folate producing starter cultures (*L. bulgaricus* CRL871, and *Streptococcus thermophilus* CRL803 and CRL415) yielded a yogurt containing high folate concentrations (263.1 ± 2.4 µg/L); a single portion of which would provide 14 % of the Recommended Dietary Allowance. This is the first report about the capacity of *L. amylovorus* to grow and produce folates, and it was successfully elaborated a yogurt naturally bio-enriched in folate.

E058

Use of lactic acid bacteria as a biotechnological strategy to increase riboflavin levels in soymilk

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Riboflavin (vitamin B_2) plays an important role in cellular metabolism participating in numerous oxidation-reduction reactions and energy usage. In this work, lactic acid bacteria that can produce vitamin B_2 in soymilk were identified from 179 strains tested that were previously isolated from a wide range of food products. Only 42 strains were able to grow in a commercial riboflavin-free medium after which the concentration of this vitamin was determined by HPLC. Five of these strains were pre-selected for their capacity to produce elevated concentrations of riboflavin. These were then inoculated in soymilk to evaluate their capacity to grow in this food matrix and increase its low riboflavin concentrations. Only *L. plantarum* CRL 725 was able to significantly increase (700 ± 20ng B2/ml) the initial concentration of riboflavin in soymilk (309 ± 19ng B2/ml) after 12h of incubation at 37ºC. Roseoflavin resistant variants of this strain were obtained and evaluated in soymilk. One of the obtained variants increased 6 times (1860 ± 20ng B2/ml) the initial riboflavin levels of soymilk. Roseoflavin-resistant strains capable of synthesizing riboflavin in soymilk constitute an interesting and economically feasible biotechnology strategy that could be easily adapted by the food industry to develop novel vitamin-bioenriched functional foods with enhanced consumer appeal.

E059

Online monitoring of lactic acid fermentation by means of Proton-Transfer-Reaction Mass Spectrometry

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Proton-Transfer-Reaction Time-of-Flight Mass Spectrometry (PTR-ToF-MS) is an analytical technique based upon the "soft"ionization of a gaseous matrix, followed by mass spectrometric separation, performed at high time and mass resolution. PTR-ToF-MS allows to monitor in non-invasive fashion the changes in volatile release induced by fermentation, as already shown for the lactic acid fermentation of milk. In the present work a matrix constituted by heat-treated (30 minutes at 80°C) low-fat milk was inoculated with different yogurt starters. For the first time, the reaction was monitored in automated fashion by means of a multifunctional autosampler, adapted to PTR-MS analysis. In a typical experimental setup, this allowed to follow four different experiments, with a time resolution of four sampling points/hour and minimal need for manual operation. Our preliminary results show the possibility to monitor the kinetics of production and depletion of volatile compounds of major importance in the manufacturing of fermented dairy products, both from technological and sensory point of view (e.g. acetaldehyde, pentanedione). This provides an example of the applicability of PTR-ToF-MS as a valuable tool for the rapid screening of lactic acid bacteria (LAB) strains of industrial interest.

E060

Sorbitol utilization by *Pediococcus parvulus* 2.6

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Pediococcus parvulus 2.6 isolated from cider secretes a 2-substituted (1,3)-β-D-glucan exopolysaccharide (EPS) synthetized by the GTF glycosyltransferase using UDP-glucose as substrate. The potential of *P. parvulus* 2.6 as a probiotic strain has previously been established. In this work, the ability of this bacterium to utilize sorbitol has been detected, its genetic determinants identified and its interplay with the 2-substituted (1,3)-β-D-glucan synthesis investigated.
Metabolomic analysis revealed that *P. parvulus* 2.6 can use sorbitol as a carbon source. DNA sequencing of the *P. parvulus* 2.6 genome and subsequent analysis showed the existence of a sorbitol utilization operon in a plasmid carried by this bacterium. The operon consists of six genes (*gutFRMCBA*). *GutF* encodes a sorbitol-6-phosphate dehydrogenase; *gutRM* genes encoding two putative regulators; and *gutCBA* genes encoding the proteins EIIC, EIIBC and EIIA which are components of a phosphoenolpyruvate-dependent sorbitol phosphotransferase system (PTSGut). Homology of these gene products with those of the *Lactobacillus casei gut* operon indicate that regulation of *gut* operon expression in *P. parvulus* could be carried out in a similar way to that described for *L. casei*.
Metabolomic analysis of *P. parvulus* grown in the presence of sorbitol and glucose revealed a co-metabolism accompanied by a diauxic growth. A inhibition ELISA assay was optimized to allow direct and specific quantification of 2-substituted (1,3)-β-D-glucan in culture supernatants. EPS quantification showed that indeed the 2-substituted (1,3)-β-D-glucan is synthesized when sorbitol was used as the only carbon source and that this synthesis is very efficient since a similar rate of production of the EPS was observed in cultures grown in media containing either glucose or sorbitol.

E061

Preliminary selection for potential probiotic *Bifidobacterium* isolated from subjects of different Chinese ethnic groups and evaluation of their fermentation and storage characteristics in bovine milk

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A total of 29 strains of *Bifidobacterium* were isolated from 18 samples of human feces in different ethnic mi-nority regions of China. All isolates were identified as *Bifidobacterium longum* (9 strains) and *Bifidobacterium pseudocatenulatum* (20 strains) based on 16S rRNA gene sequencing and phylogenetic analysis. These strains were preliminarily tested for their suitability to become probiotics by assessing their ability to survive adequately at low pH conditions and their tolerance of different concentrations of bile salts and simulated gastrointestinal juices. In vitro tests were sequentially used to predict the survival of these strains in the simulated conditions in the human gastrointestinal tract. These strains were first exposed to pH 2.5 for 3 h, and 7 out of the 29 strains were discriminated from the others by their high survival rates. Out of these 7 strains, 4 were found to grow and survive well at aneven lower pH of 2.0 and in high bile salt concentration. Apart from the gastrointestinal survival capacity, both fermentation efficiency and storage characteristics are important criteria for selecting for suitable potential probiotic strains. Therefore, the fermentation efficiency in bovine milk and the bacterial viability during the storage in the resultant fermented milk were also evaluated for these 4 selected strains. In this study, we isolated and identified 29 novel *Bifidobacterium* strains. Based on our initial evaluation, at least 4 of them may serve as valuable resources for further dairy probiotic strain selection.

E062

Genomics and transcriptomics comparison of two closely-related *Streptococcus thermophilus* strains

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The lactic acid bacterium *Streptococcus thermophilus* is an essential component of starter cultures that are used for the industrial production of yogurt and various cheeses such as emmental and mozzarella. A thorough selection process is usually required to identify the strains that will provide both the desired technological features within final products and the best yield and survival rate during biomass production. In this way, we recently noticed two genetically closely-related strains that displayed marked phenotypic differences. Whole genome sequencing using NGS technologies indicated a number of potential mutation events in coding sequences. To investigate which functions or pathways could explain these differences, we undertook a mutational approach, in which the impact of these potential genetic differences is evaluated. In addition, the regulation of gene expression was addressed by high-throughput RNA sequencing.

E063

Genetic characterization of *Weissella confusa* strains with different dextran producing capacities in cereal fermentations

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The use of lactic acid bacteria of the genus *Weissella*, and the species *Weissella confusa* in particular, has recently attracted interest as homopolysaccharide-producing starter cultures for different fermented foods. They are capable of producing the glucose polymer dextran from sucrose, which has been shown to lead to improvement in several technological and organoleptic characteristics in fermented cereal ingredients used in bakery products. They also produce a relatively low level of organic acids during fermentation, which permits a wide array of potential applications.
W. confusa strains vary greatly in their ability to produce dextran in a given set of fermentation conditions even though their dextransucrase enzymes - solely responsible for dextran biosynthesis - have so far shown high homology. In addition, dextran production levels of a strain can vary significantly between different fermentation matrixes.
We sequenced the genomes of three *W. confusa* strains with different dextran-producing properties in cereal bran fermentations to look for divergence in genetic elements. The genome information also facilitates subsequent transcriptional analysis to study gene regulation patterns taking place in both favorable and unfavorable conditions for dextran production. Preliminary transcriptome profiling in simple laboratory model conditions will be conducted for the strains and relevant findings will be discussed.

E064

Screening high-yield acid *Lactobacillus helveticus* strain and optimization of process conditions

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The major gastrointestinal medicines lactobacillin tablet has been selling for over 30 years in China. The annual output of lactobacillin tablet is 10 billion tablets, and the output value is over €300 million. However, in recent years the production of lactobacillin by the constraints of the following problems: lower production of lactic acid, slower rate of acid production, instability production between batches, and inefficient use of substrate. The high-quality strains of KLDS culture collections were used, and the logistic mathematical evaluation model was used for screening the high-yield lactic acid strains of *Lactobacillus*. The optimum conditions were explored to improve the substrate conversion rate and production efficiency. With the rate of acid production and pH changing as indicatory, five high and fast acid productions of the strains were obtained. The best process conditions of high-yield lactic acid bacteria prime fermentation were as follows: the third generation of the strain was inoculated into 20% skim milk, and the inoculation amount was 3% (v/v). The skim milk was incubated at 42°C and natural pH for static fermenting 20h. The acid production was reached 415ºT.

E065

Preliminary animal trial for a potential live vector vaccine against avian influenza virus using *Lactococcus lactis*

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Constant progress is being made in applying lactic acid bacteria (LAB) for production of heterologous proteins of therapeutic and prophylactic properties. Due to their GRAS status, LAB are of substantial interest for implementation in various research domains, including vaccine development. *Lactococcus lactis* is a model LAB bacterium, which has been shown to efficiently produce heterologous proteins of different origin, e.g. viral, bacterial, mammalian. It is also considered as an attractive antigen delivery vector for immunization by mucosal routes, and a good alternative to commonly applied attenuated pathogens.
The present study describes the use of *L. lactis* strains producing avian influenza A (H5N1) virus main antigen (H5) or its derivative forms in evoking specific immune response in birds. For this, recombinant *L. lactis* cells were injected *in ovo* or orally administered to hatched chicken. To optimize the anticipated response, we assayed different parameters, such as dose of bacterial preparations, administration schedule (i.e. boosters) or strain type. Control chickens were given preparations containing *L. lactis* strain carrying the empty vector. Sera were collected at different time points proceeding vaccination and examined by ELISA method to assess IgY and IgA levels. Our preliminary study demonstrates that oral delivery of certain recombinant strains using specific vaccination schemes may elicit mucosal immune responses in chicken. Results revealed a relation between the observed response and the type of recombinant *L. lactis* cells as well as the vaccination schedule.
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E066

Contribution of the novel sulphur producing adjunct, *Lactobacillus nodensis*, to flavour development in Gouda cheese

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The influence of the aroma-producing adjunct strain, *Lactobacillus nodensis* on the production of sulphur-containing compounds in Gouda-type cheese was evaluated. Four Gouda cheeses were produced using the following starter systems; a direct vat inoculum (DVI), a DVI with the added adjunct *Lb. nodensis*, a bulk starter culture, and a bulk starter culture with the added adjunct *Lb. nodensis*. No significant variations were observed in any of the Gouda cheeses in terms of moisture, fat, and pH during the ripening process. Seven sulphur containing compounds were detected in Gouda cheese with and without the adjunct strain. However, elevated levels of sulphur compounds were detected in Gouda cheese containing the adjunct, *Lb. nodensis*. The sulphur aroma compounds, hydrogen sulphide, (H_2S), dimethyl disulphide (DMDS) and dimethyl trisulphide (DMTS) were present in higher abundance in Gouda cheese containing *Lb. nodensis*. Principal component analysis (PCA) confirmed that DMDS, dimethyl sulfone, H_2S, and methanethiol were highly associated with cheese containing the adjunct, while DMS and dimethyl sulfone were associated with control cheeses. High levels of the volatile compound, (H_2S) were associated with cheeses containing the adjunct. Further analysis using lead acetate paper as an indicator for H_2S production confirmed that *Lb. nodensis* is able to produce H_2S in broth and within the cheese matrix. These data confirm that *Lb. nodensis* is able to produce numerous volatile sulphur compounds, including hydrogen sulphide. This suggests that the inclusion of *Lb. nodensis* as an adjunct culture has the potential to significantly alter the flavour profile of the final cheese product.

E067

Challenges in the viability assessment of commercial lactic acid bacterial cultures by flow cytmetry and standard plate counting

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Quantifying lactic acid bacteria, bifidobacteria, and propionibacteria using flow cytometry and distinguishing viable from non-viable cells can be extremely useful for culture optimization at lab scale, scale up in Pilot, and eventual commercialization in Production. In fact, use of flow cytometry to follow viability of the culture throughout the manufacturing process can be used to fine tune and adjust the process so that it is better controlled and will result in a more consistent, reliable, and higher quality product. In order for flow cytometry to be used as an effective tool for culture and process optimization, it is essential that the flow cytometer methodology is validated for each strain to have confidence in the data used to make process improvements. Examples are provided where results from flow cytometry are consistent and where results are not comparable with standard plate counts. The underlying potential causes are discussed and recommendations are presented.

E068

Diversity and evolution of bacteriophages in wheys derived from an undefined mixed DL-starter culture

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Compared to single strain or defined mixed cultures it is difficult to measure phage attack on DL-starter cultures precisely, since they are composed out of an unknown number of *Lactococcus lactis* and *Leuconostoc* strains with different impact on acidification, flavor, and texture in fermented milk products. To follow phage propagation and phage evolution in a dairy, 192 isolates from DL-starter culture A were phage-typed with whey as well as bulk starter samples in a high-throughput screening approach. Phage species were identified by a PCR method in combination with phage quantification by qPCR. Phages were furthermore distinguished by comparing the 936 receptor binding proteins by PCR-DGGE. Samples were taken over a 5-year period.
Although up to 75% of the single strain isolates were tested phage positive towards the wheys, inhibition levels on the whole culture A was negative or only weak positive. A high phage load against the single isolates was also found in bulk starter samples.
Metavirome analysis showed that the majority of the phages belonged to the 936 species, while P335 and c2 type phages were present with less than 5%.
The qPCR analysis of 936 phages revealed a phage titre between 1e08 and 1e10 in the majority of the bulk starter samples, whereas the phage titre in the whey samples was typically one to two logs lower.
Considering that the dairy did not report specific acidification problems using starter A the result indicates a surprisingly good phage resistance, even when the general phage level was very high and the majority of the single isolates found to be phage attacked.

E069

Co-cultivation of EPS synthesizing *Weissella* and *Propionibacterium* spp. for bread shelf life extension

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The shelf life of bakery products is limited by physical changes and microbial deteriorations resulting in high product losses. Exopolysaccharides (EPS), and antimicrobial metabolites produced by the trophic chain of lactic acid bacteria (LAB) and propionic acid bacteria (PAB) yielding acetic and propionic acid can improve bread quality. The aim of this study was to select EPS producing LAB and PAB to develop a co-cultivation process to extend the shelf life of bread.
Weissella confusa 11GU-1 and *Propionibacterium freudenreichii* JS15 were chosen for co-cultivation. *W. confusa* produced a high molecular weight dextran with low degree of α-(1,3)-branching and synthesized gluco-oligosaccharides (GOS) in the presence of maltose. The dextranscurase was constitutively expressed, partial sequencing of the respective gene evidenced high genetic conservation of *Weissella* dextransucrases. In addition, *W. confusa* formed a capsular polysaccharide consisting of glucose, two unidentified components and O-acetyl groups. *P. freudenreichii* produced a heteropolysaccharide composed of glucose, mannose and glucuronic acid. A co-cultivation process of *W. confusa* and *P. freudenreichii* was developed in semi-defined malt based medium supplemented with sucrose, and was optimized for EPS, acetate and propionate production. At an addition level of 15% (w/w) flour base - corresponding to 1.5 g EPS, 0.5 g acetate and 1 g propionate per kg dough - this co-culture ingredient delivered antimicrobial metabolites in the active concentration range for antifungal effects and resulted in breads with significantly softer fresh crumbs and reduced staling rate during storage.
The developed process allowed simultaneous production of GOS and EPS together with high levels of acetate and propionate in a single fermentation. LAB and PAB co-cultivation thus delivers beneficial microbial metabolites to improve the quality of bread.

E070

Use of a nisin-producing *Lactococcus lactis* strain, combined with thyme essential oil, to improve the safety and shelf-life of minimally processed lamb's lettuce

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In recent years the number of foodborne outbreaks linked to minimally processed vegetables has increased dramatically. Although chlorine is the most common decontaminant used in these products, it is quite ineffective in reducing pathogens on vegetables at the concentration used. The drawbacks of chlorinate sanitizers have stimulated the investigation on alternatives. In this context, the use of lactic acid bacteria (LAB) as biocontrol agents represents a good alternative, and numerous LAB have been identified as bioprotective agents, also due to their ability to produce bacteriocins. Furthermore the use of natural antimicrobials has been proposed as alternative to the traditional sanitization methods.
The purpose of this study was to evaluate the potential application of the nisin-producing *Lactococcus lactis* CBM21 on the safety and shelf-life of minimally processed lamb's lettuce combined or not with thyme essential oil (EO). Challenge tests in the presence of *Listeria monocytogenes* and *Escherichia coli* were also performed to assess the effects on product safety. The biocontrol agent, thyme EO, and pathogens were added in the lamb's lettuce washing solution. The products were subsequently stored at 6°C. During storage, microbiological, color and sensory analyses were performed.
Data obtained highlighted the good performance of strain CBM21, combined or not with thyme, to inhibit both the inoculated pathogenic species and the total mesophilics. The addition of the biocontrol agent did not affect the quality parameters of lamb's lettuce. These results suggest that the considered alternative "hurdles" can represent a new strategy to ensure the safety and quality of minimally processed vegetables.

E071

Biotechnological production of D-lactic acid - Specific supplementation and process optimization

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Polylactic acid (PLA) provides an ecological and economical alternative to petrochemical plastics. It is biodegradable and can be produced biotechnologically from renewable resources. This makes PLA to one of the most promising biopolymers which is already widely used in the packaging and food industry. However, the applications are limited by the thermal stability of PLA. An improvement is provided by the isotactic stereocomplex of poly-L-and poly-D-lactate, increasing the melting point. For this reason, there is a high demand for optically pure D-lactic acid. Therefore the development of a highly productive and cost-effective biotechnological process is necessary.
The most cost-intensive components are the nitrogen source and the carbon source, such as yeast extract and glucose. Yeast extract used as a complex nitrogen source in the majority of biotechnological processes is expensive and influences the cultivation in an undefined way. In this study, various yeast extracts, hydrolysates from renewable resources and molasses are completely analyzed for their composition. A statistical evaluation of the analytical data in combination with cultivation experiments of two *Sporolactobacillus* stains should give an insight into the nutritional requirements of the lactic acid bacteria. In this way, the optimal medium composition can be achieved through specific supplementation.
For this purpose, various parameters have to be examined, such as optimal pH values, growth temperatures and carbon source concentrations in order to determine the ideal cultivation conditions of the lactic acid bacteria and to establish a reproducible experimental procedure for further studies.

E072

Development of a fermented quinoa-based beverage with health-promoting properties

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Quinoa is a crop originated from the Andes. It has a high nutritional value, outstanding agro-ecological adaptability and low water requirements. Quinoa is an excellent crop alternative to help to overcome food shortages, and it could also have a role in the prevention of developed world lifestyle diseases, such as type-2 diabetes, cardiovascular diseases, osteoporosis, inflammatory and autoimmune diseases, etc. In order to expand the traditional uses of quinoa and to provide new, healthier and more nutritious food products, a fermented quinoa-based beverage with potential probiotic bacteria was developed. Two quinoa varieties (Rosada de Huancayo (RH) and Pasankalla (PK)) were studied. Technological aspects such as the fermentation process, prevention of syneresis, and the bacterial stability and viability were monitored. Both quinoa varieties proved to be suitable as base for fermented products. However, the product made from PK flour had higher protein concentration, viscosity and bacterial exopolysaccharide content than the product made with RH flour. These results suggest that the differences between quinoa varieties may have substantial effects on food processes and on the properties of final products. This study describes a protocol for the preparation of a quinoa-based beverage fermented with potentially probiotic bacteria. The product would be a good source of protein, fibre, vitamins and minerals, and contribute to a balanced microbiota due to the potential probiotic bacteria. The beverage and related products could be excellent snacks for the coeliac and lactose intolerant population, and also suitable as a novel and exotic alternative to consumers in general.

E073

Screening for lactic acid bacteria active against piglet-pathogenic *C. perfringens* strains

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526 LAB strains were isolated from the upper and lower gastrointestinal tract of the piglets, and from faeces obtained through gastrointestinal stomata. It was established that the isolated strains are characterized by high specificity of action and a narrow range of activity anti-*C. perfringens*. More than a half of the isolates exerted the antagonistic activity towards only one *C. perfringens* toxotype. On average, only one in every three LAB isolates acted antagonistically against two *C. perfringens* toxotypes; and only one in every ten - was active against three *C. perfringens* toxotypes. Most of LAB species belonging to the native intenstinal microbiota of piglets exhibited weak antagonistic activity towards *C. perfringens*. Only 10 of the isolates displayed strong antagonistic effect on *C. perfringens*. The detected antagonistic activity was attributed to both competition as well as synthesis of exogenous metabolites, mainly organic acids and bacteriocins. The strains with the strongest activity towards *C. perfringens*, represented 6 species: *E. faecium*, *E. hirae*, *E. avium, L. mesenteroides* and *C. divergens*. Amongst the identified strains, the strongest antagonistic activity towards *C. perfringens* and the broadest activity range was exhibited by *L. mesenteroides* strains. All of the most active against *C. perfringens* strains were devoid of antibiotics-resistance, unable to produce hemolysins, and were characterized by strong adhesive properties. Furthermore, the strains were highly resistant to acidity and bile salts, resulting in high survival rate during gastrointestinal transit, and fulfilled most of the criteria for probiotic microorganisms. Three strains with the most beneficial physiological and technological properties were selected for further use as components of a novel eubiotic preparation for piglets.

E074

Characterization of the microbial populations in atole agrio, a traditional Mexican fermented beverage

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In Latin America, fermented foods are an important part of the diet. These foods are made of plants or animal materials by spontaneous fermentation driven by the microbiota naturally present in raw materials. Fermentations are cost-effective ways to improve the microbiological safety, quality, nutritional value and organoleptic properties of perishable materials. However, these processes should be controlled and predictable. This could be accomplished by using defined starters together with the spontaneous fermentation. The aim of this study was to characterize the microbial populations of a traditional fermented Mexican beverage, atole agrio. Atole agrio is prepared at ambient temperature through a 6-12 hour spontaneous fermentation of maize and water. After fermentation the end product is filtered and boiled to achieve creamy texture. There is no previous publication on the fermentation of atole agrio and thus this research provides the first results. Total mesophilic microbes, LAB, yeasts, molds and Enterobacteriaceae were measured by plating technique from start materials, during fermentation and from end products, as well as the pH. The results showed relatively low levels of LAB (6.7 log cfu/ml) throughout the fermentation. The average level for total mesophilic bacteria was 8.3, yeasts and molds 6.9 and Enterobacteriaceae 5.8 log cfu/ml. The pH decreased from 7.5 to 4.5. Due to the short period of fermentation, the microbial community remains relatively stable. However, increasing the initial levels of LAB could alter the total microbial community and improve the organoleptic properties of the product. The research will continue by identification and characterization of the isolated LAB strains and applying the LAB strain embossing the best growth properties as a starter to improve the organoleptic properties of atole agrio.

E075

Activity of lactic acid bacteria against pathogenic microorganisms prevalent in farm animals in Poland

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Introduction. Most probiotic strains occur among lactic acid bacteria, particularly *Lactobacillus, Bifidobacterium* and *Enterococcus*. These bacteria are part of the normal intestinal microflora of animals and can be used as probiotics. Their biological activity is closely linked with the ability to produce bioactive metabolites.
Aim. The aim of this study was to isolate active probiotic bacteria strains characterized with targeted pro-health action against pathogenic strains of *Clostridium perfringers* and *Escherichia coli*.
Materials and methods. The screened bacterial isolates were obtained from the upper and lower gastrointestinal tract of piglets, calves and from poultry manure. Their activity against pathogenic strains of *E. coli* and *C. perfringers* indicators, specific for each animal group, were determined. Screening was performed by measuring the diameter of the clearing zones around the isolates deposited on the agar medium inoculated with bacteria indicator. The next step of research was indication the level of adhesion and hydrophobicity of LAB cultures.
Results. We examined 1267 lactic acid bacteria cultures and 480 yeast cultures collected from upper and lower gastrointestinal tract of piglets, calves and from poultry manure. It was found that LAB colonizing gastrointestinal tract of piglets and piglets have a high specificity of action and narrow spectrum of activity against *C. perfringers* and *E. coli*. The level of adhesion and hydrophobicity was high in those groups of animal too.
Conclusions. Lactic acid bacteria have been identified in the gastrointestinal tract of piglets, calves and in poultry manure, which bacteria can be used as an effective probiotic for each group of animals and can become healthy alternatives for antibiotic additives.

E076

Temperate bacteriophages induced from an undefined mixed DL-starter culture

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Bacteriophages (phage) attacking starter cultures cause serious problems in dairies. Bulk starters are among the potential sources of phage problems in dairy production facilities. New phages may be introduced in to dairies via bulk starters in the form of temperate phages released either spontaneously at lower frequencies or in the presence of certain inducers. We present work on spontaneous induction of phages and induction by Mitomycin C from an undefined mixed DL-starter culture and induction by Mitomycin C from 192 isolates of same starter culture using a high-throughput screening approach. We characterized the induced phages by high-throughput screening of their abilities to infect individual indicator host strains, by electron microscopy and by high-throughput sequencing combined with a metavirome sequencing approach. Our data show that approx. 17 % of isolates from the DL-culture could be induced by Mitomycin C and that the complete induced DL-starter culture predominantly contains lactococcal P335 type phages. Other types of phages have also been occasionally detected.

E077

Thermal stability of *Lactococcus lactis* bacteriophages: evaluation of phage inactivation in a pilot plant pasteurizer

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Bacteriophages are a frequent cause for fermentation problems in dairies. When bacteriophages infect lactic acid bacteria, slow or incomplete fermentation may occur resulting in significant financial losses. It has previously been shown that phages of *Lactococcus lactis* starter strains may reveal a remarkably high thermal stability under laboratory conditions (i.e., in 1.5-ml stainless-steel test tubes in a water bath), and the most resistant phages were still detectable in skim milk after heating for 5 min at 95°C and 97°C, respectively [1].
The aim of this study was to determine the thermal inactivation of lactococcal phages suspended in high titers in raw milk using a pilot plant pasteurizer (sample volume: 30 l, continuous flow principle). Phages of two phage species with highest thermal stability (small isometric-headed phages P680 and P1532 of the 936 phage species, prolate-headed phage P635 of the c2 species) were included, in addition to the heat-sensitive reference phage P008. The titer of the later phage decreased after heating for 25 sec at 75°C by 5 log units, whereas phages P635, P680, P1532 required for significant inactivation (≥ 99.99% after 25 sec heat treatments) temperatures of 80°C (6-log units reduction), of 95°C (5-log units reduction) and of 97.5°C (4-log units reduction), respectively. Thus, thermal inactivation of phages in the pilot plant pasteurizer is notably more efficient than treatment under laboratory conditions used for screening of phages with high thermal resistance. However, even under pilot plant conditions, high temperature & short time pasteurization does not appear to be a hurdle for temperature-insensitive lactococcal phages.

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E078

Susceptibility of wine lactic acid bacteria to zinc, silver and silver/copper nanoparticles

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The aim of this study was to evaluate the antimicrobial activity of nanoparticles of various metals on several species of lactic acid bacteria related to the winemaking process: *Lactobacillus brevis*, *Lactobacillus fructivorans*, *Lactobacillus hilgardii*, *Oenococcus oeni*, *Pediococcus damnosus* and *Pediococcus pentosaceus*. These nanoparticles could be an alternative to the sulphites in order to control the development of undesired wine microorganisms. The effect on bacterial growth of four different concentrations of zinc, silver and silver/copper nanoparticles was investigated. As the effect of antimicrobials depends on of the microorganism's concentration, we evaluated the action of nanoparticles over two different lactic acid bacteria concentrations of: 10^3 and 10^7ufc/mL. The results show that the silver nanoparticles and the silver-copper mixture did not allow the growth of any bacteria whatever the concentration tested. Zinc nanoparticle were unable to inhibit growth neither at 10^3 nor 10^7ufc/mL. From the results obtained, it seems possible that silver or silver/copper nanoparticles can replace the use of sulphites in wine, although it should be tested.

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E079

Effect of different ice-cream matrices on *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* Bb-12 viability and their survival to *in vitro* gastrointestinal stresses

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The use of non-dairy raw materials or ingredients with low lactose content has been encouraged for the development of probiotic food products. Whey protein isolate (less than 0.5% lactose) and soymilk may be alternatives for lactose intolerant individuals. Therefore, the effect of replacing milk (M) by soymilk (S) and/or whey protein isolate + inulin (PI) for the production of probiotic apple ice-creams on the survival of *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* Bb-12 in the product and under *in vitro* simulated gastrointestinal conditions was investigated throughout 84 days of storage at -18 °C. Employing a simplex-centroid mixture design, seven ice-cream-making trials were produced and supplemented with La-5 and Bb-12: T1: M; T2: S; T3: PI; T4: M + S; T5: M + PI; T6: S + PI; and T7: M + S + PI. On each sampling day (days 7, 42, and 84) the probiotic survival was investigated on 4 periods (0h, 2h, 4h, and 6h) under simulated gastrointestinal conditions. All ice-creams proved to be good vehicles for La-5 and Bb-12 and showed satisfactory populations during the whole storage period, remaining always above 7.4 log cfu/g. The Bb-12 survival under simulated gastrointestinal conditions was at least 1 log cycle higher than the La-5 survival, except in T3, which showed the lowest protective effect on both probiotic strains. However, the other mixtures may expand the range of probiotic products for individuals with varying degrees of lactose intolerance. In conclusion, the results show that gastrointestinal tolerance of probiotic strains can potentially be affected by the choice of the food matrix.

E080

Selection of *Lactobacillus* strains to induce biological acidification in low acidity wines

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In oenology, one of the consequences of global warming is the steady decline in acidity in musts and wines of many warm weather regions. That generates early crops with low values of total acidity and malic acid, and high sugar concentration. Musts with low acidity and high pH show disrupted important organoleptic properties, and are very susceptible to microbial spoilage. The most commonly used solution to this phenomenon is the chemical acidification with organic acids such as malic, tartaric and lactic acid. Another alternative is physical acidification by methods such as cation exchange resins or electrodialysis treatments. These strategies have disadvantages and are subject to strict legislation. However, the biological acidification in not legislated and it is also applicable to organic wines. The goal of this study was the selection of *Lactobacillus* strains, which show a high potential as biological acidification starters for winemaking. Different selection criteria were used such as ability to grow in must, carry out malolactic fermentation, must acidification, synthesis of lactic acid from sugars, resistance to lysozyme and sulfur dioxide, and biogenic amines' forming inability. Two strains have been selected and characterized which are suitable as starter cultures for biological acidification in low acidity wines. The use of these strains ensures the microbiological stability by lowering pH and providing a faster winemaking by early malolactic fermentation - a prompt stabilization of wines can be made just after the end of the alcoholic fermentation.

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E081

Development of multi-strain rye sourdough starter

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Sourdough is a mixture of flour and water that is fermented with lactic acid bacteria (LAB) and yeasts. Sourdough fermentation can be initiated with either spontaneous fermentation of raw materials or by inoculation with starter cultures. Starter LAB guarantee desired quality and properties of sourdough, but can be outcompeted by indigenous LAB during sourdough propagation at selected conditions (fermentation temperature and time, inoculum size and dough yield).
The aim of this study was to test performance of a "universal starter" composed from nine LAB strains isolated from mature industrial and laboratory rye sourdoughs with different technological parameters. Laboratory rye sourdough with high dough yield was started in three parallels. For a comprehensive overview both chemical (pH, total titratable acidity, production of lactic acid and utilization of carbohydrates) and microbiological properties (DGGE and pyrosequencing of partial 16S rRNA gene amplicons) of the sourdoughs were characterized during the experiment. It was observed that four out of nine introduced LAB strains (*Lactobacillus plantarum*, *Lactobacillus brevi*s, *Lactobacillus paralimentarius* and *Lactobacillus crustorum*) formed the dominant community in studied sourdoughs during at least 13 renewal cycles. Selection of LAB strains occurred already during the first fermentation cycle. Inoculated sourdoughs reached maturity over the first two cycles and their chemical parameters were more stable throughout the study compared to the spontaneously started sourdoughs. Also, lactic acid production was higher in the inoculated sourdoughs starting from the first fermentation step.

E082

Characterization of an anti-staphylococcal bacteriocin enterocin MK3

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In this study, a lactic acid bacterium which produces a bacteriocin inhibiting pathogenic *Staphylococcus aureus*, was isolated. Optimal conditions for the bacteriocin production were investigated, the partially purifiedbacteriocin was characterized, and finally the bacteriocin was purified. The lactic acid bacterium (MK3) was isolated from kimchi and it was identified as *Enterococcus faecium* by 16S rRNA gene sequencing and named *Enterococcus faecium* MK3. The bacteriocin named enterocin MK3 did not inhibit the growth of Gram-negative *Escherichia coli*, whereas it inhibited the Gram-positive pathogenic *Listeria monocytogenes* and *Staphylococcus aureus*. MRS broth, 25℃, and pH 6.0 under anaerobic condition were confirmed to be optimal conditions for production of enterocin MK3. Partially purified enterocin MK3 was stable at various pHs (2.0-10.0), heat (60-100°C), organic solvents (ethanol, methanol, acetonitrile, etc.) and enzymes (RNase A, S1 nuclease, Lysozyme, etc.) treatments. Enterocin MK3 showed bactericidal action mode against *Staphylococcus aureus*. Enterocin MK3 was purified by acetone extraction and HPLC as 94.9-fold. The molecular mass of the purified enterocin MK3 was approximately 4.0 kDa on a tricine SDS-PAGE gel. In the near future, it will be subjected to N-terminal amino acid sequencing and the corresponding gene will be cloned.

E083

Exploring the influence of variable NaCl content on microbial viability, autolysis and cell distribution during maturation of semi-hard cheese.

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Salt is essential for human health but too much can be harmful leading to high blood pressure, heart disease and even strokes. Reducing NaCl in cheese is a major challenge for the dairy industry. NaCl influences the cheese structure, texture, flavour and the microbial stability, which is of importance for the matured cheese.
The aim of this study was to investigate how variable NaCl content influenced the starter culture in a semi-hard cheese. Cheeses were made with 0.0, 0.8, 1.3 and 1.8 % (w/w) NaCl using two different starter cultures (C1 and C2). The cheeses were analysed during production and at 1, 2, 7 and 11 weeks of maturation. They were analysed with respect to viable count, level of cell autolysis and cell distribution using confocal laser scanning microscopy (CLSM) combined with LIVE/DEAD staining.
The NaCl content in the cheese did affect the microbial development of the starter culture. The impact on the microbial development was larger in C1 compared to C2. The first two weeks of maturation, the cell number declines faster in the samples with NaCl than in the sample with 0% NaCl. However, after 7 weeks there were no differences between the samples. For C1, the autolysis of the cells was significantly affected by the NaCl content. A decrease in NaCl content lead to an increase in autolysis. The same tendency was observed by CLSM where cell dead was more pronounced with 0% NaCl.
This study shows that starter cultures are affected in different ways by a decrease in NaCl. This knowledge is important when designing specific starter cultures for cheeses with reduced NaCl content.

E084

Role of gene clusters encoding extracellular polysaccharides in *Streptococcus thermophilus*

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Polysaccharides (PS) are omnipresent components of Gram-positive bacteria surfaces. *Streptococcus thermophilus* is well known for its ability to synthesize exopolysaccharides (EPS) that are secreted in the environment such as milk. EPS have a major contribution to texture, mouth-feel, taste perception and stability of fermented dairy products. Genomic analyses have revealed that up to three gene clusters (*eps*, *rgp*, *hasAB*) could be responsible for the production of extracellular PS in this species. The *eps*, *rgp*, *hasAB* gene clusters show respectively some similarity in terms of organization with capsular PS (CPS), rhamnose-glucose PS (RGP), and hyaluronic acid (HA) capsule encoding clusters of other streptococci. All the *S. thermophilus* genomes contain both *eps* and *rgp* gene clusters while the presence of the *hasAB* locus is more variable.
Our aim was to investigate the functionality of these three gene clusters and evaluate their contribution to the physiology of *S. thermophilus*.
For this purpose, simple and multiple deletions of these gene clusters were performed in two *S. thermophilus* strains, DGCC7710 and LMD-9, which are considered as texturing and non-texturing strains, respectively. In addition, LMD-9 contains the three gene clusters while DGCC7710 only possesses *eps* and *rgp* clusters. Interestingly, the multiple deletions of these gene clusters were not lethal but a growth defect was generally observed when two clusters were inactivated. The phenotype of these mutant strains was analysed for various properties including their importance for cell wall functionality (charge, autolysis, biofilm formation, cell-surface morphology), competence, and their water retention capacity during milk fermentation. From these analyses, we conclude that all these three gene clusters are functional and contribute at different levels to the physiology of *S. thermophilus*.

E085

Improved sensitivity of phage detection in dairy processing by loop-mediated isothermal amplification

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Bacteriophage populations capable of infecting lactic acid bacteria used as starter cultures in dairies are a persistent challenge for fermentation processes, in particular in dairies. Early phage identification is crucial for preventing fermentation failures and can thus save time and reduce financial losses. We adapted for detection of dairy phages loop-mediated isothermal amplification (LAMP) [1], a method for highly sensitive detection of specific DNA allowing rapid and easy performance at low costs.
In LAMP, a polymerase with strand displacement activity is applied, which allows to keep the temperature constantly at 60-65°C during amplification. Besides the polymerase, a set of four specially designed primers is required. A typical LAMP-assay is carried out within one hour and can be performed in laboratories with standard equipments (i.e., heating block or water bath, microcentrifuge tubes).
A LAMP-assay was developed for *Lactococcus lactis* phage P680, a thermo-resistant phage of the widespread 936 phage species [2]. We present results for detection of pure phage isolates by LAMP compared to a standard PCR-based assay. We further tested LAMP with DNA extracted directly from whey and compared its sensitivity with that of the standard PCR assay.

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E086

Lytic lactococcal bacteriophages with morphological similary to the reference phage BK5-T are a heterogeneous group of phages

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Many dairy starter strains are susceptible to infection by lytic bacteriophages. For *Lactococcus lactis* cultures, the most predominent phages belong to three distinct phage species and represent typical *Siphoviridae* phages with either isometric heads (phage species 936 & P335) or prolate heads (phage species c2). Within the last years, we have also noticed that other types of isometric-headed and long-tailed (240 nm) phages infecting lactococcal starter strains have emerged in different dairies. They reveal morphotypes similar to the well-known lactococcal phage BK5-T originating from a lysogenic starter culture in New Zealand. BK5-T-like phages were isolated from samples obtained from German, Swiss, and Swedish cheese factories and furthermore from raw milk samples.
Data are presented that these phages represent a heterogeneous group of lactococcal phages with four distinct sub-populations revealing different host ranges and baseplate structures. Sequence analysis of 14 phages revealed dsDNA genomes with sizes ranging from 33.4 to 55.6 kb. The majority of the phages used a *cos*-type packaging system - though one of the genomes was circularly permuted. Bioinformatic analysis indicated the presence of 49 to 82 coding sequences per genome unit. The genomes of one of the phage groups were similarly organized as those of the rare 1706 phage species.

E087

Characteristics of exopolymers produced by *Oenococcus oeni* cells adhered to oak

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Malolactic fermentation takes place in a stainless steel tank or directly in the oak barrels where the wine is aged. This fermentation, performed by *Oenococcus oeni*, enables deacidification, microbiological stabilization and the development of organoleptic qualities of the wine. Since they are present in the wine environment, the indigenous bacteria can trigger a spontaneous fermentation, therefore their adhesion capabilities have an impact on wine quality. In addition, the interface between the wood and the wine is the place of reciprocal interchanges between the oak compounds and the wine.
At this wood-wine interface, the development of a biofilm may interfere or promote transfers of aromatic molecules. The biofilm development has been studied in other types of lactic acid bacteria, but little is known about adhesion properties and biofilm development of *Oenococcus oeni*. The biofilm matrix would be mainly composed of homopolymers such as b-glucans, hexopolymers rich in glucose, galactose and rhamnose. It also contains polymers with nucleic acid and amino acid components. Some enzymes trapped in this matrix catalyze the conversion of wood components in molecules with organoleptic properties (such as vanillin). After analyzing the adhesion properties and the kinetics of development of four strains of *O. oeni* on stainless steels or wood chips, our study focuses on the analytical monitoring of the matrix exopolymeric contents. This step is carried out by molecular and macromolecular characterization.
Thus, a deeper understanding of the development and the composition of these biofilms will be important for an improved control of the process of winemaking.

E088

Characterization of *Lactococcus lactis* strain expressing a small heat shock protein from *Oenococcus oeni*

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Lactococcus lactis is a lactic acid bacterium widely used in cheese and fermented milk production. During fermentation, *L. lactis* is submitted to acid stress that impairs their growth capacities. In this work, we expressed in *L. lactis* a small heat shock protein (sHsp) named Lo18 from the acidophilic strain *Oenococcus oeni*. This sHsp is known for playing important roles in protein protection and membrane stabilization in *O. oeni*. The role of this sHsp could be fully studied in *L. lactis,* since no gene encoding for sHsp has been detected in this species.
The strain *L. lactis* subsp. *cremoris* MG1363 was transformed with the plasmid pDL/JM. This plasmid is derived from the plasmid pDL278 and contains *hsp18* gene (encoding for Lo18) and its promoter sequence. The production of Lo18 during stress conditions was checked by immunoblotting and the cellular location of Lo18 in *L. lactis* cells after heat shock was elucidated. Our results clearly indicate that Lo18 participates to cytoplasmic proteins protection and membrane stabilization during stress. The sHsp expression in *L. lactis* improves its tolerance to heat and acid conditions. Finally, milk fermentation performed by *L. lactis* producing this sHsp was performed.
This study suggests that a *L. lactis* strain expressing a sHsp presents a better resistance to stress and consequently, improving technological performances.

E089

Aeration reduces stress in *Lactococcus lactis* MG1363

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Lactic acid is the main end product of *Lactococcus lactis* that causes growth inhibition at acidic pH finally resulting in complete growth arrest in fermentations. Lactic acid is produced to regenerate NAD^+, which is needed for on-going glycolysis. The aim of this work is to improve fermentation capacity of lactococci by reducing low pH-induced lactic acid stress. In aerobic conditions, the conversion of oxygen to water by NADH-oxidase offers an alternative pathway to regenerate NAD^+, with concomitant changes in final product composition reducing lactic acid stress. Comparative growth performance in M17 medium revealed that the maximum specific growth rate of the aerobically grown cells was about 14 % higher than anaerobically incubated cells. To assess activation of genes associated with adaptive stress response, we performed a comparative transcriptome analysis of *L. lactis* MG1363 cells harvested in different growth phases from anaerobically and aerobically grown cultures. In anaerobically grown cells, stress related genes were induced when cells entered the end-exponential or stationary phase, while at the same time genes necessary for growth such as pyrimidine and purine biosynthesis were down regulated. On the contrary, aerobically grown MG1363 cells showed a significant lower expression of stress related genes in the end-exponential as well as in the stationary phase. These results show that aerated MG1363 cells have a higher growth rate and are less stressed in transition and stationary phase of growth compared to anaerobically grown cells. Since intracellular pH homeostasis is regarded as an active process that is crucial for maintenance of cell activity and viability, we are currently validating the use of intracellular pH as a stress indicator in aerobically and anaerobically grown *L. lactis* cells.

E090

Assessment of wild non-dairy lactococcal strains for flavour diversification in a mini-Gouda type cheese model

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Wild lactococci possess enhanced metabolic capabilities in comparison to industrial dairy strains, including increased amino acid-converting enzyme activities. A bank of *Lactococcus lactis* strains isolated from different non-dairy environments exhibited wider carbohydrate fermentation profiles in comparison to dairy lactococcal strains. In addition, these non-dairy lactococci had the ability to ferment lactose and produce diverse aroma profiles when grown in milk. Based on volatile analysis, five of these non-dairy strains were selected and investigated as adjuncts to diversify cheese flavour using a model mini-Gouda cheese-making process and compared to a cheese manufactured with a commercial adjunct. In total, 8 different cheeses were evaluated in duplicate and ripened for 14 days at 12 °C, followed by 8 °C for 84 days thereafter. Physicochemical analysis of cheeses was performed at day 14 and sensory evaluation at day 84. Viable counts, intracellular enzyme activity and indices of proteolysis were monitored over ripening. The ability of the non-dairy strains to survive, lyse, and release intracellular enzymes and alter proteolysis was strain-dependent. Some strains performed as well as the commercial adjunct in terms of secondary proteolysis although others were associated with bitterness and development of off-flavours and off-aromas. Attenuation of DPC6853 positively reduced its association with bitterness during ripening. It is evident that non-dairy strains have potential as adjuncts in semi-hard type cheeses, and could be harnessed to diversify flavour profiles in semi-hard cheese varieties.

E091

Immobilization of *Oenococcus oeni* and co-immobilization of *Oenococcus oeni* and *Saccharomyces cerevisiae* in wood sawdust for winemaking process

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Malolactic fermentation (MLF) is considered a simple phenomenon but with a practical relevance in wine. The main significance of the MLF in vinification is the biological de-acidification, which results from the transformation of L-malic acid into L-lactic acid by lactic acid bacteria (LAB), mainly by *Oenococcus oeni*. The starter cultures used to induce MLF ensure a faster fermentation and reduce the potential spoilage by other LAB. Moreover, the use of immobilized LAB as starter cultures provides advantages compared to free cells because the supporting matrix protects the cells against the effects of pH, temperature, ethanol, etc. In the present study, *O. oeni* was successfully immobilized in wood sawdust, a material of food-grade purity, cheap and abundant in nature. Besides, *O. oeni* and *Saccharomyces cerevisiae* could be successfully co-immobilized together, confirmed by fluorescence and electron microscopy. The ability of the immobilized bacteria or yeast/bacteria mixture to carry out the MLF and alcoholic fermentation (AF) was evaluated in terms of malic acid degradation and sugars' consumption in red grape musts. The immobilization of *O. oeni* permitted a faster MLF in wine, with a complete malic acid consumption in less than 5 days after the AF. The co-immobilization of both microorganisms provides a novel biocatalyst procedure that facilitates and shortens the whole winemaking process, as only one co-immobilized starter is inoculated. Furthermore, as both fermentations occur simultaneously, the whole vinification time becomes shorter. The results indicate that wood sawdust can be used to immobilize mixtures of yeasts and bacteria '*à la carte'*, depending on the needs of the industry or the cellar.

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E092

Cytofluorometric detection, identification and quantification of *Lactobacillus plantarum* in winemaking samples by fish and flow cytometry

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Lactobacillus plantarum is a lactic acid bacterium commonly isolated of different environments such as cattle dung, human mouth, gut and faeces, residual water, fermented dairy products (yogurt), fermented vegetables (sauerkraut) and fermented beverages (wine). *Lactobacillus plantarum* is found in a natural way in must and wine and can be inoculated as starter culture in winemaking too. In this work, we have adapted previous fluorescence *in situ* hybridization (FISH) protocols for *Lactobacillus plantarum* to flow cytometry. For this aim, we optimized a liquid hybridization protocol with specific fluorescein-labelled probes targeted to the 16S-rRNA of *L. plantarum*. By this technique, we analyzed winemaking samples (must and wine) to detect, identify, and quantify *Lactobacillus plantarum* by flow cytometry and epifluorescence microscopy. This methodology results very accurate, reliable, fast and easy, and could be regarded as a powerful and emerging tool in wine microbiology.

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E093

Development of low-calorie fermented milk with chia flour

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Foods with antioxidant properties are important because of their ability to react with the free radicals from the cells and stabilize them. Chia seed presents high protein (20%) and lipid (30%) contents with considerable antioxidant activity, and high water holding capacity, which confers the property of forming gel. Its addition into fermented milks can also increase the cell viability of lactic acid bacteria (LAB) used in the fermentation, besides providing bioactive compounds to the product. Currently, there are few studies dedicated to verify the antioxidant activity in fermented milks, especially promoted by chia flour. In this study, the aim was to study the effect of chia flour on physicochemical characterization of low-calorie fermented milk. Two treatments were conducted in two separate independent trials: one of them was not fortified (control) and 1% of chia flour was added to the other. Milk was fermented by *Streptococcus thermophilus* at 42 °C up to a pH of 4.6. The acidification kinetic parameters were determined during fermentation and physicochemical characteristics, antioxidant activity and LAB viability were evaluated during 14-day storage at 4 °C. The addition of chia flour accelerated the fermentation process, resulting in higher acidification rate (V_{max}) values, lower time to reach pH 4.6 ($T_{pH\ 4.6}$), increased acidity and provided higher antioxidant capacity during storage. The development of low-calorie fermented milk with chia flour showed interesting results for further studies and may present potential commercial value besides provide health benefits for the consumers.

E094

***Listeria monocytogenes* biofilm inhibition and/or displacement by bacteriocinogenic *Lactobacillus sakei* CRL1862**

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Biofilms are complex microbial communities widespread in nature. Since biofilms are very difficult to eradicate, the ability of bacteria to form biofilms poses a major problem in industrial and medical settings, being a persistent source of contamination and/or infection. Recently, major advances have been made in the prevention and control of pathogens biofilm by lactic acid bacteria. Therefore, the objective of this study was to investigate the ability of *Lactobacillus sakei* CRL1862 and/or its bacteriocins to compete, exclude or displace *L. monocytogenes* FBUNT (Facultad de Bioquímica Universidad Nacional de Tucuman) during biofilm formation on stainless steel (SS) and polytetrafluoretylene (PTFE) surfaces at 10 ºC. Assayed inhibitory strategies involved (i) displacement (*L. sakei* CRL1862 planktonic cells, its supernatant and partially purified bacteriocin on pathogen biofilm), (ii) exclusion (*L. monocytogens* FBUNT planktonic cells on *L. sakei* CRL1862 biofilm) and (iii) competition (biofilm formed by *L. sakei* CRL1862 + pathogen planktonic cells). *L. monocytogenes* biofilm displacement showed partially purified bacteriocin addition as the most effective antimicrobial treatment compared with supernatant and planktonic bacteriocinogenic cells, reducing pathogen biofilm in 3.5 log on SS and PTFE surfaces. Biofilm formation by *L. monocytogenes* in the exclusion assay was prevented by *L. sakei* CRL1862 biofilm showing 4.0 and 2.7-log reductions on PTFE and SS, respectively. However, the greatest inhibition of *L. monocytogenes* biofilm cells was observed during competition assay; pathogen cells in the biofilm were significantly reduced by 5.5 and 4.5 log on PTFE and SS, respectively. Moreover, a greater inhibition of *L. monocytogenes* biofilm on PTFE than on SS was found. These results shows the effectiveness of bacteriocinogenic *L. sakei* CRL1862 for the prevention and control of *L. monocytogenes* on abiotic surfaces.

E095

Sugar addition affects the final product of *Lactobcillus paracasei* subsp. *paracasei* CRL 207 on soy solid state fermentation

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Soy is a great substrate for its high nutritional value and low cost. In previous studies the behavior of *Lactobacillus (L.) paracasei* subsp. *paracasei* CRL 207 on soy solid state fermentation (SSF) with different moisture content, temperatures and inoculums sizes was analyzed. These results demonstrated that this strain is able to develop on proposed SSF and the optimized conditions were 65% moisture, 37ºC and 2% of inoculum. The aim of this study was to analyze the addition of sugars (glucose or sucrose) on the β-glucosidase and proteolytic activities of this strain on soy SSF previously optimized. Pastes were made from commercial soy flour and distillated water, sugar was added at 2%; then were sterilized and inoculated. These were incubated at 37ºC for 24 and 72 h. The pH reached at 4,65 in presence of glucose but was practically maintained respect to the control with sucrose and without sugars at 24 h, in these both conditions the acidification increased at 72h. On the other hand, the β-glucosidase activity was evidenced in all tested conditions at 24 and 72h. It was higher in presence of sucrose at 24 h. Besides, an increase of amino acids amount was found. The addition of glucose enhanced the amount of free aminoacids at 24h. The proteolytic activity of this strain was evidenced with SDS-PAGE. We can conclude that for this strain the addition of sugar in the soy substrate affects their metabolism and it is important to lead the fermentation to a high β-glucosidase activity or proteolytic activity.

E096

Lactococcal surface properties and their role in microbe-matrix interactions in dairy products

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Microbial surface properties are highly variable and they are considered to be important for the interactions of the bacterial cell with the product matrix. Lactic acid bacteria are industrially used for the fermentation of milk to produce, e.g., yoghurt or cheese. In fresh milk, bacteria encounter particles such as casein micelles or fat droplets, while in fermented milk products, the proteins form a semi-solid casein matrix, in which fat globules and whey pockets are present. However, the extent to which the interaction of dairy starter cultures with the various matrix components is important for the result of fermentations is largely unknown. Here, we analyzed the surface properties of 55 *L. lactis* strains and found big variations in bacterial cell-charge, surface hydrophobicity and cell attachment to milk proteins. As we also have the full genome sequences of these 55 strains, genotype - phenotype matching was used to identify mechanisms involved in the described surface properties. The identified properties and mechanisms and their relevance in relation to microbe-matrix interactions will be discussed.

E097

Virulence factors and antibiotic resistance in lactic acid bacteria from Pico cheese

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Enterococci are part of the dominant flora in traditionally fermented cheeses made with raw milk and play a relevant role in the development of the organoleptic characteristics of the final product. Because of their role in ripening, flavour development and bacteriocin production in cheeses, it has been suggested that *Enterococci* with desirable technological and metabolic traits could be included in starter cultures for various cheeses. However, the *Enterococcus* genus is not regarded as GRAS and safety concerns may be raised for certain strains.
The aim of the present work was to characterise 27 strains *Enterococcus faecalis* isolated from Pico cheese with emphasis on those aspects of greater concern such as the incidence of virulence factors, antibiotic resistance traits and production of biogenic amines.
PCR reactions were used to detect the presence of genes involved in the expression of gelatinase (*gelE*), hyaluronidase (*hyl*), aggregation substance (*asa1*), enterococcal surface protein (*esp*), cytolysin (*cylA)*, endocarditis antigen (*efaA*), collagen adhesion (*ace*), vancomycin resistance (*vanA* and *vanB*), aminoglycoside resistance [*aac(6')-Ie-aph(2')-Ia*, *aph(2')-Ib*, *aph(2')-Ic*, *aph(2')-Id*, *aph(3')-IIIa*, and *ant(4')-Ia*], histidine decarboxylase (*hdc1* and *hdc2*), tyrosine decarboxylase (*tdc*), and ornithine decarboxylase (*odc*).
None of the tested strains carried vancomycin resistance genes. In the case of aminoglycosides, 8 isolates tested positive for just one of the six resistance genes tested. The most prevalent virulence genes were *efaA, gelE* and *ace*. The *tdc* gene was also common in the tested strains. All of the tested isolates were *efaA* positive. The prevalence of virulence genes may be a hurdle when considering their application as starter cultures.

E098

Molecular basis of *Oenococcus oeni* stress response to inhibitory compounds produced by wine yeasts during fermentation

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Oenococcus oeni is the most important lactic acid bacterium species involved in the induction of malolactic fermentation (MLF) at the end of the alcoholic fermentation (AF) during wine-making process. MLF is a critical step to obtain a high quality final product. However, bacterial growth and MLF are not always successful due to the harsh environmental conditions of wine. The viability of *O. oeni* in wine depends on its resistance to several stress factors such as acidity, SO_2, ethanol and other inhibitory metabolites produced by wine yeasts. To study the possible mechanisms that *O. oeni* X_2L utilizes to survive and carry out the MLF in stressful wine conditions, a proteomic approach (2D-E) and RT-PCR were performed. *O. oeni* X_2L was grown in unfermented (control) and fermented grape juice. Forty three proteins spots showed significant changes in expression during growth in fermented juice: 23 spots were up-regulated while 18 proteins down-regulated. One spot (aminotransferase/fructokinase) was specific to fermented medium while another spot (aspartate carbamoyltransferase) was completely inhibited in this condition. Enzymes involved in carbohydrate, lipid, nucleotide and amino acids metabolism, as well as chaperone proteins (DnaK/DnaJ) and two peptidases (oligopeptidase F and endopeptidase O) were over-expressed while enzymes related to lipid biosynthesis, glycolysis, amino acid metabolism, hydrolases and dehydrogenases were down-regulated. Also, differential expression of genes involved in malic acid and citrate metabolism was observed when *O. oeni* grew in fermented medium. This study contributes to the knowledge of the molecular mechanisms used by *O. oeni* to tolerate stressful wine conditions as well as to the selection of the best adapted starter culture to carry out successfully the MLF during wine-making.

E099

Use of semi-purified lacticin 3147 and essential oils against food-borne pathogens

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Lantibiotics are ribosomally-synthesized antimicrobial peptides that undergo extensive post-translational modifications resulting in the formation of unusual amino acid residues. Lacticin 3147 is a two-peptide lantibiotic with a broad spectrum of activity. It is one of the most well studied lantibiotics and exerts its activity through a dual mode of action by binding lipid II, an intermediate involved in peptidoglycan biosynthesis, followed by insertion into the target cell membrane. Essential oils are secondary metabolites extracted from plants. They often possess antimicrobial activities and are thought to be important in plant defense. Essential oils are now primarily used in the food industry as flavourings but there has been increased interest in recent years in their application as natural food preservatives. Here, we investigate the activities of semi-purified lacticin 3147 and the essential oils, thymol, carvacrol and cinnamaldehyde against food-borne pathogens of interest. Semi-purified lacticin 3147 and the essential oils are both effective at limiting bacterial growth however, a greater potency is seen when the antimicrobials are used in conjunction.

E100

The dominance of *Lactobacillus sanfranciscensis* in backslopped sourdough fermentations depends on defined process parameters

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Sourdough, a fermented flour-water mixture, represents a complex ecosystem of yeasts and/or lactic acid bacteria (LAB). Spontaneous sourdough fermentations are initiated with microorganisms originating from the flour itself, other dough ingredients, or the environment. It often concerns *Lactobacillus fermentum* and *Lactobacillus plantarum* as LAB species As *Lactobacillus sanfranciscensis* was initially associated with wheat sourdough fermentations only, probably selected for by the applied technology for sourdough production, the present study aimed at the detection of *Lb. sanfranciscensis* during spontaneous teff sourdough fermentations carried out under defined conditions. Therefore, two spontaneous teff sourdough fermentations were carried out through backslopping for ten days under laboratory conditions (teff flour as the only non-sterile ingredient, sterile fermentors, high dough yield, high temperature) and three under bakery conditions (small bakery, open vessels, low dough yield, low temperature). A microbiological analysis was performed by culture-dependent and culture-independent methods. The results showed that spontaneous sourdough fermentations based on teff did allow the growth of *Lb. sanfranciscensis*, solely or in combination with other LAB species, albeit not reproducibly. For instance, during the laboratory teff sourdough fermentations, this LAB species was not always competitive enough to dominate the fermentation process. In contrast, the bakery conditions, which allowed a slower acidification of the cereal matrix at low temperature, favoured the growth and dominance of *Lb. sanfranciscensis*. It turned out that its presence in the flour allowed its outgrowth when the right technological process parameters, such as temperature, dough yield, pH and backslopping time, were applied.

E101

Expression and secretion of hydrolases in *Lactobacillus plantarum* CD033 - development of a tool kit, bottlenecks and first results

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Lactobacillus plantarum is, due to its "generally regarded as safe" status, an emerging host for the expression of heterologous genes in the fields of industrial, agricultural as well as medical biotechnology. In agricultural biotechnology, *L. plantarum* is frequently used as a starter culture in ensiling processes; the strain used in our study, *L. plantarum* CD033, was isolated from stable grass silage too.
Hydrolase activity, which allows a bacterium to degrade cellulose, hemicellulose, inulin or phytic acid would be a beneficial feature to a silage starter strain. By glycanase activity, rapid fermentation and acidification of the substrate might be achieved without any addition of soluble carbohydrates. Phytase would liberate phosphorus bound in phytic acid of cereal-based feed and would make it available for animal nutrition. Such accelerated and improved ensiling processes would significantly improve quality and stability of the inoculated silage.
In order to obtain high titer hydrolase-producing and -secreting *L. plantarum* strains, the genes encoding various bacterial endo- and exo-1, 4-β-glucanases, a levanase gene derived from *Bacillus subtilis*, and genes encoding phytases derived from different bacteria were cloned into the high copy vector pCDLbu1 or the low copy plasmid pCD256. A set of transcriptional- and translational regulatory elements as e.g. constitutive as well as inducible promoters and various ribosomal binding sites were tested regarding their suitability for recombinant protein production in *L. plantarum*. Different leader sequences were examined for improved protein secretion. Based on the obtained data we identified feasible strategies but also yet unsolved problems and bottlenecks for high-level gene expression and protein secretion in *L. plantarum*.

E102

Safety and *in vitro* study of beneficial properties of lactic acid bacteria isolated from Portuguese fermented meat products

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Many lactic acid bacteria (LAB) produce bacteriocins with rather broad spectra of inhibition and could offer potential applications in food preservation. The bacteriocin production by starter cultures may have an advantage for these strains in competitive interactions with the pathogenic bacteria from the food matrix. The objective of this study was to determine the safety of beneficial strains *Lactobacillus pantarum* ST202Ch and ST216Ch, *Enterococcus faecium* ST211Ch, *Lactobacillus sakei* ST22Ch, ST153Ch and ST154Ch previously isolated from fermented meat products and characterized as bacteriocin producers. Auto-aggregation is strain-specific, and values 28.97%, 27.86% and 28.56% were recorded for *Lactobacillus sakei* ST22Ch, ST153Ch and ST154Ch, 16.95% and 14.58% for *Lactobacillus pantarum* ST202Ch and ST216Ch and 12.77% for *Enterococcus faecium* ST211Ch. Various degrees of co-aggregation between 28.85% and 44.76% with *Listeria monocytogenes* 211, *Listeria monocytogenes* 409 and from 23.60% to 34.96% with *Enterococcus faecium* ATCC 19553 were observed. According to the results of the diffusion method, studied strains demonstrated susceptibility to penicillin G, ampicillin, amoxicillin, amoxicillin/clavulonic acid, imipenem, linezolid and tetracycline. In addition susceptibility of these 6 strains to various non-antibiotic commercial medicaments was examined. Production of β-galactosidase by *L. sakei* ST22Ch, ST153Ch and ST154Ch, *Lactobacillus plantarum* ST202Ch and ST216Ch and *Enterococcus faecium* ST211Ch was confirmed by employing sterile filter paper disks impregnated with o-nitrophenyl-b-D-galactopyranose. A statistically significant ($p<0.001$) repression of *Mycobacterium tuberculosus* growth by bacteriocins produced by *Lactobacillus pantarum* ST202Ch (38.3% inhibition), *Lactobacillus pantarum* ST216Ch (48.6% inhibition), *Enterococcus faecium* ST211Ch (21.7% inhibition), *Lactobacillus sakei* ST153Ch (16.2% inhibition) and *Lactobacillus sakei* ST154Ch (16.1% inhibition) was observed. The tested six strains showed a low virulence gene profile as was determined by PCR.

E103

Kinetics of pectin and polygalacturonic acid degradation using polygalacturonase (PG) isolated from cassava retting microorganisms

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Five strains including two lactic bacteria (KSLAB30, KSLAB32) and three yeasts (KSY4, KSY5, KSY9) had shown their ability to produce the PG were grown on two substrates, pectin and polygalacturonic acid (PGA) to assess the ability of these strains to degrade these substances. The solutions from the two substrates were used at pH 5 and mixed with enzyme extracts from each strain then the whole was incubated at 30°C for 25 min. At regular time intervals, PG activity was determined. The results showed for each strain, amounts of galacturonic acid (GA) on the pectin substantially equal to 10 g/l polygalacturonic acid and 20 g/l. The solutions obtained from the two substrates were used pectic to pH 5 and mixed with enzyme extracts from each strain then the whole was incubated at 30 ° C for 25 min. At regular time intervals samples of different samples were made and PG activity was determined. The results showed for each strain, amounts of galacturonic acid (GA) on the pectin substantially equal to 10g/l polygalacturonic acid and 20 g/l. It appears from this study that the structure and concentration of the substrate influence the polygalacturonase activity of microorganisms.

E104

GABA production by *Lactobacillus brevis* Qr150, a strain isolated from Andean grains, is influenced by growth media peptide content

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Gamma-aminobutyric acid (GABA), a non-protein amino acid, possesses well-known physiological functions such as neurotransmission, induction of hypotension, diuretic and tranquilizer effects. This compound is synthesized by glutamate decarboxylase (GAD), a pyridoxal 5'-phosphate-dependent enzyme that catalyzes the irreversible α-decarboxylation of L-glutamate to GABA. Several reports have shown the presence of GAD in lactic acid bacteria (LAB), whose decarboxylation has also been coupled with energy synthesis in *Lactobacillus.* In this study, twenty-two strains isolated from Andean grains and fermented flours (sourdoughs) were tested for their GABA production ability. Among the screened strains, *Lactobacillus brevis* Qr150, identified by 16S rDNA sequencing, yielded the highest levels of GABA (~300 mM) after 144 h of incubation in MRS broth containing 5% monosodium glutamate (MRSG). GABA production by this microorganism was remarkably higher in cells grown in a peptide-rich medium (MRSG) compared with those found in a chemically defined medium (CDM) in the presence of 5% monosodium glutamate (CDMG). When cells were grown in CDMG supplemented with Casitone, GABA production was considerably enhanced suggesting that GABA synthesis was influenced by the growth media peptide content. These results were confirmed by GAD transcriptional expression. GABA production by *L. brevis* Qr150 during flour fermentation will contribute to obtain new healthy enriched food products.

E105

Metabolism of β (1,4) glucoside substrates by *Lactobacillus casei* 12A

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Due to increase energy demands and environmental pressures, conversion of plant biomass from renewable resources and agriculture wastes to industrial byproducts is an area receiving intense research attention. The goal of this research area is to study the metabolism of β (1,4) linked glucoside substrates by *Lactobacillus casei* 12A. Growth of *L. casei* 12A occurred on cellobiose, the disaccharide derived from cellulose, arbutin, salicin and amygdalin. *In silico* analyses indicated six β-glucosidase gene clusters, defined by the presence of a β-glucosidase gene (*bgl*), are present in the *L. casei* 12A genome. Comparison of the transcriptome from *L. casei* 12A cells grown on glucose to *L. casei* 12A cells grown on cellobiose by RNA-seq analysis revealed significant (P<0.05) changes in the expression of 78 genes. Of the differentially expressed genes, 42 genes were involved in carbohydrate metabolism. These included seven phosphotransferase system transporters which were up-regulated during growth on cellobiose, with one of these predicted to transport cellobiose. Of particular interest, one of the six cellobiose clusters was upregulated. This organism metabolizes hexoses through the Embden-Meyerhof-Parnas pathway, producing predominately lactate from pyruvate metabolism, with the potential to produce low levels of formate, acetate, and ethanol. During growth on cellobiose, an increase in expression of a lactate dehydrogenase (*ldh2*) and pyruvate formate lyase (*pfl*) was observed. This is in agreement with lactate and formate being present as *L.casei* 12A metabolic end products during growth on cellobiose. Carbon catabolite repression (CCR) by glucose was also observed. These results reveal details concerning the mechanism by which *L. casei* 12A utilizes β (1,4) linked glucoside substrates, including cellobiose, the disaccharide derived from cellulose.
Keywords: *Lactobacillus casei*, cellobiose, β-glucosidase

E106

Enumeration of viable *Lactobacillus plantarum* in wine by PMA-qPCR

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Malolactic fermentation (MLF) is a longstanding process in winemaking. The complexity and diversity of the metabolism of lactic acid bacteria (LAB) make that MLF affects positively or negatively the quality of the wines. *Lactobacillus plantarum* has resistance mechanisms, enabling it to survive and proliferate in wine. The fast enumeration of viable cells of this bacterium is necessary, both to verify the level of inoculum and the survival of cells when MLF is desired, as to monitor the presence and growth of bacteria when MLF is an unwanted process, or acidification from sugars undesired. Traditional methods for enumerating bacteria in wine involve time-consuming plating techniques. Molecular methods, as qPCR, are unable to discriminate between viable and dead cells, overestimating frequently the microorganisms. Furthermore, the PCR reaction is frequently inhibited by substances present in wine, typically but not only phenolic compounds. In the present study we have developed a fast and reliable qPCR method in conjunction with propidium monoazide (PMA) - PMA-qPCR. PMA, after photoactivation, inhibits the PCR amplification in DNAs isolated from compromised membrane cells. Altogether, the optimized method involves the removal of the wine inhibitors and the ability to distinguish between live and dead cells by their different responses to the PMA-qPCR reaction. Conventional total and viable (cfu) counting were also performed as controls for the wine samples, showing that the assay is specific for *L. plantarum*, reproducible and highly robust. A rapid enumeration of viable *L. plantarum* cells by PMA assay can improve the control of MLF in wine allowing prompt corrective measures to regulate the bacterial growth.
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E107

Genomic analysis of dominant *Lactobacillus curvatus* non-starter bacteria isolated from Cheddar cheese

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Microbiological studies of Cheddar cheese made at Utah State University (USU) and the University of Wisconsin-Madison (UW) show *Lactobacillus curvatus* has become the predominant species of non-starter lactic acid bacteria (NSLAB). To explore the basis for its emergence, we collected draft genome sequences from *L. curvatus* strains WSU1 and LFC-1, which were isolated from aged cheese manufactured at USU and UW, respectively. Comparisons between predicted CDS in the two strains showed overall genome content was similar but not identical; strain WSU1 had 312 unique predicted proteins, and LFC-1 had 297 unique proteins. Many of the unique genes are predicted to encode proteins of unknown function or phage components, but they also include proteins associated with the ability to ferment different carbohydrates. *L. curvatus* LFC-1, for example, has genes for maltose and trehalose fermentation*,* as well as for citrate utilization, which are absent from strain WSU1. Because both strains are dominant NSLABs, we reviewed the shared gene set for traits that may be important for this attribute. Both NSLABs have genes to ferment ribose and N-acetylglucosamine, as well as the arginine deiminase pathway, which may facilitate growth in ripening cheese. Interestingly, both strains carry genes for a putative sakacin-like bacteriocin and for pathway predicted to convert 1,2-propanediol to propanol and propionic acid. Screening for bacteriocin-like activity showed inhibition of three lactic acid bacteria, *Listeria innocua,* and *Salmonella typhimurium* by *L. curvatus* WSU1. Additionally, propionic acid was found to accumulate during ripening of Cheddar containing *Lb. curvatus* WSU1 as the dominant NSLAB, and tests are underway to confirm its production. Combined, these factors may promote the ability of *Lb. curvatus* to dominate the NSLAB biota of Cheddar.

E108

Use of lactic acid bacteria for the fermentation of cv. Kalamon olives processed by the Greek-style method

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“Kalamata type table olives”I s a very well known type of Greek-style natural black olives, mainly due to the excellent characteristics of the raw material used, which is the Greek olive cultivar “Kalamon”. The processing method for their production remains artisanal and empirical despite its economic importance. During this study, laboratory fermentations using specific LAB strains as starter cultures were performed in the presence of low salinity brines. More precisely, the wild strain *Leuconostoc mesenteroides* subsp. *mesenteroides* (Lm139), isolated from the brine of Kalamon olives laboratory fermentation as well as the lactic acid bacterium *Lactobacillus pentosus* DSM 16366 isolated from fermenting green olives, were separately used as starters in laboratory Kalamon table olives fermentation, in 5% w/v NaCl brines. As a control, spontaneous olives fermentation was also performed. Microbiological and physicochemical analyses of the brines revealed that the use of both starters had a significant effect on the fermentation of the olives, leading to a faster and more efficient consumption of soluble sugars and acidification of the brines. The final pH value reached by each starter culture used indicates a successful lactic fermentation. The production of lactic acid by the starters and the concomitant drop of the pH value, led to the inhibition of enterobacteria in a shorter period of time compared to the spontaneous fermentation. Concluding, the use of these lactic acid bacteria as starters in Kalamon olives fermentation could lead to a more controllable fermentation resulting in a product of high and constant quality being safer for the consumers and having a longer lifetime.

E109

Development of reduced-fat Cheddar and Swiss-type cheeses enriched in hypocholesterolemic heteropolysaccharide-producing *Lactobacillus mucosae* DPC 6426

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Previous work has demonstrated the promising hypocholesterolemic effects of heteropolysaccharide-producing *Lactobacillus mucosae* DPC 6426 in the atherosclerosis-prone apolipoprotein-E deficient murine model (London *et al.* in review). The aim of this study was to investigate the suitability of reduced-fat cheese as a functional food matrix for the delivery of this strain, while assessing its compositional, textural and sensory properties.
Cheddar and Swiss-type cheeses were manufactured in triplicate and cheese milk was inoculated with 10^6 CFU/ml *Lb. mucosae* and standard starter cultures. Viability of *Lb. mucosae* DPC 6426, all starters and NSLAB was assessed throughout 182 day ripening period. The impact of the use of *Lactobacillus mucosae* DPC 6426 as adjunct culture during cheese manufacture on starter performance and product quality was assessed. Parameters measured were cheese yield, moisture, pH profile, proteolysis, total free amino acids and exopolysaccharide content as well as texture profile and sensory properties of the ripened cheeses. Finally, confocal laser scanning microscopy was employed to visualize the microstucture of the cheese, including the distribution of the exopolysaccharide.
The EPS-producing adjunct culture remained viable at 10^7 CFU/g in Swiss-type cheese following 154 days ripening. Although Cheddar is dry-salted and reaches a lower pH than Swiss-type, *Lb. mucosae* demonstrated comparable viability at 10^7 CFU/g. Textural analysis revealed no significant differences in the maturation of the cheese when compared with the control. Results suggest that Swiss-type and Cheddar cheeses are suitable functional food matrices for the delivery of *Lb. mucosae* DPC 6426, with no apparent reduction in technofunctional quality.

E110

Control of Enterococci and Pseudomonads in industrial production and storage of Mozzarella cheese from raw cow milk

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“Fior di Latte” is a soft, high-moisture raw milk Mozzarella cheese (RMM) which is highly susceptible to proliferation of bacteria originating from cow milk or contaminations. This study aimed to optimize single steps in the industrial production process which should reduce bacterial proliferation during this process and after shelf-life storage. Predominant spoilage bacteria were identified by molecular biological methods and microbial properties of the RMM evaluated over 27 days of storage at 4 and 8°C. Curd stretching at 85°C was identified as a critical control step to suppress the initial microflora reducing enterococci from 3.8-4.9 to 2.4-2.7 $\log_{10}$ cfu g^{-1},whereas *Enterobacteriaceae* and *Pseudomonas* spp. were reduced below the detection limit of 2.0 $\log_{10}$ cfu g^{-1}. Enterococci were predominant throughout production and storage; they originated from the original raw milk according to their rep-PCR fingerprints. Enterococci were dominant in the end product, showing great temperature dependency below 10°C. At a storage temperature of 8°C, enterococci counts up to 7.2 $\log_{10}$ cfu g^{-1} were recorded after 27 days, whereas at 4°C cell counts in the analyzed four batches were between 2.2-3.2 $\log_{10}$ cfu g^{-1} over 27 days of storage. *Pseudomonas* spp. were only detected in one out of four batches in storage tests indicating a low extent of contamination in the production chain of this modern enterprise.
Conclusively, post-process contamination by *Pseudomonas* spp. and survival of *Enterococcus* spp. may be critical for storage which should be kept at 4-5°C for this cheese. We propose pseudomonads and enterococci as critical marker indicator bacteria for monitoring safety and quality of RMC during production and storage.

E111

Strain composition robustness of a *Lactococcus* multi-strain starter culture produced with varying substrate batches and fermentation profiles

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Many fermentations in the dairy industry are performed by multi-strain cultures containing a varying number of strains. Examples range from cheddar cultures with 3-4 strains to up to 100 different strains in undefined LD-cultures, as they are used in continental cheeses. The production of multi-strain cultures can pose a challenge with respect to the consistency of the strain composition with varying substrate batches or under different fermentation regimes. Until recently the available methods for determining culture compositions at strain level were so called fingerprinting methods based on chromosomal DNA restriction fragments (PFGE) or PCR-fragments (REP-PCR, RAPD). They are performed on isolates, have a small dynamic range and are far too laborious for detecting and quantifying specific strains in investigations with increased sample number.
The recent developments of low cost genome sequencing technologies, so called next-generation sequencing (NGS), have enabled sequencing of bacterial strains on a routine basis. One possible application amongst many is the identification of strain specific sequences. Quantitative PCR targeted to these strain specific sequences allows rapid detection and quantification of specific strains in a high number of complex samples - without the need for isolation and purification of strains.
In this study we present an investigation of the composition robustness of a multi-strain *Lactococcus* starter culture with varying medium ingredients batches, fermentation volumes and conditions.

E112

***Lactobacillus casei* 12A as a biocatalyst for the production of isobutanol**

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An increasing demand for petroleum-based transportation fuels has led to a need for new fuel sources. Alcohols can serve as replacements for petroleum-based fuels, and can be produced by microbial fermentation. Isobutanol has numerous advantages over ethanol and hence its production by microbial fermentation is an area of intense research interest. *Escherichia coli*, *Saccharomyces cerevisiae* and *Clostridium cellulolyticum* have been examined for the production of isobutanol; unfortunately features like low alcohol tolerance, sensitivity to a variety of substrate related stresses makes them unsuited as biocatalysts for isobutanol production from lignocellulosic substrates. However *L. casei* has numerous advantages, such as high innate tolerance to alcohols and lignocellulosic related stressors. Five *L. casei* strains were screen for tolerance to isobutanol; *L. casei* ATCC 334 and 12A exhibited the highest isobutanol tolerance, with 1.96 and 1.89% isobutanol required to reduce their maximal growth rates by 50%, respectively. Additionally, *L. casei* 12A can be transformed at high efficiency and is highly tolerant of lignocellulose-derived inhibitors.The aim of this study is to employ *L. casei* 12A as a biocatalyst for production of isobutanol. Therefore, we have integrated 1) *Bacillus subtilis* acetolactate synthase (AlsS); 2) *E. coli* ketol-acid reductoisomerase (IlvC) and 3) dihydroxy-acid dehydratase (IlvD); 4) *Lactococcus lactis* 2-ketoacid decarboxylase (Kdc); and 5) *L. lactis* alcohol dehydrogenase (AdhA), enzymes needed for isobutanol production. Additionally, a genome-scale metabolic network for *L. casei* 12A has been constructed to facilitate the construction of a 12A derivative in which metabolic flux to lactate has been redirected to isobutanol. The long-term objective is to construct a *L. casei* 12A derivative that produces isobutanol as its primary metabolic end product.

E113

Population dynamics and strain selection for a malolactic starter in a biodynamic cellar

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Biodynamic wines are made embodying the ideal of ever-increasing ecological self-sufficiency just as with modern agro-ecology. This type of vitiviniculture views the farm and the cellar as a cohesive, interconnected living system, and has become increasingly popular in the recent years all over the world. The use of commercial starters, single or not, is not welcome in those habitats, as the natural equilibrium in these environments must be respected. But application of microbiology is possible though, if the use of a mixed culture of autochthonous microorganisms - isolated from a biodynamic environment and propagated under biodynamical conditions -- is contemplated.
In the present work, the diversity and population dynamics of lactic acid bacteria (LAB) in a biodynamic cellar has been studied during two consecutive years. Isolates were identified by 16S-ARDRA, and typed by using M13-RAPD and VNTR. In general, no big differences were observed compared to conventional (non biodynamic) spontaneous wines. However, a low level of LAB were found in the initial grape must. Later, these populations increased to regular levels, following normal dynamics during all the winemaking process. *Oenococcus oeni* became the predominant species in malolactic fermentation with a scant diversity of other LAB species.
A selection program was established to choose the most suitable LAB strains among the isolates, namely *O. oeni*, to constitute the mixed starter culture for this particular cellar. Among other criteria, commonly used to select malolactic bacteria (as ability to metabolize malic acid, resistance to pH, ethanol or SO_2, etc.), the adaptation to the particular conditions of these wines was considered, as the ability to coexist.
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E114

Redirecting metabolic flux from lactate to ethanol in *Lactobacillus casei* 12A

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The production of biofuels and commodity chemicals from plant-derived biomass is of considerable interest due to concerns related to the use of fossil fuels. *Lactobaillus casei* 12A, a facultatively heterofermentative lactobacilli isolated from corn silage, is highly resistant to lignocellulosic stressors and biofuels. The objective of this study was to redirect metabolic flux in *L. casei* 12A from having lactate as its primary metabolic end-product to producing primarily ethanol. This organism metabolizes hexoses through the Embden-Meyerhof-Parnas pathway and converts pyruvate to lactate via a variety of different lactate dehydrogenases (LDHs); including four L- lactate dehydrogenases (L-LDHs), one D-LDH, and one D-hydroxyisocaproate dehydrogenase (D-HIC). Deletion of *L-ldh1* and *L-ldh2* was found to be the most effective for reducing L-lactate production while deletion of *D-hic* inhibited D-lactate production. A mutant lacking *L-ldh1*, *L-ldh2*, and *D-hic* genes reduced lactate yield to 11% of compared to 80.5% in wild type, while little improvement of ethanol production was observed showing at 5.1% ethanol yield. To increase flux to ethanol, codon optimized Production of Ethanol (PET) cassette from *Zymomonas mobilis* encoding pyruvate decarboxylase (PDC) and alcohol dehydrogenase II (ADHII) was expressed into different *ldh* deletion mutants. The expression of PET enzymes was designed to be under the control of native *L. casei* 12A *pgm* promoter and *kdgR* transcriptional terminator. The highest ethanol yields were found in Δ*L-ldh1*/Δ*L-ldh2* Δ*D-hic* mutant giving at 89.7% in plasmid born PET and 83.4% in chromosomal PET, with mol ethanol to mol lactate ratio of 23.2 and 9.0, respectively. Results from this study demonstrate that redirecting metabolic flux in *L. casei* from lactate to ethanol can be readily achieved.

E115

Isolation of lactic acid bacteria from Okinawan biological resources and their antibacterial activity

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Okinawa islands locate in southern part of Japan with a subtropicalclimate. It has been reported that there are much higher variation of animal and plant species than other regions, such as Japan mainland. Therefore, it could be possible for us to find another higher degree of microorganism variety, enabling us to obtain local lactic acid bacteria (LAB) with unique characteristics. We have isolated acid-producing, Gram-positive and catalase-negative bacteria from natural resources in Okinawa, including fruits, plant leaves and flowers, vegetables and seaweeds, to obtain LAB with antibacterial activity for possible food processing. In total 44 strains of acid-producing, Gram-negative and catalase-negative bacteria have successfully been isolated from 133 resources. We could isolate 3 strains from seaweeds, in addition to 10 strains from leaves, 6 strain from flowers and 15 strains from fruits. One strain, namely IT-1 strain isolated from plant origin, out of the 44 strains could inhibit growth of *Lactobacillus delbruekii* subsp. *bulgaricus* (JCM1002) and its antibacterial activity was almost 8 times higher than that of *Lactobacillus gasseri* LA39 (JCM11657), known as gassericin A producing strain and about 4 times higher than that of *Lactobacillus gasseri* LA158 (JCM11046), known as gassericin T producing strain. Antibacterial activity produced by IT-1 strain in a culture supernatant was quite heat stable and there was no decrease in activity after treatment at 63 degrees C for 30 min. One-fifth of the initial activity was remained after an autoclave treatment at 121 degrees C for 20 min. Identification of IT-1 strain is in progress by sequencing of the 16S rRNA gene.

E116

Formation of novel pyranoanthocyanidins and vinylphenol-adducts of deoxyanthocyanidins during fermentation of sorghum sourdough.

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Sorghum (*Sorghum bicolor* [L.] Moench) has a high content of phenolic compounds. During sorghum sourdough fermentation, the phenolic acids and flavonoids are modified (1), but the fate of the deoxyanthocyanidins present in red sorghum is presently unknown. This study determined the fate of deoxyanthocyanidins in sorghum sourdoughs. Sourdoughs were prepared from pure cultivar sorghum var. 'Town', a red variety, and fermented for 48h with a binary strain combination consisting of the caffeic acid decarboxylating *Lactobacillus plantarum* FUA3171 and the caffeic acid decarboxylase negative *L. casei* FUA3166. Deoxyanthocyanidins were recovered by liquid-liquid extraction and analysed by LC-MS/MS.
Prior to fermentation apigeninidin and methoxyl-apigeninidin were the major deoxyanthocyanidins in ´Town´ sorghum; luteolinidin and methoxyl-luteolinidin were additionally present. The concentration of these deoxyanthocyanidins decreased during fermentation and novel deoxyanthocyanidins were formed. Two of the novel deoxyanthocyanidins were purified by preparative LC; analysis by NMR spectroscopy and high resolution MS identified the compounds as a 3-deoxyanthocyanidin-vinylphenol adduct and pyrano-3-deoxyanthocyanidin. Pyrano-3-deoxyanthocyanidin was previously identified in sorghum but its presence was not linked to microbial activity (2). To identify possible pathways of their formation, sorghum was fermented with single strains, *L. plantarum* or *L. casei*. Remarkably, 3-deoxyanthocyanidin-vinylphenol and pyrano-3-deoxyanthocyanidin were formed only during fermentation with the caffeic acid-decarboxylating *L. plantarum* FUA3171, indicating that the decarboxylated product, 4-vinylphenol, is required for their formation. Chemical synthesis with apigeninidin and either vinylphenol or *p*-coumaric acid confirmed that 3-deoxyanthocyanidin-vinylphenol and pyrano-3-deoxyanthocyanidin are rapidly formed with vinylphenol but not with *p*-coumaric acid. In conclusion, the products of microbial decarboxylation of hydroxycinnamic acids convert apigeninidin and methoxyl-apigeninidin to pyrano-3-deoxyanthocyanidins and vinylphenol adducts.
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E117

Characterization of *Lactobacillus wasatchii* WDC04, a novel species associated with late gas production in Cheddar cheese

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We propose the name *Lactobacillus wasatchii* for a new obligate heterofermentative *Lactobacillus* species associated with late gas production defect in cheese. Originally isolated from aged Cheddar cheese, *L. wasatchii* WDC04 has a very limited ability to ferment carbohydrates, indicating an oligotrophic nature. The strain shows the highest growth rate in MRS broth with 1.5% ribose as the carbohydrate source, and slower growth rates when glucose or galactose is provided as the energy source. Gas production is observed when one of the hexose sugars is added to the media, but gas production is greatly increased when the culture is grown with both galactose and ribose. In MRS broth with 1.5% ribose, the bacterium was able to grow at pH (5.2) and salt concentrations (2.5-4.5%) found in ripening Cheddar cheese. To learn more about the metabolic capabilities of *L. wasatchii* WDC04, total genomic DNA was sequenced and assembled into a 1.90 Mbp draft genome and annotated. Genome analysis showed the absence of any ortholog of 6-phosphofructokinase and fructose-1,6 bisphosphatase, but the presence of glucose-6-phosphate dehydrogenase. Thus, *L. wasatchii* WDC04 is obligately heterofermentative, using the pentose phosphate pathway for energy production and it is dependent on exogenous carbohydrates for essential anabolic precursors. Cheesemaking experiments with added *L. wasatchii* WDC04 confirm the bacterium promotes gassy defect in Cheddar cheese, particularly when cheese is ripened at elevated temperature (12°C).

Section F: Emerging Technologies

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F001

Anti-oomycete potential of *Lactobacillus amylovorus* JG2 against the potato blight pathogen *Phytophthora infestans*

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Phytophthora infestans is an oomycete responsible for late blight; the most devastating disease in potato production today. This study aimed to assess *in vitro* antifungal potential of lactic acid bacteria against *P. infestans*. Five strains which had initially exhibited strong antifungal activity against *Penicillium*, *Aspergillus* and human dermatophytes were selected and tested against Irish potato blight *P. infestans* strains. All strains caused inhibition and the strongest was *Lb. amylovorus* JG2. Cell-free culture and freeze-dried supernatants of *Lb*. *amylovorus* JG2 and the non-antifungal *Lb*. *amylovorus* DSM20531 were used to access and compare antifungal activity on agar and microtitre plate assays. When freeze-dried cell-free supernatant powder from *Lb. amylovorus* JG2 was incorporated in culture medium at concentrations greater than 1%, *P. infestans* mycelial radial growth was inhibited. Additions of 12.5 % caused complete inhibition of *P. infestans* growth on the basis of turbidity. Cell-free supernatants of *Lb. amylovorus* JG2 and DSM 20531 were analysed by Liquid Chromatography Fourier Transform Mass Spectrometry (LC-FTMS) and High-Resolution Gas Chromatography-low resolution Mass Spectrometry (HRGC/MS). Fifteen antifungal acids as well as four cyclic dipeptides were detected. This study has demonstrated that biological control, accomplished by beneficial microorganisms, may be a viable “green approach” to reducing late blight.

F002

Kinetics and qualities of stress response of *Lactobacillus paracasei* ssp. *paracasei* analyzed by MALDI-TOF MS

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Lactic acid bacteria (LAB) are widely used as starter cultures and probiotics in dairy products. Although LAB are fastidious with respect to fermentation substrates and may exhibit low survival rates in preparation processes, resource-saving production of LAB and fermentation processes are advised. This can be achieved through the identification of biomarkers and stress proteins related to their fitness and survival, and subsequently exploit these for pre-conditioning of strains. In order to identify responsive conditions, we subjected *Lactobacillus (L.) paracasei* ssp*. paracasei* to different stress qualities (osmotic stress, oxidative stress, temperature stress, pH stress and starvation). Using MALDI-TOF MS we analyzed the kinetics of its stress responses along the expression of stress proteins. It was possible to monitor the expression of stress proteins, identify a specific time point of 60 minutes when the expression of stress proteins reached its maximum, and statistically differentiate types of stress responses into groups. These results demonstrate the discriminatory power of MALDI-TOF MS to characterize stress response dynamics and types of *L. paracasei* ssp*. paracasei* and enable a knowledge-based focus for the identification of these biomarkers and stress proteins. Moreover, we generally propose MALDI-TOF MS as an easy and quick method to characterize responses of microbes to different environmental conditions, to focus efforts of more elaborate approaches on responsive time points and stress qualities.

F003

***Lactobacillus* as delivery vector for a novel Tuberculosis vaccine -Specific targeting of dendritic cells yields strong immune responses**

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About one third of the world's population is infected by *Mycobacterium tuberculosis* (Mtb), the causative agent of tuberculosis (TB). New vaccines with simplicity, safety and low cost, and effective against all forms of TB are urgently needed. Microbial vaccine delivery vehicles have the potential to become the cheapest and safest option for vaccination, because they are needle-free, and easy to produce and distribute. Their ability to induce mucosal immunity is an additional advantage.
We work on developing lactobacilli, which are natural inhabitants of the human gastrointestinal tract, as producer and delivery vector of such vaccines.In the present work *Lactobacillus plantarum* has been manipulated to direct and surface anchor Ag85B-ESAT6, a fusion of two major Mtb antigens. The antigen was fused to dendritic cell (DC) binding peptides. The surface exposed location was confirmed by flow-cytometry and fluorescence microscopy. Fusion of the DC-targeting peptide to the displayed antigens resulted in a significantly higher uptake of the recombinant lactobacilli by DCs.
The recombinant bacteria were administrated to mice via the nasal route. The immune response was evaluated in Ag85-ESAT6 or BCG stimulated peripheral blood mononuclear cells (PBMCs). IFN-g and IL-17 release by stimulated PBMCs from immunized mice showed that the DC-targeting bacteria are highly immunogenic. The developed expression systems allow precision display of antigens and targeting to immune cells, without harming the viability of the lactobacilli. The results obtained with Ag85B-ESAT6 are promising.

F004

Searching for anti-allergic function of lactic acid bacteria

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Okinawa has a subtropical climate, it is warm with a lot of rain, so a variety of bioresources that have adapted to this climate exists. This variety also includes microbes, we focused on those microbes that potentially have useful functions.In particular, we chose to focus on lactic acid bacteria (LAB), it has recently been reported that LAB can serve as a probiotics, help in intestine regulating, and has anti-allergic functions. LAB acquired from Okinawan bioresources has been shown an especially inhibitory effect on a mountain cedar pollen allergy. LAB samples were added to IgE antibody induced human peripheral blood lymphocyte, a decrease of IgE was used as an indicator of anti-allergic properties. As a result, six strains of LAB isolated from Okinawan bioresources such as mango and pineapple showed depressed production of IgE. These strains were identified by 16S rRNA-based analysis as in *Lactobacillus paracasei* and *Enterococcus faecalis*. Proposed industrial applications will be based upon our findings and the genus and species of the LAB.

F005

Bioinformatics and real functions of a lactic acid bacteria isolated from the Okinawan natural environment

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It is thought that a variety of bioresources are existing in Okinawa have adapted to the peculiar subtropical climate of this area. Lactic acid bacteria (LAB) of these bioresources are considered to be useful microbes. We found out one LAB named IM-1. So, we attempted a genome sequencing of this IM-1(*Lactobacillus casei* IM-1 strain) using next-generation sequencers. As a result, it was predicted that within the IM-1 strains many amino-acid-metabolism genes would be conserved, rather than the candidate used as a comparison. Pathway reconstruction was carried out using the Kyoto Encyclopedia of Genes and Genomes (KEGG). KEGG is a database resource for understanding high-level functions and utilities of the biological system. From amino-acid-biosynthesis pathway analysis, there was some suggestion that the IM-1 strain biosynthesized six kinds of amino acids. First, we examined amino acids natural demands, having prepared the *Lactobacillus casei* complete synthesized culture medium. We next removed each amino acid. Samples were prepared that added all 16 amino acids without Gly, Ala, Gln, Asn as a positive control and without amino acid as a negative control. Prepared LAB liquid was inoculated and it was cultivated for 48 hours. As a result, eight kinds of amino acids, Gly, Ala, Pro, Met, His, Lys, Asp, and Ser, became 50% or more of control number of the LAB. The result suggested the possibility of the IM-1 strain biosynthesizing six types of amino acids. And it is considered carrying out industrial use of the IM-1 strain.

F006

Comparative analysis of polysaccarides encapsulated *Lactobacillus plantarum* with cell wall anchored heterologous protein

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The oral administration of microencapsulated probiotics carrying heterologous proteins through the Gastro intestinal tract (GIT) can be a novel approach for vaccine delivery. To date, immunogenecity profile of encapsulated *Lactobacillus plantarum* with cell wall anchored antigen is still unknown. The encapsulation of recombinant vaccine is believed to enhance the viability of the cell in the intestine, therefore, up-regulated the immune system. The purpose of study is to compare the coating ability and immunogenecity of the encapsulated *L. plantarum* with cell wall anchored antigen. The lactic acid bacteria, *L. plantarum* with or without cell wall anchored heterologous protein were encapsulated into calcium alginate beads which were coated with chitosan and alginate layer. The beads produced were tested for the cell survivability under stress conditions designed to simulate the GIT passage. Encapsulated and unencapsulated (free cell) cells were then incubated in Simulated Gastric Juice (SGF) solution (pH1.8) followed by incubation in Simulated Intestinal Juice (SIF) solution at pH7.45 and then tested for its viability. Three percent alginate coated chitosan beads provided the best protection for *L. plantarum* in all treatments. While free cell only survive first 60 minutes with 2.99×10^2 cell viability compared to encapsulated cells with 6.25×10^8 cell viability after 120 minutes of the SGF solution treatment.

F007

Evaluation of the composition of bifidobacterial human gut communities through an ITS-metagenomics protocol

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The precise appraisal of the composition of the human gut microbiota still represents a challenging task. The advent of next generation sequencing approaches has opened new avenues in dissecting the microbial biodiversity of this ecosystem through the use of 16S metagenomics approaches. However, the detailed representation of specific members of the human gut such as bifidobacteria may be largely influenced by the PCR primers employed in the amplification step of 16S metagenomics pipeline. Here, we define the internal transcribed spacer (ITS) sequences of all the taxa harbouring the genus *Bifidobacterium*, providing a *Bifidobacterium*-specific primer set that amplify an hyper-variable region within the ITS sequences suitable for precise taxonomic identification of all the 48 so far recognized taxa harbouring the *Bifidobacterium* genus. In addition, we present an enhanced protocol for ITS sequence-based profiling based on QIIME software, allowing accurate reconstruction of the composition of the bifidobacterial communities residing in the human gut reaching subspecies accuracy.

F008

FlowFisH in the assessment of multiple-species starter cultures

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Commercial starter cultures consisting of different species are applied in the fermented food industry worldwide. During the manufacturing of such cultures the monitoring and final evaluation of the bacterial flora composition are crucial to guarantee consistent quality and functionality of the product.
Up to date traditional plate counting on selective agar media is applied to evaluate the percentages of the different species. However, these methods hold some disadvantages such as long incubation times, much lab work, low selectivity of media, and underestimation of cells due to clumping, specific growth requirements and more.
Given these constraints, flow cytometry (FCM), primarily developed for medical applications, has become of high interest in biotechnological fields offering a highly precise quantification of cells and analysis of cell parameters in nearly real time.
However, no discrimination of the species is possible by FCM. This can be achieved by combining Flow cytometry and Fluorescent *in situ* hybridization (F*is*H). Flow-F*is*H analyses are based on *in situ* hybridization of whole cells with fluorescently labelled selective oligo-nucleotides followed by flow cytometric analysis. Oligo-nucleotides can be specifically designed for the species of interest.
The labelling of oligo-nucleotides with different dyes even allows a simultaneous detection of two different species within the same sample preparation.
In the current study different oligo-nucleotide probes targeting different species were designed and applied to multiple species cultures. The poster will reflect on advantages and challenges of FlowF*is*h and present its application in the analysis of thermophilic and mesophilic multiple species cultures.

F009

Chromosomal expression of the *Clostridium botulinum* serotype A neurotoxin heavy chain antigen by *Lactobacillus acidophilus*

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Clostridium botulinum produces potent neurotoxins that cause botulism in humans. In this study, we expressed the non-toxic receptor binding domain of the heavy chain of *C. botulinum* serotype A neurotoxin in conjunction with the dendritic cell (DC) targeting peptide in *Lactobacillus acidophilus* NCFM. Two genetic cassettes, each consisting of the signal peptide from the S-layer protein A of *L. acidophilus*, the serotype A1 neurotoxin heavy chain receptor binding domain (BoNT/A-Hc) and either the DC targeting peptide or the DC control peptide sequence were codon optimized for *Lactobacillus*. These cassettes were cloned into pTRK882 which controls expression from a constitutive phosphoglycerate mutase promoter. Additionally, both cassettes were cloned into the integration vector pTRK1038 to facilitate chromosomal expression in *L. acidophilus* downstream of LBA0889, a highly expressed enolase gene. Western blot analysis confirmed heterologous expression of the BoNT/A-Hc antigen from both plasmid and chromosomal locations. Additionally, higher expression was observed by Western blot when the recombinant strains were grown in a glucose semi-defined medium (GSDM) compared to MRS medium. Reverse transcriptase quantitative-PCR (RT-qPCR) confirmed higher BoNT/A-Hc mRNA copy numbers in GSDM compared to MRS grown strains. Additionally, RT-qPCR analysis demonstrated comparable BoNT/A-Hc mRNA copy numbers from the chromosomally expressed strains and the plasmid expressed strains. This study demonstrated that the chromosomal integrative recombinant strains are promising vaccine delivery vehicles to circumvent plasmid instability issues and undesired requirements for antibiotic selective maintenance of recombinant plasmids.

F010

Quantitative proteomic investigation employing stable isotope labeling by peptide dimethylation on protein changes of *Lactococcus lactis*

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Bacteriocinogenic lactic acid bacteria (LAB) can be affected by various environmental stresses such as temperature, presence of chemical substances, acidic condition and nutrient limitation. To ensure the effectiveness of bacteriocins in food systems, we need to consider the adequacy of the environment for bacteriocin stability and production. The quantitative proteomic approach is an invaluable tool for understanding the adoption of LAB to stressful environments.
Lactococcus lactis (7.17) isolated from mung bean sprout was found to have anti-listerial property in simulated medium of lettuce or cantaloupe juice. A quantitative proteomic investigation employing stable isotope labeling by peptide dimethylation was conducted on *Lact. lactis* cultured in lettuce or cantaloupe juice at 10 °C for 0, 3 and 7 days. Two biological replicates were prepared and analyzed. Our results indicated that 718 and 832, or 912 and 620 proteins were identified and quantified for two replicates samples of *Lact. lactis* cultured in lettuce juice or cantaloupe juice, respectively. Furthermore, 48 or 30 proteins were quantified as the significantly changed ones, respectively. Using the normalized ratio of protein abundance changes, proteins that changed two- fold or more were identified as up- or down- regulated ones for *Lact. lactis*. Interesting proteins were further grouped into four clusters based on their quantitative changes. Among the up-regulated proteins were the ones involved in translation, carbohydrate metabolic process, oxidation-reduction and methylation while the down-regulated proteins were those that are responsible for carbohydrate metabolic process, glycolysis, translation and metabolic process. This study provides useful information on the regulation of *Lact. lactis* proteins under nutrient starvation conditions that lay the foundation for further researches and application.

F011

Screening for texturing *Lactococcus* strains

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Texturing *Lactococcus lactis* strains are of interest for mesophilic dairy products. A screening assay was developed, combining small scale 96-well microtiter plates and automated liquid handling, to find strains that give good texture in fermented milk.
Over 1100 *Lactococcus* strains were screened. Less than 1% of the strains analyzed were found to enhance milk texture significantly, the majority belonging to the subsp. *cremoris*. In order to elucidate mechanisms behind the texture-enhancing properties of these strains, selected strains were genome sequenced. As exopolysaccharide (EPS) production is believed to positively contribute to fermented milk texture, mining for EPS genes was performed. Several plasmid-associated EPS gene clusters were identified and characterized in the *L. lactis* subsp. *cremoris* texturing strains, whereas the *L. lactis* subsp *lactis* EPS gene clusters seem chromosome encoded. All the texturing strains contained *epsR* to *epsD* genes, which encode for regulation, polymerization, chain length determination and transfer of glycosyl groups, while the strains giving highest texture contained several additional glycosyltransferases and other genes related to EPS production.

F012

Development of recombineering tools in *Lactococcus lactis*

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Recombineering as an *in vivo* genetic engineering method was first developed in *Escherichia coli* using the prophage Rac or bacteriophage λ generalized recombination systems. It allows introducing any modifications into any part of the bacterial chromosome or plasmid DNA without an intermediate cloning step by mediating direct homologous integration of ssDNA or dsDNA fragments into the chromosome. Recombineering has been exploited in numerous ways, including mutagenesis - point mutations, deletions, insertions, as well as gene knock-outs and *in vivo* cloning. Moreover, it provided new potential for large scale approaches, such as high throughput tagging of proteins. Up to date recombineering techniques have been successfully applied for manipulations in Gram-negative bacteria, but are limited for the Gram-positive branch. Thus, recombineering systems based on phage-encoded recombination functions would be an attractive alternative to traditional methods for the genetic manipulation of Gram-positive bacteria, especially those that are not naturally competent. To analyze the possibility of applying recombineering in *Lactococcus lactis* Gram+ bacterium, we identified by a large-scale computational analysis of bacteriophage and prophage genomes from Gram-positive bacteria specific phage Recombination Modules (RMs). To avoid difficulties of cloning and maintaining phage RMs, an original expression system based on the universal for Gram-positive bacteria regulon controlling copper homeostasis has been developed. The RMs from lactococcal phages bIL309, bIL67 and bIL285 were examined for their ability to promote locus-specific integration of linear dsDNA fragments carrying an antibiotic-resistance marker (EmR). Efficiency of integration cassettes depending on the length of DNA homology and target in the *L. lactis* IL1403 chromosome has been tested. The work is in progress to test the functionality of other phage RMs.

F013

Development of a method for tracking industrially important strains of *Lactococcus lactis*.

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Lactococcus lactis is an AT-rich, Gram-positive bacterium that is extensively used for cheese production worldwide. Majority of cheese production is initiated with starter cultures. Mesophilic starter cultures are composed of a number of strains of *Lactococcus (L.) lactis*, sometimes supplemented with other species of lactic acid bacteria (LAB). During the starter propagation and the fermentation process, strains composition undergoes large fluctuations, which can be triggered by several factors e.g. phage infection. However, tracking of fluctuations of *L. lactis* strains is problematic to perform using standard phylogenetic tools like e.g. 16S amplicon sequencing. This is because most of these tools are limited to the species level.
We have investigated the potential of four gene regions that could be used for tracking of *L. lactis* strains. Regions were localized within so-called pellicle or cell wall polysaccharide operon. The method relies on sequencing amplicons using the MiSeq Illumina technology and has potential to be used for tracking lactococcal strains within dairy fermentation environment in a high throughput manner.

F014

Leaderless Leading: Introducing post-translational modifications into leaderless bacteriocins

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Ribosomally-synthesized and post-translationally-modified peptides (RiPPs) are well known for their potential to provide various modification systems for peptide stabilization. Bacteriocins, including many kinds of RiPPs, are antimicrobial peptides produced by diverse Gram-positive and -negative bacteria. Generally, bacteriocins are translated as inactive precursors with N-terminal extensions termed leader peptides, which are required as recognition sites in a maturation process including post-translational modification and/or secretion, and the matured active bacteriocins are formed after cleavage of leader peptides.
Lantibiotics are defined as post-translationally modified bacteriocins containing dehydrated residues and thioether bonds i.e. lanthionine (Lan) and β-methyl lanthionine (MeLan), which enhance their stability and are required for their activity. Nisin is the best known lantibiotic and is already applied for food preservation in more than 80 countries, as a safe and powerful preservative to eliminate food spoilage bacteria.
In contrast to the majority of bacteriocins including the RiPPs, there has been reported a group with a very simple maturation process, so called leaderless bacteriocins, that contain no post-translational modification and even no leader peptide, allowing them to be immediately active after translation inside cells. In this project, in order to create promising new-to-nature peptides with enhanced antimicrobial activity and/or novel bioactivity, we utilize leaderless bacteriocins as leading substrates for several modification systems. Now we are introducing lantibiotic modifications into leaderless bacteriocins using the nisin leader fused to the leaderlees peptide and NisBTC modification system in *Lactococcus lactis* or *Escherichia coli*. First results show the feasibility of this approach, since we can produce and process new to nature peptides, after cleavage of the leader of leaderlees bacteriocins containg at least one lanthionine ring.

F015

Optimization of heterologous surface display of DARPINs on various species of non-genetically modified lactic acid bacteria

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Lactic acid bacteria (LAB) are attractive hosts for surface display of heterologous proteins due to their long and safe usage in food industry and their potential health-promoting properties. Surface display on non-genetically modified organisms (non-GMO) is preferred for therapeutic and food applications. Non-GMO display has already been demonstrated by fusing the recombinant proteins to the C-terminal part of AcmA (cA) which contains three peptidoglycan-binding lysine motif domains (LysM). The goals of the present study were to select optimal lactobacilli carrier for heterologous non-GMO surface display, and to improve LysM-mediated surface display in *Lactococcus lactis*.
As a model binding protein for the display on the bacterial surface, we introduced designed ankyrin repeat proteins (DARPins) I_07 and I_19, which bind Fc domain of human IgG. We expressed DARPins as fusion proteins with Usp45 secretion signal on the N-terminus and cA on the C-terminus. We have shown high expression of DARPin_cA fusion proteins on the surface of *L. lactis* with flow cytometry, fluorescent microscopy and whole cell ELISA. Non-GMO LAB were incubated with the DARPIN_cA fusion protein-containing growth medium of recombinant L. lactis to achieve heterologous binding. We tested ten non-GMO *Lactobacillus* strains for non-covalent binding of I07_cA fusion protein to their surface. Among them, *Lactobacillus salivarius* exhibited increased surface display ability of several orders of magnitude higher in comparison to other strains. *Lactobacillus salivarius* is therefore a good candidate for heterologous non-GMO surface display. Additionally, we observed increased heterologous surface display on *L. lactis* when antibiotics were added to the growth medium during bacterial growth.
Effective display of DARPINs on the surface of non-GMO LAB opens up several new application possibilities.

F016

Occurrence and diversity of CRISPR-Cas systems in Bifidobacteria

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Clustered regularly interspaced short palindromic repeats (CRISPR), together with CRISPR-associated proteins (Cas) constitute the CRISPR-Cas system, which provides adaptive immunity against exogenous genetic elements in bacteria and archaea. Typically, DNA from invasive elements is captured in CRISPR loci and subsequently transcribed into small interfering RNAs that guide Cas nucleases for sequence-specific targeting and cleavage of complementary DNA. We analyzed the genome sequences of 35 bifidobacterial species and identified a total of 56 distinct CRISPR-Cas loci. Each of the three main types of CRISPR-Cas systems were observed, represented by 43 Type I, six Type II and seven Type III systems, respectively. Overall, occurrence (35/47 genomes, 75%) and diversity were much higher than what is generally observed in the genomes of bacteria, of which approximately 46% contain CRISPR loci. We further observed diversity in terms of array size with CRISPR spacer number ranging from 4 to 172, displaying an unusually high average of 60 spacers per locus. Noteworthy, we observed CRISPR loci in all the major phylogenetic groups of bifidobacteria, indicating these systems are evolutionarily widespread throughout this genus. Matches between CRISPR spacer sequences and those of bacteriophages and plasmids imply these systems may be functional in providing adaptive immunity against exogenous DNA in bifidobacteria. Altogether, these results suggest that diverse CRISPR-Cas immune systems are widespread in the genomes of bifidobacteria and that they may be leveraged for applications in typing and engineered phage resistance in these backgrounds.

F017

Lactic acid bacteria isolation from fermentative reactors producing hydrogen fed with agro-industrial substrate

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Biohydrogen production by fermentative consortium allows combinations of metabolic pathways and use of several substrates. In the present study, it was isolated and quantified viable population of lactic acid bacteria (LAB) from bioreactors fed with whey-milk or cassava for H_2 production aiming to understand how LAB and/or their bacteriocins influence H2 production in fermentative process.
Continuous anaerobic fluidized bed reactors (AFBRs) fed with whey-milk or cassava were operated during 160 days for fermentative H_2 production. Colonies (218) were selected on MRS agar and 94 strains were designated as presumptive LAB. Whey-milk reactors (RW1 and RW2) had LAB count ranging from 20 to 2.2 $x10^9$ CFU/ml and cassava reactors (RC3 and RC4) count of LAB ranged from <10 to 1.5 x 10^9 CFU/ml. Sequencing of 16S rRNA gene identified *Lactococcus lactis* (c.a. 70%), *Leuconostoc pseudomesenteroides*, *Lactobacillus casei* and *Bifidobacterium* sp. in whey-milk reactors. For cassava-fed reactor (RC3) it was found *Lactobacillus* sp. (45.4%), followed by *L. lactis* (31.8%), *B. subtilis* (13.6%) and *Leuconostoc citreum* (9.1%). Reactor RC4 operated with an increased organic loading (15 g COD L-1) had increased prevalence of *Lactobacillus* sp. (78.6%), *Leuconostoc citreum* (7.14%), *Lactococcus* spp. (7.14%) and *Bifidobacterium* (7.14%). Inhibitory activity was observed for 46.6% and 16.3% of the strains isolated from whey-milk and cassava reactors, respectively, with *L. lactis* as the main bacteriocinogenic genera (76.2%). It was observed that the LAB and their bacteriocins modulated the fermentative process of H_2 production from agro-industrial wastewater. Whey-milk reactors had two points of reduction of H_2 yields with simultaneous high numbers of bacteriocinogenic strains, whereas, in cassava reactors was observed absence of bacteriocin-producing strains when H_2 yields were maximum.

Author		Abstract code				
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Abe	F.	B045	B048			
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Adamberg	K.	SL 09	E046			
Adepehin	J.	E034				
Agback	P.	B099				
Agrawal	A.	D005				
Aguiar E. Câmara	S.P.	E097				
Aguilar	A.	E078				
Ahl	D.	D043				
Ahn	Y.T.	D052				
Ait Addi	E.H.	E033				
Ait Ben Oumar	A.	E033				
Aktas	B.	D041				
Alard	J.	D046				
Aleksandrzak-Piekarczyk	T.	B025				
Alexander	T.	SL 15				
Alexandraki	V.	A032				
al-Hindi	R.	A031				
Alkema	W.	A024	E050			
Aller	K.	E046				
Altermann	E.	A027	B083	D053		
Amaral	M.F.	B078				
Ammerlaan	B.C.H.	SL 04				
Amoresano	A.	SL 12				
Anastasiou	R.	A030	A032	E108		
Anderegg	J.	A019				
Andersen	H.S.	E111				
Andersen	K.L.V.	E111				
Anderson	R.C.	D053				
Andreevskaya	M.	B004				
Anekella	K.	E022				
Aoudia	N.	D040				
Aoyama	H.	F005				
Arakawa	S.	F005				
Ardö	Y.	E024				
Arena	M.P.	E026	E031	B023	D018	E037
Arendt	E.K.	B063	B064	E003	F001	
Arike	L.	SL 09				
Arioli	S.	B009				
Aristimuño	C.	A021				

Aristimuño Ficoseco	M.C.	B086				
Armalyte	J.	B006	B052	D029		
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Arroyo-López	F.	E040				
Aryantini	N.	D011				
Asami	Y.	B094				
Atamer	Z.	E085				
Atarashi	H.	B014				
Auburger	C.	D007				
Auvinen	P.	SL 13	B004	B071		
Axel	C.	F001				
Axelsson	L.	B061				
Azcarate-Peril	M.	B083				
Azevedo	V.	B001	D037			
Aznar	R.	A021	B079	C004		
Bachmann	H.	A024	B037	B080	E096	
Backus	L.	B073				
Bai	Y.P.	E116				
Ballal	S.A.	D006				
Balolong-Parungao	M.	D020				
Balzarini	J.	D042				
Bansal	N.	SL 07				
Bardowski	J.K.	A020	B008	B025	B053	B068
		B084	E065	F012		
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Barrangou	R.	SL 03	A018	A026	B020	F016
Bartowsky	E.J.	SL 02				
Bastard	A.	E087				
Bastin	G.	B046				
Bayjanov	J.R.	B018	B073	E050		
Béal	C.	D006				
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Beerthuyzen	M.	A024	B080	E096		
Beganovic	J.	D027				
Begley	M.	B098				
Behr	J.	A003	D003	F002		
Belkum, van	M.J.	B011				
Benavent-Gil	Y.	B067	E113			
Benjakul	S.	E016				
Bennedsen	M.A.	B072				
Benozzi	E.	E059				
Berbegal	C.	E091				
Beresford	T.P.	E109				
Bergsveinson	A.	B003	E063			
Berlec	A.	SL05	F015			

Bermudez-Humaran	L.G.	D015	D037			
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Berthoud	H.	A011	A034	B033	B034	
Bessmeltseva	M.	E081				
Beyjan	J.R.	D001				
Bidnenko	E.	A020	F012			
Birkelund	M.	E068				
Biswas	I.	B087				
Biswas	S.	B087				
Björkroth	J.	B004	D022			
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Blom	B.	A030				
Böck	D.	A019				
Bockelmann	W.	B075				
Bockhorn	H.	F008				
Boeijen, van	K.H.	E009				
Boekema	E.J.	B069				
Boekhorst	J.	A024				
Bogovic Matijasic	B.	E021				
Bonacina	J.	B042				
Bonvini	B.	A004	SL 10			
Boreczek	J.	B068				
Borneman	A.R.	SL 02				
Bosma	T.	B090				
Bottacini	F.	A002	A022	B020	B029	
Bottari	B.	A014	B060			
Boudyach	E.H.	E033				
Boutillier	D.	D014				
Boyaval	P.	E084				
Brandsma	J.	B054	E066	E089		
Brazeilles	R.	D005				
Briner	A.	F016				
Brinks	E.	E085				
Brix	S.	D039				
Broadbent	J.R.	B035	C005	E105	E107	E112
		E114	E117			
Broberg	A.	B099				
Brockmann	E.	E111				
Broek, van den	M.	D051				
Bron	P.A.	B070	E050			
Brosnan	B.	F001				
Brown	L.	B044	B082			
Bruggmann	R.	A011	B034			
Bruno-Barcena	J.M.	B083				
Budde-Niekiel	A.	E067	F008			
Buist	G.	B006	B022	B090		

Buivydas	A.	F014				
Bulajic	S.	C003				
Bumgardner	S.	D008				
Buntin	N.	A013				
Butinar	M.	SL 05				
Cabrera-Ostertag	I.J.	E054				
Caggianiello	G.	B018	E026	B023	E037	
Call	E.	D032				
Callejón	S.	B095				
Campbell	L.	F010				
Campion	A.	E099				
Capozzi	V.	B018	E026	E031	E032	B023
		D018	E037	E059		
Cappellin	L.	E059				
Capronnier	S.	D015				
Capuani	A.	D007				
Carminati	D.	A004	C001	E015	SL 10	
Carrizo	L.	A028				
Carroll	I.	D038				
Casarotti	N.	B078				
Casey	E.D.	A022				
Castelain	M.	D029				
Castellano	C.	E094				
Castiglione Morelli	M.A.	A007				
Castioni	A.	A014				
Catarino	T.	B092				
Cattivelli	L.	SL 10	B018			
Cavanagh	D.	E090	A027			
Celebioglu	H.U.	D039				
Centurión	D.	E074				
Cepeda	A.P.	B092				
Chain	F.	D015				
Chambers	J.	SL 02				
Chamlagain	B.	SL 13				
Chapot-Chartier	M.	B006	B052	B038	D029	
Chat	S.	B006	D029			
Chaves Ribeiro	S.I.	B081				
Chen	J.	B039				
Chen	S.	D030	F010			
Chen	X.	D033				
Chen	Y.	B049	E044	E061		
Chervaux	C.	B029				
Chifiriuc	C.	B016				
Chluba	J.	D040				
Chmielewska-Jeznach	M.	B053				
Chodzik-Kozlowska	J.	D047				

Choi	I.D.	D052				
Christiansen	L.D.	E111				
Chudzik-Kozlowska	J.	D044				
Cioffi	S.	E028				
Cirrincione	S.	E049				
Ciusa	M.L.	E043				
Claes	I.	D051				
Clark	A.	B029				
Clerens	S.	D053				
Cocconcelli	P.S.	A021	B079	C004		
Coelho	C.	E020	E087			
Coffey	A.	B063	B064	E003	F001	
Connelly	L.E.	E029				
Constandse	T.	E051				
Cortes-Perez	N.	D029				
Cosentino	S.	A033	E043			
Costa Gomes Silva	C.	E020				
Costello	J.	SL 02				
Cotter	P.D.	A027	B077	B096	B098	E099
Courtin	P.	B006	B038	B052		
Cousin	F.J.	B021				
Coûté-Monvoisin	A.C.	A029				
Cruz-Pio	L.E.	B067				
Culumber	M.	E107	E117			
Czyzowska	A.	E056				
Dague	E.	D029				
Dal Bello	F.	A022				
Dalmasso	M.	D002				
Daly	K.M.	B096	B098			
Dandoy	D.D.	B074	E084			
Daniel	C.	D014				
Dapkevicius	M.L.E.	E020	E097			
David	B.L.	E084				
De Vrese	M.V.	B075				
De Vuyst	L.	A015	B076	E100		
De Wolfe	T.J.D.	D041				
De Wouters	T.	A019				
Dean	G.	D008				
Del Carmen	S.	D037				
Delplace	B.	B074				
Delvigne	D.F.	E103				
Dennin	V.	D014				
Deplano	M.	A033	E043			
Deptula	P.	SL 13				
Derks	E.	SL 04				
Deschuyteneer	G.	E084				

Desclée de Maredsous	C.	D015				
Díaz-Ruiz	G.	E074				
Dijkstra	A.R.	E050				
Dijl, van	J.M.	B090				
Dillen, van	S.	A029				
Dimitrijevic	R.	F003				
Dobrowolska	D.A.	E073	E075			
Dobson	A.E.	E066	B054			
Domek	M.J.	E107				
Domingos-Lopes	F.P.	E020				
Donath	M.	E114				
Doolgindachbaporn	S.	E002				
Doorn, van	J.M.	SL 04				
Doucette	C.	D030	F010			
Douillard	F.P.	B100	D004			
Draper	L.A.	B077				
Drosinos	E.F.	E031				
Drucbert	A.S.	B040				
Du	P.	E064				
Duar	R.	SL 08				
Dubar	M.	B074				
Duchêne	M.C.	B013				
Dufrêne	Y.	D029	E084			
Dupres	V.	D029	E084			
Duranti	S.	A002	A008	A009	B020	D016
		D017	F007			
Durmaz	E.	E030				
Duviau	M.P.	D029				
Dybka	K.	B043				
Eckhardt	T.H.	B057	B069			
Edema	M.O.	E034				
Ehrlich	S.D.	D005				
Ehrmann	M.A.	D003	D007			
Eijsink	V.	D026				
Eijsink	V.G.H.	F003				
Ekundayo	F.O.	E006				
El Khoury	M.	E032				
Elizaquível	P.	A021				
Elke	B.R.	B072				
Elmoslih	A.	E033				
Endo	A.	B014	B021			
Engelhart	E.	E114				
Espinosa	J.	E074				
Eugster	E.	A011	B034			
Fabiszewska	A.	E018				
Fadda	M.E.	A033	E043	E098		

Fan	L.	D030	F010			
Faulhaber	K.	B066				
Faurie	J.M.	D005				
Felis	G.E.	A014	A016	A017		
Fenster	M.	E067				
Fernández Ramírez	M.D.	B028				
Ferranti	P.	E052				
Ferrario	C.	A009				
Ferreira	P.	E079				
Ferreira	S.	A030				
Ferrer	S.	B067	E078	B095	E080	E091
		E092	E106	E113		
Fett	B.	E067				
Feuchtenhofer	L.	E101				
Field	P.	B096	B098	E099		
Fillmore	S.	D030				
Findlay	B.	E116				
Findrik	K.	D028				
Fiocco	D.	E026	B023	D018	E037	
Fitzgerald	G.F.	E090	A027	E109		
Fléchard	M.	B040				
Florys	K.	E065				
Foksowicz-Flaczyk	J.	E073	E075			
Folkertsma	B.	SL 04				
Fontaine	L.	B031	B040	B046	B074	E084
Fontana	C.	B079				
Fornasari	M.E.	A004	E015	SL 10		
Förster	J.	B041				
Fotschki	J.	D048	D049			
Foulon	C.	B040				
Fracchetti	F.	A017				
Franco	B.	E059				
Franco	W.	E029				
Frank	S.	B085				
Franken	L.E.	B069				
Frantzen	C.A.	B059				
Freitas	A.S.R.	E020				
Fremaux	C.	E062	E084			
Fujiwara	D.	D009				
Fujiwara	S.	SL 14				
Fukiya	S.	B015	B047			
Fukuda	K.	D011				
Funane	K.	D021				
Furey	A.	F001				
Furlan	S.	B006	B052	D029		
Fusetti	F.	B069				

Gaenzle	M.	SL 08				
Galano	E.	SL 12				
Gallini	A.	D006				
Gänzle	M.	SL 11	B049	D033	E116	
Garault	P.	B029	D006			
García Torres	M.	E072				
Gardini	F.	A010	B058	B097	E045	E053
		E070				
Garnier	F.	E010				
Garofalo	C.	E032				
Garrett	W.S.	D006				
Garrido	C.	D040				
Garro	M.S.	E095				
Gaspar	P.L.	B041				
Gatti	M.	A014	B060	B062		
Gatto	V.	B058	E053			
Georgalaki	M.	A032	E108			
Gerritsen	J.	B055				
Gestel, van	J.	B037				
Ghazi	F.	A001				
Gijtenbeek, van	L.A.	B007				
Gioiosa	L.	D016	D017			
Giraffa	G.	A004	C001	E015	SL 10	
Glickman	J.N.	D006				
Gloor	G.	D042				
Goh	Y.J.	D031	D032			
Goin	M.	D029				
Golomb	L.	B019				
Gombossy de Melo Franco	B.F.	E102				
Gomes	B.	F017				
Gorecki	R.K.	B008	B053	E065		
Gottardi	D.	B097				
Gottlieb	C.T.	E039				
Gougeon	R.	E087				
Grabherr	R.	E101				
Grajek	K.	E073	E075			
Grangette	C.	D046				
Grattepanche	F.	E069				
Griffin	C.	B054				
Griffiths	M.W.	E035				
Grolli	S.	C001				
Gromadzka	B.	E065				
Groof, de	B.M.J.	E047				
Grosu-Tudor	S.S.	B044	E011			
Grynberg	M.	F012				
Guerzoni	M.E.	B097				

Guggenbühl	B.	A011	B033			
Guidone	A.	E028				
Guillot	A.	D029				
Guinane	C.	B063				
Guo	J.	F001				
Guo	T.	B002	B012			
Guo	X.	B091	B094			
Gurakan	G.	A001				
Guyonnet	D.	D005				
Guzzo	J.	E087	E088	D040		
Hadjilouka	A.	E031				
Haller	D.	D003				
Hallet	B.	B013				
Hamad	M.	E004	E005			
Hammer	P.	E077				
Hamodrakas	S.	A030				
Hanemaaijer	L.	A023	B065	D035	E007	
Hannon	J.H.	E066				
Hansen	G.	B027				
Hansen	L.	E068	E076	E086	F013	
Harris	M.B.	B021				
Harth	H.	E100				
Hartung	C.	D007				
Hashikura	N.	B045	B048			
Hattori	A,	F005				
Haustenne	L.	B046				
Hebert	E.M.	A015	B044	B082	C004	E052
		E104				
Hee, van	P.	A023	B065	D035		
Heidenreich	J.M.	E105				
Heinl	S.	E101				
Heiss	S.	E101				
Heller	K.	A022	B066	B075	E076	E077
		E085	E086			
Heras	J.M.	E080				
Hijum, van	S.A.F.T.	A024	B018	B023	B073	B093
		D001	E050			
Hill	C.	B054	B077	B096	B098	D002
		E066	E099			
Hinrichs	J.	E085				
Hiratou	S.	B047				
Hirayama	Y.	B015				
Hiroko	K.	E038				
Hiroshi	H.	E038				
Höll	L.	A003				
Hollander, de	H.	E007				

Holm	L.	B071				
Holms	L.	D043				
Holo	H.	B059				
Hols	P.	B013	B031	B040	B046	B070
		B074	D029	E084		
Hongpattarakere	T.	A013				
Hoogeboom	M.J.	B010				
Hooiveld	G.	D034				
Horigome	A.	B045				
Horvath	P.	A029	E062	E084		
Houghton	L.A.	D005				
Hu	Y.	E030				
Hugenholtz	F.	B098	D034			
Hugenholtz	J.	E050				
Hughes	E.	B035				
Hughes	T.	D030	F010			
Huh	C.S.	D052				
Huo	G.	E064				
Huppertz	T.	E096				
Huttinga	H.	E054				
Hwang	J.E.	E048				
Hylckama Vlieg, van	J.E.T.	B029	D005	D006	D015	
Hynönen	U.	D024	D027			
Iacomino	G.	B024				
Ianniello	R.	A007	A010	B024		
Ibrahim	M.	D011				
Ichinose	H.	D021				
Ikeda	T.	D012				
Ikehara	S.	B014				
Ikematsu	S.	E115	F004	F005		
Illeghems	K.	A015	B076			
Inglin	R.C.	SL 01				
Inoue	Y.	F004				
Irisawa	T.	B021				
Irmler	S.	A011	B033	B034		
Isomura	N.	F004				
Jääskeläinen	E.	B004				
Jaatinen	A.	D024				
Jacob	F.	E003				
Jacobsen	S.	D039				
Jagusztyn-Krynicka	E.K.	B088				
Jakava-Viljanen	M.J.	D022				
Jans	C.	A019	E110			
Janzen	T.	E068	E076			
Jego	G.	D040				
Jensen	H.M.	E041				

Jensen	P.R.	B039		
Jeong	J.W.	D052		
Jespersen	L.	B027	E083	
Ji	G.E.	E048		
Jiang	Q.	D023		
Jimenez	E.	A021	B079	C004
Jiménez-Díaz	R.	E040		
Johansen	C.L.	E062		
Johansen	C.V.	E010		
Johansson	P.	B004		
Johnson	B.R.	D038	A026	
Johnston	C.	B064		
Jong, de	A.	SL 06	B080	
Jonsson	H.	D043		
Jonsson	H.S.	B099		
Jounai	K.	D009		
Juarez del Valle	M.	A028	E057	E058
Juvonen	R.	E063		
Kadooka	Y.	D025		
Kajala	I.O.	E063		
Kajikawa	A.	D008		
Kanayama	M.	D009		
Kang	D.	D020		
Kankainen	M.	B071		
Kant	R.	D010	D022	
Kari	K.	D023		
Karimi	S.	D043		
Karna	B.	E036		
Kasuga	G.	B094		
Katina	K.	E063		
Kaufmann	N.	E071		
Kawahara	H.	F004		
Kawai	T.	SL 14		
Kawai	Y.	B091	B094	
Kaya	H.I.	E023		
Kazou	M.	A032		
Kekus	D.J.	E021		
Kennedy	S.P.	D005		
Kepa	K.	E056		
Kerrebroeck, van	S.	E100		
Kihal	M.	A001		
Kilcawley	K.N.	A027	E066	E090
Kim	J.H.	D006		
Kim	G.B.	D052		
Kim	S.	E082	D052	
Kim	T.Y.	D052		

Kimura	K.	D021				
Kinner	M.	SL 11				
Kinouchi	T.	B091				
Kishino	K.	D019				
Klaenhammer	T.R.	SL 03	A018	A026	D008	D031
		D032	D038	E030	F009	
Kleef, van	C.	SL 04	E017			
Kleerebezem	M.	B070	D001	D034		
Kleiveland	C.R.	D026	F003			
Kliche	T.	B075				
Kline	M.	E041				
Klotz	S.	E071				
Klumpp	J.	A025				
Kneževic Jonjic	N.	D028				
Kobatake	E.	D025				
Kobayashi	I.	D021				
Koberg	S.	B066				
Kobierecka	P.A.	B088				
Koedijk	D.G.A.M.	B090				
Koguchi	H.	B047				
Kok	J.	SL 06	B006	B007	B010	B022
		B037	B052	B069	B080	E096
Kong	J.	B002	B012			
Koort	J.M.K.	D022				
Kördikanlioglu	B.	E023				
Korhonen	H.J.	E072				
Korlach	J.	B029				
Koryszewska-Baginska	A.	B025				
Kos	B.	D027				
Kot	W.	E068	E076	E079	E086	F013
Kouhounde	S.H.S.	E103				
Kouwen	T.	A023	B065	D035	E007	
Kovacek	I.	D028				
Kowalczyk	M.	B053	B084			
Krieger	S.A.	E080				
Kroplewski	B.	D049				
Ku	K.H.	D052				
Kuczkowska	K.	D026				
Kuczynski	J.	A002				
Kuenz	A.	E071				
Kuipers	O.P.	B006	B022	B037	B052	B069
		F014				
Kulakauskas	S.	B006	B038	B052	D029	
Kuprys-Caruk	M.	E018				
Kuraya	E.	F005				
Kurek	D.	B022				

Kuwano	Y.	SL 14				
Kwok	L.Y.	E061				
Lacorre	D.A.	D014				
Lacroix	C.	SL 01	A019	E069	E110	
Lähteinen	T.L.	D022				
Lahtinen	S.	D039				
Lahtvee	P.J.	SL 09				
Laine	P.	B004				
Laiño	J.	A028	E057	E058		
Lamontanara	A.	SL 10	B018	B023		
Lanciotti	R.	B058	E045	E053	E070	
Landstorfer	R.	D003				
Lange	K.	D034				
Langella	P.	B036	B084	D015	D037	
Lanigan	N.	B017				
Laurent	J.	E088				
Laval	L.	D015				
Lavoy	A.	D008				
Lazzi	C.	B060	B062			
Le	L.	D029				
Lea	T.	F003				
Lebeer	S.	D042	D050	D051		
Leblanc	J.G.	A028	D037	E057	E058	E095
		E102	E104			
Leboš Pavunc	A.	D027				
Leclercq	S.	B001				
Leduc	S.	E010	E062			
Lee	I.	B070				
Leelawatcharamas	V.	E025				
Lehrter	V.	D046				
Lenoci	L.	B093				
León-Romero	A.	E040				
Lepek	K.	E065				
Leulier	F.	D001	D013			
Levante	A.	B062				
Levonen	K.	D022				
Li	B.	B075				
Liang	M.	B085				
Liang	Z.	SL 07				
Lievens	E.	D050				
Lillevang	S.K.	E008				
Lim	K.S.	E035				
Lima	F.A.	B001				
Lin	B.	SL 08				
Lindenstrauß	A.G.	D003				
Lindholm	A.	D024				

Lindlbauer	K.	E014				
Lindvald Johansen	C.	B027	E041			
Liscia	A.	A033				
Liu	W.	B012				
Lo Bianco	G.	E049				
Locci	F.	E015				
Lohans	C.	SL 08				
Lohans	C.T.	B011				
Lolkema	J.S.	B032				
Looijesteijn	E.	E042				
Lopez	P.	E060				
López-López	A.	E040				
Loren van Themaat, ver	P.E.	B065				
Loubiere	P.	B084	D029			
Lucas	P.	E032				
Lucio	O.	E080				
Ludena Urquizo	F.E.	E072				
Lugli	G.A.	A002	A008	A009	B020	D016
		D017	F007	F016		
Lynch	D.B.	B021				
Lynch	K.M.	B063	B064			
Lynch	S.	B021				
Lytra	I.	E108				
Macklaim	J.	D042				
Magalhães	A.F.	E020				
Magri	M.	A005				
Mahony	J.	A022	A023	A025	D035	
Maitre	M.	E088				
Makki	K.	D046				
Malang	S.	E069				
Målbakken	N.	D026				
Malcata	F.X.	E097				
Malik	S.	D042	D050			
Maljaars	C.E.P.	SL 04				
Maljaars	L.	E017				
Mancabelli	L.	A008	B020	F007		
Mancha Agresti	P.	B001				
Mangiapane	E.	SL 12	E049			
Mangifesta	M.	D016	A008			
Mangifesta	M.	A009	B020	D017	F007	
Mangin	I.	D046				
Manolopoulou	E.	A032	E108			
Marco	M.L.	B019				
Margolles	A.	B020				
Mariadassou	M.	A020				

Markiewicz	L.	D048	D049			
Marten	G.	E041	E055			
Martin	R.	D015	D037			
Martínez	B.	B092				
Marutescu	L.	B016				
Marx	H.	E014				
Marzohl	D.	A034				
Mastrigt, van	O.	E008				
Masuda	T.	B091	B094			
Masuda	Y.	F014				
Mathiesen	G.	D026	F003			
Matias	N.S.	E079				
Matos	C.	D001				
Mattanovich	D.	E014				
Mavrogonatou	E.	A030				
Mawatari	T.	D012				
Mcauliffe	O.	E090	A027			
Mccann	A.	B021				
Mccarthy	K.	B085				
Mcdonnell	B.	A023				
Mcleod	A.	B061				
Mcmahon	D.J.	E107	E117			
McNabb	W.	D053				
Meeuws	S.	B055				
Meidong	R.	E001	E002			
Meier	F.	E110				
Meijer	W.C.	B054	E009	E066	E089	
Meile	L.	E110				
Meile	L.	A019				
Mendoza	L.	E098				
Menton	J.F.	E054				
Mercier-Bonin	M.	B084	D029			
Merrall	A.V.	B089				
Meske	D.	B075				
Mesnage	S.	B006				
Meucci	A.	E015				
Meulen, van der	S.B.	SL 06				
Meurman	J.	D023				
Meyrand	M.	D029				
Michaud	M.	D006				
Mierau	I.	E042				
Miettinen	M.	D045				
Mignolet	J.T.R.	B031				
Milani	C.	A002	A008	A009	B020	D016
		D017	F007	F016		
Milder	F.	B090				

Mills	S.	E066	B054			
Minami	J.	B048				
Miocinovic	B.	E021				
Miquel	S.	D015				
Mirkovic	L.	E021				
Mirkovic	N.	C003				
Mishra	V.	E036				
Misset	T.	E007				
Mitchell	M.	D013				
Miura	Y.	D009				
Miyagi	M.	E115	F004			
Miyaji	T.	B014				
Miyamoto	M.	D019				
Miyoshi	A.	B001	D037			
Miyoshi	M.	D025				
Modl	J.	E014				
Mohamadzadeh	M.	F009				
Mohamed	E.	E004	E005			
Mohamed	M.	B066				
Mohedano	M.L.	E060				
Moltzau	J.A.	E024				
Mommsen	A.	F008				
Montanari	C.	B058	B097	E045	E053	
Monteagudo-Mera	A.	B083				
Monti	L.	E015				
Moon	G.S.	E082				
Mora	D.	B009	B020			
Moreno de Leblanc, de	A.	D037				
Moreno-Baquero	J.	E040				
Morone	G.	E028				
Morovic	W.	A035				
Morsomme	P.	B070				
Mosleth	E.F.	B061				
Moughan	P.J.	D053				
Mozzi	F.	A015	B042	B044	B082	E052
Muhammed	K.	E068	E076	E079		
Mukai	T.	B005	B026	D011		
Mulder	L.	B055				
Mulder	N.L.	B032				
Murphy	J.	A025				
Murtini	D.	D011				
Nagai	K.	SL 14				
Nakagawa	J.	B014				
Nakamura	T.	D021				
Narvhus	J.	A005				
Natividad	J.	D015				

Nauta	A.	A025	E042			
Nedelcu	I.	B016				
Neef	J.	B090				
Neve	H.	A022	A023	B066	E076	E077
		E085	E086			
Neves	A.R.	B041				
Neviani	E.	B060	B062			
Nhiep	T.	SL 07				
Nielsen	D.S.	E068	E076			
Nierop Groot	M.N.	B028				
Nieuwenhuijzen	H.	E009				
Nishijima	T.	D012				
Nishimura	J.	B091	B094			
Nishiyama	K.	B005				
Nitisinprasert	S.	E013				
Noben	J.P.	A022				
Noens	E.E.E.	B032				
Noirot	P.	F012				
Nyman	T.	SL 13				
Oberg	C.J.	E107	E117			
Oberg	T.S.	E107	E117			
Obert	J.	E010	E062			
O'Brien	E.	E066				
O'Connell	K.J.	A002				
O'Connell Motherway	M.	A002	A022	A025	B017	B029
O'Connor	M.	B098				
Odamaki	T.	B045	B048			
Oeregaard	G.O.R.	F011				
O'Flaherty	S.	D031	D032	F009		
Ogawa	A.	D025				
Ogawa	O.	D019				
Ogrodowczk	A.	D048	D049			
Oh	S.	E035				
Ohnesorge	S.	E041				
Ohnishi-Kameyama	M.	D021				
Ohshio	K.	D009				
Oijen, van	A.M.	B007				
Ojala	T.	B071				
Okada	S.	B014	B021			
Oliveira	J.R.	D035				
Oliveira	P.	E003				
Olsen	M.L.	E076				
Oltuszak-Walczak	E.B.	B043	E056			
Oozeer	R.	D005				
Øregaard	G.	A004	B057			
Orrù	L.	SL 10	B018	B023		

Ortakci	F.	E117				
Ortiz	M.E.	E052				
Oshima	S.	B091				
Oskolski	P.	B025				
Ossowski, von	I.	D024				
O'Sullivan	M.	E090				
Otlewska	A.	B043				
O'Toole	P.W.	B021				
Overbeck	T.J.	C005				
Øverland	L.	F003				
Paalme	T.	E081				
Paixão Domingos Lopes	M.F.	D036				
Palanza	P.	D016	D017			
Pallin	A.	B099				
Palva	A.	B100	D004	D010	D022	D024
		D027				
Pannella	G.	B024				
Papadelli	M.	A032	E108			
Papadimitriou	K.	A030	A032	E108		
Papandreou	N.	A030				
Paramithiotis	S.	E031				
Pardo	I.	B067	B095	E078	E080	E091
		E106	E113			
Parente	E.	A007	A010	B024	E027	E028
Parisi	F.	E037				
Park	D.J.	E035				
Park	M.S.	E048				
Park	P.	D019				
Passolungo	L.	E015				
Patrignani	F.	E053	E070			
Paulin	L.	SL 13	B004	B071	D010	
Paunovic	D.	C003	E021			
Péchoux	C.	D029				
Pedersen	T.B.	E024				
Peebo	K.	E046				
Pelinescu	D.	B016	E011			
Penna	A.L.B.	E093	B078			
Pereira	V.	B001				
Pérez	A.	A021				
Pérez Ibarreche	M.	E094				
Perez-Diaz	I.	E029				
Pérez-Rámos	A.	E060				
Pessione	A.	SL 12	E049			
Pessione	E.	SL 12	E049			
Petrov	K.	B030				
Petrova	M.	D042	D050			

Petrova	P.	B030		
Petrusic	M.	C003	E021	
Peucelle	V.	D046		
Peyer	L.	E003		
Pflügl	S.	E014		
Pham	T.	SL 07		
Phrommao	E.	E105	E112	E114
Picard	F.C.	E062		
Picariello	G.	E052		
Pietilä	T.E.P.	D027		
Piironen	V.	SL 13		
Pillidge	C.J.	B089		
Pisano	M.B.	A033	E043	
Plucienniczak	G.	E065		
Plumed Ferrer	C.	B079	E072	E074
Poedenphant	H.	E111		
Poiret	S.	D014		
Polo	L.	E078	E113	
Pons	N.	D005		
Poolman	B.	B007	B069	
Porcellato	D.	A005		
Portilla	S.	B092		
Portmann	R.	B033		
Pot	B.	A030	D014	D046
Poulsen	V.K.	F011		
Powell	I.B.	B089	E036	
Prentice	M.B.	B085		
Prestel	E.	A020	F012	
Price	C.E.	E017		
Prosperi	C.	B001		
Pumpuang	L.	E016		
Puri	P.	B069		
Puzia	W.	B068		
Qiao	M.	B051		
Quéré	G.	D006		
Ra	J.H.	D052		
Radulovic	T.	E021		
Radulovic	Z.	C003		
Radziwill-Bienkowska	J.M.	B084		
Rahkila	R.	B004		
Ramoni	R.	C001		
Ramos Vásquez	E.	C004		
Rasinkangas	P.T.	B100	D004	
Raya	R.	B086	E052	E098
Razny	M.	E073		
Regulski	K.	B038		

Reid	G.	D042				
Remagni	M.C.	B009				
Renault	P.	A030				
Renckens	B.	B018				
Repo-Carrasco-Valencia	R.	E072				
Reunanen	J.	B100				
Ricciardi	A.	A007	A010	B024	E027	E028
Riedel	C.	A002				
Rieu	A.	E088	D040			
Rintahaka	J.	D024				
Rinttilä	T.	D022				
Ritari	J.	B100				
Robert	V.	B084				
Roberts	J.	B029				
Rocha	C.	B001				
Rodrigues	M.G.	E079				
Rodriguez	M.C.	B086				
Rodríguez	A.	B092				
Rodriguez de Olmos	A.	E095				
Rokutan	K.	SL 14				
Rolain	T.	B013	B070			
Rollan	G.	A028				
Romaniello	A.	E028				
Romano	A.R.	E059				
Roos	S.	B099	D043			
Rosa	J.D.	E020				
Rosa	P.R.F.	F017				
Ross	P.	B054	B077	B081	B096	B098
		D002	D036	E066	E099	E109
Rossetti	L.	A004	C001	E015	SL 10	
Roubos	J.A.	B065				
Roy	N.C.	D053				
Roytrakul	S.	E016				
Rud	I.	B061				
Ruiz	L.	B017				
Ruiz Rodríguez	L.G.	A015				
Rumjuankiat	K.	E013				
Russo	P.	E026	E031	B023	D018	E037
Ryan	P.M.	E109				
Ryan	S.P.	E066				
Ryssel	M.	E083				
Saad	M.I.	E079				
Saavedra	L.	B042				
Sabalza Gallues	M.	E030				
Sabidi	S.	F006				
Saczynska	V.	E065				

Sainz	T.	E074			
Saito	K.	D021			
Saito	T.	B091	B094		
Saitoh	S.	F005			
Sakamoto	I.K.	F017			
Sakanaka	M.	B015	B047		
Salusjärvi	T.	D045			
Salvetti	E.	A016	E070		
Samtlebe	M.	E085			
Sanchez Maldonado	A.F.	E116			
Sangsuwan	P.	SL 13			
Sanozky-Dawes	R.	A018			
Santos	A.C.	B001			
Santos	F.	B061			
Saraiva	T.	D037			
Sarand	I.	E081			
Sarbu	C.	B016			
Saris	P.E.J.	B051	E023		
Sartor	R.B.	D003			
Sasaki	R.	B026			
Sasaki	Y.S.	E038			
Sauer	M.	E014			
Savage	B.	SL 04			
Savijoki	K.	SL 13			
Savo Sardaro	M.L.	B060	B062		
Savoy de Giori	G.	E057			
Savoy de Giori	G.	E058	E104		
Sawada	D.	SL 14			
Scaltriti	E.	C001	SL 10		
Schieber	A.	E116			
Schleper	C.S.	SL 15			
Schofield	L.	D053			
Scholtz	S.	F008			
Schott	A.	F002			
Schwab	C.	E069	SL 15		
Schwarzer	M.	D013			
Segata	N.	B020			
Seiman	A.	SL 09			
Selle	K.M.	SL 03	A018	D032	F016
Sendra	R.	B095			
Sengupta	R.	D053			
Serafini	F.	A002			
Serrano	L.M.	B050	B054	E066	
Serrazanetti	D.I.	B097			
Serror	P.	B006			
Servais	F.	B040			

Sesma	F.	B042				
Seto	Y.	D025				
Setyawati	M.C.	E050				
Sgarbi	E.	A014	B060			
Shani	N.	A034				
Sheehan	J.J.	E109				
Shimomura	Y.	D025				
Shinzato	N.	F005				
Shiwa	Y.	B014				
Siebring	J.	B010				
Siezen	R.J.	A006	A024	B073	B080	D001
Silva	C.C.	B081	D036	E097		
Silva	E.L.	F017				
Silva	F.	B078				
Sim	J.H.	D052				
Simsek	O.	E023				
Sinderen, van	R.	A002	A008	A009	A022	A023
		A025	B017	B020	B029	D016
		D017	D035	F007		
Sip	A.	E073	E075			
Siroli	L.	E070				
Smid	E.J.	B028	C002	E008	E047	E051
Smidt	H.	D010	D034			
Smokvina	T.	B029	D015			
Snipen	L.	B061				
Soares Dos Santos	V.	E106				
Soerensen	J.	E068				
Sokol	H.	D015				
Solari	P.	A033				
Solem	C.	B039				
Solheim	M.	B059				
Solopova	A.	B037	B052			
Somda	M.	E103				
Søndergaard	L.	E083				
Song	J.	D030	F010			
Song	Y.	A012	E035			
Sonomoto	K.	E016				
Sørensen	K.I.	A004	C001			
Sørensen	J.	E076	F013			
Souza	B.M.	B001				
Spano	G.	B018	E026	E031	E032	B023
		D018	E037	E059	E060	SES1
Spus	M.	C002	E047			
Stahl	B.	A029				
Stahl	L.	A035				
Stamatova	I.	D023				

Stancu	M.M.	E011				
Stanton	C.	B081	D036	E109		
Staring	G.T.A.J.	E009				
Starrenburg	M.J.C.	E009	E050			
Stecka	K.M.	E018	E019			
Steele	J.L.	C005	D041	E105	E107	E112
		E114				
Štefanac	M.	D028				
Stefanelli	E.	A017				
Stevens	M.J.A.	SL 01				
Stoffolano	J.R.J.G.	A033				
Stoica	I.	B016				
Storelli	G.	D001				
Streekstra	H.	E017				
Strukelj	B.	SL 05				
Štrukelj	B.	F015				
Stuart	M.C.	B069				
Suarez	N.	B042				
Sugahara	H.	B045	B048			
Sugimura	T.	D009				
Sujaya	I.	D011				
Sun	T.	E061				
Supply	P.	A030				
Šuškovic	J.	D027				
Suzuki	H.	B094				
Suzuki	S.	D019				
Svensson	B.	D039				
Swam, van	I.I.	B070				
Szatraj	K.	B025				
Szatraj	K.	E065				
Szczepankowska	A.	A020	B053	E065	F012	
Szczesny	P.	B084				
Szeliga	M.	B022				
Szewczyk	B.	E065				
Szymczak	P.	F013				
Tabanelli	G.	A010	B058	B097	E045	E053
Tada	I.	F004	F005			
Takala	M.	B051				
Takami	K.	D012				
Takao	M.	E038				
Takeshima	K.	E115				
Tamai	S.	B015				
Tanabe	T.	D019				
Tanaka	H.	E115				
Tanaka	N.	B014				
Tanaka	Y.	D012				

Tandee	K.	D041				
Tarazanova	M.	E096	B080			
Tauer	C.	E101				
Tempel, van den	T.	SL 04				
Terán	L.	B086				
Teusink	B.	B037				
Thomas	G.	D029				
Thomas	M.	B084				
Thonart	P.	E103				
Tilborg, van	M.W.E.M.	SL 04				
Tilmann	M.	E059				
Timarova	V.	E046				
Tjåland	R.	F003				
Todorov	S.D.	E102				
Tomiduka	K.	D012				
Tomita	S.	B070				
Tongpim	S.	E002				
Torriani	S.	A014	A017	B058	E045	E053
		E070				
Tosukhowong	A.	E016				
Tramutola	A.	A010				
Tran	T.L.	D029				
Traore	T.A.	E103				
Trip	H.	B032				
Trugnan	G.	D029				
Truszkowska	Z.	B068				
Tsakalidou	E.	A030	A032	E108		
Turk	B.	SL 05				
Turner	D.L.	B092				
Turner	S.	SL 07				
Turroni	F.	A002	A008	A009	B020	D016
		D017	F007	F016		
Tynkkynen	S.	D045				
Uenishi	H.	D025				
Uotinen	V.	B100				
Urashima	T.	D011				
Urich	T.	SL 15				
Uroic	K.	D027				
Usvalampi	A.	B051				
Vågesjö	E.	D043				
Väkeväinen	K.S.	E074				
Valderrama	A.	E074				
Valeriano	D.	D020				
Vanderleyden	J.	D042	D050			
Vania	M.	B018				
Vannini	L.	B097				

Varesche	M.B.A.	F017				
Varmanen	P.	SL 13				
Vassu	T.	B016				
Vederas	J.C.	SL 08	B011	E116		
Veiga	P.	B052	D005	D006		
Velikova	P.	B030				
Ventura	M.	A002	A008	A009	B020	D016
		D017	F007	F016		
Vera Pingitore	E.	B079				
Verce	M.	B076				
Verdu	E.F.	D015				
Verhoeven	T.L.A.	D042				
Viale	S.	E043				
Viappiani	A.	A008	A009	B020	D016	D017
		F007				
Viggiani	F.	A007				
Vignolo	G.	A021	A028	B079	B086	C004
		E094	E098			
Viiard	E.	E081				
Viitala	R.	D045				
Villegas	J.M.	B082	E104			
Vilu	R.	SL 09	E046			
Vinay-Lara	E.	E112				
Vindeloev	J.	E111				
Visessanguan	W.	E016				
Visweswaran	G.R.R.	B022				
Vitali	S.	A019				
Vliet, van	M.J.	SL 04				
Vogel	R.F.	A003	D003	D007	F002	
Vogensen	F.K.	E024	E068	E076	F013	E086
Vorlop	K.D.V.	E071				
Vos, de	W.M.	A013	B100	D004	D010	
Vu	T.	SL 07				
Waanders	J.M.	SL 07				
Wacher	C.	E074				
Wagner	N.	E077	E085			
Wahl	A.W.	B046	B040			
Wakai	T.	B056				
Walczak	P.P.	B043	E056			
Waligora-Dupriet	A-J.	D046				
Walter	J.W.	SL 08				
Wang	S.	B012	E112			
Wang	Y.	SL 07				
Warren	M.J.	B085				
Wasilewska	E.	D044	D047	D048	D049	

Watson	D.	B029			
Wechsler	D.	B034			
Weckx	S.	A015	B076		
Wegkamp	A.	E009	E089		
Weidmann	S.	E087	E088		
Weissing	F.J.	B037			
Welker	L.	B035			
Wels	M.	A024	B080	E051	E096
Wenjun	W.	E061			
Werning	M.L.	E060			
Westerlund-Wikström	B.	B071			
Whorwell	P.J.	D005			
Wiid	I.J.	E102			
Wijsman	M.	E042			
Wilmes	J.	E041			
Wind	A.	B072			
Wismmer	W.	SL 11			
Wojtania	A.	B088			
Wolfgruber	S.	E101			
Wolken	W.	B055			
Wolkers-Rooijackers	J.C.M.	C002	E047		
Wolowczuk	I.	D046			
Woodward	A.	D033			
Woraprayote	M.R.	E016			
Wright, von	A.	B079	E072	E074	
Wroblewska	B.	D048	D049		
Wu	P.	E112			
Wüthrich	D.	A011	B034		
Wyszynska	A.	B088			
Xiao	J.Z.	B045	B048		
Yamamoto	N.	B056			
Yamamoto	Y.	B005	B026		
Yankova	D.	E101			
Yépez	A.	A021	C004		
Yokota	A.	B015	B047		
Yoshikawa	H.	B014			
Yousfi	N.	D040			
Yu	X.	D024			
Yuji	Y.	E038			
Zadravec	P.	F015			
Zago	M.	A004	C001	E015	SL 10
Zagórski-Ostoja	W.	E065			
Zakri	Q.	E033			
Zamfir	I.	E011			
Zamfir	M.	B044			
Zang	L.	B002			

Zannini	E.	E003	E012			
Završnik	Z.J.	SL 05				
Zendo	T.	E016				
Zhang	C.	B012				
Zhang	H.	A012	D030	E044	E061	F010
Zhang	L.	D008				
Zhang	W.	E044				
Zhao	J.	SL 11				
Zhao	W.	E044				
Zheng	J.S.	SL 08				
Zhihong	S.	A012				
Zhu	Y.	SL 07				
Zhurina	D.	A002				
Zielinska	K.	E018				
Zijlstra	R.	D033				
Ziola	B.	E063				
Ziola	R.	B003				
Zlotkowska	D.	D044	D047			
Zomer	A.	A002				
Zongo	Z.C.	E103				
Zotta	T.	A007	A010	B024	E027	
Zoumpopoulou	G.	A032	E108			
Zuber Bogdanovic	I.	C003				
Zúñiga Dávila	D.	C004				
Zurita Turk	M.	B001	D037			
Zwietering	M.H.	E089				
Zylinska	J.	B008	B053	B068		