Students and Fellows Association of ISAPP

ANNUAL ISAPP-SFA CONFERENCE 2019

Participant Abstracts

May 14th – 16th, 2019
Antwerp, Belgium

About the SFA

The ISAPP Students and Fellows Association (SFA) was created in 2009 as an initiative to link trainees working in fields related to probiotics, prebiotics and health effects of commensal microbes. We operate as a trainee-led branch of our parent group, ISAPP.

Our goal is to create an interactive network of graduate students and postdoctoral fellows across the globe working on probiotics, prebiotics or related fields, and thus promote real-time interactions, intellectual and technical exchanges, and other networking opportunities for our members. We intend to act as a resource for ISAPP and the industry, providing a communication platform to facilitate scientific discussions, internships and employment opportunities among qualified researchers in the field.

Annual Meetings

The SFA began as an initiative by Gregor Reid and the students and fellows at the 2009 ISAPP meeting in Irvine, California. The SFA annual meetings are held in conjunction with the ISAPP annual meetings. Our first meeting began in 2010 in Barcelona, Spain, followed by: 2011 in Berkeley, California, USA; 2012 in Cork, Ireland; 2013 in New York, New York, USA; 2014 in Aberdeen, Scotland; 2015 in Washington D.C., USA; 2016 in Turku, Finland; 2017 in Chicago, Illinois, USA; 2018 in Singapore; and this year, in Antwerp, Belgium.

On behalf of the SFA committee, we welcome all SFA attendees to the 2019 annual SFA meeting, and we hope you enjoy your time in Antwerp!

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2018-2019 ISAPP Students and Fellows Association (SFA) Executive Committee and Acknowledgements

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The SFA Executive Committee would like to thank:

Mary Ellen Sanders, Gregor Reid, and the ISAPP Board of Directors for integrating the SFA into the ISAPP annual meeting, and for their continued financial support of the SFA organization. We also thank all our meeting attendees who make the annual meeting so thought-provoking and fun.

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P1. Sex-specific effects of microbiome perturbations on cerebral amyloidosis and microglia phenotypes

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Previously, we demonstrated that antibiotic (ABX) perturbated gut microbiome reduces amyloid-β (Aβ) plaque pathology and gliosis in male mice of Aβ amyloidosis, termed APPSWE/PS1ΔE9. To extend these findings, we employed the highly aggressive APPPS1-21 mouse model wherein the Thy1.2 promoter drives co-integrated APPSwe and PS1L166P. ABX was performed to evaluate the sex-specific role of gut microbiota in APPPS1-21 mice. To establish a causal relationship, we performed FMT experiments in ABX-treated male APPPS1-21 mice. Gut microbiota profiles, peripheral cytokines and various brain inflammation/pathology readouts were evaluated.

Similar to our previous reports, ABX perturbed microbiome was associated with a reduced Aβ burden and altered microglia phenotypes only in male mice. We observed reduced species diversity and similar microbiome profiles in both sexes immediately after postnatal ABX, but interestingly, the microbiota exhibited sex-specific differences by 7 weeks. While ABX had a significant impact on microglia morphology in male mice, ABX treated female mice did not show these alterations. Furthermore, ABX led to pronounced alterations in microglia transcripts in male cortex compared with female mice. Finally, we demonstrate that fecal microbiota from age-matched APPPS1-21 male mice transplanted into ABX-treated male mice partially restored Aβ plaque and microglial morphologies establishing a causal relationship between microbiota and AD pathology. Additionally, FMT experiments using ABX-male and ABX-female feces were transplanted into female APPPS1-21 mice to investigate if female mice exhibit similar beneficial effects from ABX-treated male fecal microbiota. The data from the later experiment is currently being evaluated and will be presented at the conference.

P2. Using a CRISPR-Cas based mutagenesis system for the functional characterization of "genomic dark matter" in novel *Lactobacillus* isolates

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The bacterial "genomic dark matter" includes all genetic material for which no functional characterization is known. This includes for example non-coding DNA and RNA, and uncharacterized genes. This genomic dark matter contains interesting and useful genes to improve food production and medicinal applications. For example, the genes for the production of the outer exopolysaccharide layer (EPS), a key factor in the probiotic function of lactic acid bacteria, are often easily identifiable based on sequence homology, but their exact function is still greatly unknown. For many of the involved glycosyl transferases there is only limited research on their genetics, structure, substrate specificity, and mechanism of action. Often their substrate specificity is not further investigated, assuming that the gene sequence is reflected in the sequence of the monosaccharides in the repeat unit of the heteropolysaccharides. Another example involves the biosynthesis genes of bacteriocins. These gene cluster can be detected, but gaps in our fundamental knowledge on their biochemical mechanism hampers their functional annotation.

In the study, we have isolated novel *Lactobacillus* strains from many different ecological niches, ranging from humans and animals (nasopharynx, vagina, inner ear), and plants (spontaneous fermented vegetables). We aim to optimize a CRISPR-Cas based mutagenesis system in these strains to obtain site-specific and knock-out mutants of different functionally uncharacterized genes. In a first phase, our interest will be focused on the gene clusters responsible for the EPS and bacteriocin production.

P3. The Fermented Food Microbiome

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With increasing evidence highlighting the microbiome as an important contributor to host health, attention has turned to modulating microbiota composition and function, including through enhancing diversity. There has been a particular emphasis on the gastro-intestinal tract (GIT), which is home to the greatest proportion and diversity of the human microbiome. Importantly, diet has a major impact on this microbiome, and there is an ever-greater interest in the potential role of fermented foods in enhancing human health through GIT-mediated activities. While the mechanism for improved health via fermented foods is not fully understood in all instances, it is thought that the large numbers of bacteria with potential health-promoting characteristics, and associated bioactive molecules, are responsible and, thus, investigations that provide a greater understanding of the microbiota of fermented foods, and their potential functions, may be of great value. 58 fermented foods from artisanal producers in 8 countries were collected. DNA was extracted for shotgun metagenomic sequencing. Bray-Curtis analyses showed clustering of samples according to type of fermentation (Brine, Dairy and Sugar).Alpha diversity was calculated using Shannons Index, Simpsons Diversity Index and Total Species Count. Brine- and sugar-based fermented foods had higher diversities than dairy food across all alpha diversity metrics. Heatmaps were used to visualise and compare the taxonomic, genetic and bacteriocin content of the foods. Kruskal-Wallis analyses revealed taxa and gene families that were different between the foods. Ultimately, this study presents a large body of new information on a range of fermented foods facilitating future investigations to uncover the health-promoting potential of these foods.

P4. Probiotic potential of lactic acid bacteria isolated from plant material

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Background: Probiotics are increasingly being used in commercial animal production to advantageously influence intestinal microbiota functioning. In a previous work, we obtained 129 isolates of lactic acid bacteria (LAB) from oat, alfalfa, rice, barley, wheat, sorghum, maize, white clover, ryegrass, sunflower, moha, *Melilotus albus*, Gatton panic and soy. In this work, the immunopotentiating capacity of 52 of those strains was determined in order to select the most promising ones for probiotic application.

Methodology: 52 strains of LAB isolated from grass and forages were co-cultured with murine macrophages (RAW 264.7). A total of 105 macrophages per well were co-cultured for 8 h with LAB cultures (18 h, 37°C, ratio 1:100). LPS (0.1 µg/ml) were used as activation control. IL-10 was measured in the supernatant by ELISA. In base on the induction of IL-10, some strains were selected and IL-1β, IL 12p70, IL 2, IL-6, IL-10, INF-γ and TNF-α were further quantified by MULTIPLEX ELISA.

Results: 18 out of 52 LAB strains induced the highest amounts of IL-10. The selected strains belong to the species *Lactobacillus plantarum, L. paracasei, Pediococcus pentosaceus, L. rhamnosus* and *Leuconostoc pseudomesenteroides*. The co-culture supernatants of the selected strains, assayed by MULTIPLEX showed different capacities to induce TNF-α secretion, and a very low capacity to induce other proinflammatory cytokines (IL-1β, IL-12p70, IL-2, IL-6, or INF-γ).

Conclusion: These results let us narrow down the number of LAB strains, from 52 to 3: *Lactobacillus plantarum* LpAv (oat), *Lactobacillus paracasei* LcAv (oat) and *Pediococcus pentosaceus* PpM (maize), based on their potential anti-inflammatory (IL-10) and epithelial barrier promotive (TNF-α) capacities.

P5. Exploring carrot fermentation as a novel carrier for probiotic strains

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Previous work in the lab has shown that the spontaneous carrot fermentation is a robust man-made ecosystem dominated by lactic acid bacteria. The high diversity and abundance of lactobacilli makes this vegetable fermentation a good candidate for a non-dairy alternative probiotic carrier. This could be especially beneficial for people suffering from allergies to milk proteins or severe lactose intolerance. The carrot fermentation, however, is to this date mainly a spontaneous process, characterized by an unwanted initial growth of *Enterobacteriaceae*. This points towards the need of better controllable fermentations, *e.g.* with starter cultures. In this work, the potential of *L. plantarum* and *L. casei* as starter cultures was explored. From each of these species, different strains were chosen from various origins. Both fermentation and human isolates were chosen, since the latter could possess more probiotic properties and serve as functional starter cultures. In general, the autochthonous strains showed superior capacities to dominate the ecosystem, with only a selected number of allochthonous strains shown to be able to dominate the fermentation process. The well-documented probiotic *Lactobacillus rhamnosus* GG however, showed to be amongst the few allochthonous strains able to persist in the fermentation. Altogether, our work further strengthens the use of the carrot fermentation as a novel carrier for probiotics.

P6. Probiotic potential of *Lactobacillus* isolates from Irish Cheddar Cheese

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As the non-starter microbiota of cheese is composed largely of *Lactobacillus* species, some of which have been exploited for the most commonly applied commercial probiotic strains, this study was undertaken to assess the probiotic potential of non-starter bacteria from Cheddar cheese. The Food and Agriculture Organisation/World Health Organisation have defined a range of characteristics required by bacteria being proposed as probiotics and these were used to assess non-starters from Cheddar. The initial screening was based on the capacity of the non-starter microbiota to survive simulated gastric digestion (including low pH, digestive enzymes and bile). Of the 240 strains which initially survived this step, a single strain each of *Lactobacillus casei/paracasei* and *Lactobacillus rhamnosus* was selected based on further criteria including bile tolerance, bile salt hydrolase activity, and the ability to adhere to a mammalian cell model of the human intestinal epithelium. The *L. casei/paracasei* and *L. rhamnosus* strains displayed adhesion rates of 64% and 79%, respectively. The ability of these two strains to inhibit the adhesion of a pathogenic *Escherichia coli* strain was tested and both exhibited a >20% exclusion rate in a co-culture intestinal epithelium model. Additionally, growth on ruthenium red milk agar revealed exopolysaccharide production for both strains, a desirable trait for some industrial applications and for human health. These two strains have previously shown antibiotic sensitivity within the accepted European Food Safety Authority limits, meeting that criterion for acceptability as food additives. Thus, two *Lactobacillus* strains from Cheddar cheese have been identified that exhibit probiotic-related traits, which can safely be added to food.

P7. Fructo-oligosaccharides and inulin induce specific changes on gut microbiota with decreased butyrate in Chinese healthy adults: a randomized, double-blind, placebo-controlled trial

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Objective: The purpose of this study was to assess the effect of daily fructo-oligosaccharides (FOS) and inulin (INU) intake for 4 week on glucose response, lipid profile, gut microbiota and fecal metabolites in Chinese healthy adults.

Design: 135 healthy adults aged 18-60 were randomly assigned to maltodextrin (MAL) control group, FOS group and INU group (15g/d) for 4 week in a parallel-arm design. Blood and fecal sampling, OGTT and anthropometry were performed before and after intervention. 16S rDNA microbiota profiling and Flow-fish was applied to assess the composition and quantity of gut microbiota. 1H-NMR was used to profile the fecal metabolites and GC was used to quantify the concentration of SCFA in feces.

Results: 4 week intake of FOS and INU markedly increased the abundance of *Bifidobacterium* for 5-fold ($P < 0.05$) with significant effects on overall microbial richness ($P < 0.05$) and diversity ($P = 0.001$) when compared with MAL. The manipulation of FOS and INU on gut microbiota was consistently manifested in the decreased *Roseburia*, *Eubacterium*, *Feacalibacterium* and *Phascolarctobacterium* (P adjust < 0.05) at week 4. However, the concentration of acetate, propionate and butyrate all decreased ($P < 0.05$). Plasma triglyceride (TG) concentration were lower after FOS than after MAL ingestion ($P < 0.05$). No significant alterations in peripheral cholesterol profile, blood glucose response, insulin sensitivity and host anthropometric were found.

Conclusions: 4-week supplementation of FOS and INU led to ecosystem-wide microbiota shifts with decreased SCFA concentration. Further mechanism investigation is warranted to determine how the observed changes of gut microbiota and their metabolites translated into the effect on host metabolism.

P8. Adherence, acceptability and preference of an oral/vaginal probiotic to treat bacterial vaginosis (BV) in South Africa

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BV is associated with genital inflammation, increased HIV risk and adverse reproductive outcomes. Antibiotics are standard of care but recurrence is high. Adjunctive probiotics may improve treatment efficacy and durability.

The first South African Health Products Regulatory Authority (SAHPRA)-approved randomized single-blind trial in STI- BV+ women (n=30) with vaginal discharge compared topical metronidazole (control) to a combination of metronidazole with a commercially available probiotic (intervention; oral capsules/vaginal spray; *L. acidophilus*, *L. rhamnosus*, *B. bifidum* and *B. longum*, $2x10^9$ CFU/dose, 15 days).

64.9% of screened women had BV, confirming that clinical symptoms poorly predict BV. All women completed the course of metronidazole. Almost half (45.8%) experienced adverse events (AEs), including vaginal itching (33.3%), discomfort (12.5%) or bleeding (4.2%). All women in the intervention group completed the course of oral capsules and the majority (85.7%) the vaginal spray. Reported AEs included vaginal itching (14.3%), discomfort (7.1%), nipple sensitivity (7.1%) and nausea (7.1%). The majority would recommend the probiotic to other women (92.9%) and use it again (85.7%). Reportedly, oral administration was easier than vaginal application (80% vs 20%), and participants generally preferred oral to vaginal administration (75% vs. 25%). For vaginal application, participants would prefer tablets (34.8%) or gel (30.4%) over spray (13%), tampon (13%) or capsule (4.3%).

This first SAHPRA-registered probiotic trial has laid the path for future probiotic trials in South Africa. Probiotics for BV treatment were acceptable in South African women and associated with few AEs. Oral delivery was preferred to vaginal application. Further refinement of probiotic administration could improve acceptability.

P9. *Lactobacillus rhamnosus* GG cell surface molecules contribute to its probiotic action against *Staphylococcus aureus* in the skin niche

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The anti-pathogenic and immunomodulatory properties of *Lactobacillus rhamnosus* GG (LGG) make it a promising candidate for skin probiotic applications. However, its molecular interactions with pathogens and host cells in the skin niche is currently underexplored. Here, we screened several LGG knock-out mutants in cell surface molecules to unravel their role in probiotic action against the prominent skin pathogen *Staphylococcus aureus*.

In competitive adhesion assays, LGG adhered to primary human keratinocytes (NHEK) at ~2.85% efficiency and reduced *S. aureus* adhesion by half. The LGG mutant in SpaCBA pili showed drastically diminished adhesion to NHEK and significantly less efficient reduction of *S. aureus* adhesion. These results were confirmed by fluorescence microscopy. The LGG *spaCBA* mutant also showed less co-aggregation with *S. aureus* compared to wild-type LGG. Interestingly, the LGG *welE* mutant deficient in cell wall exopolysaccharides adhered significantly better to NHEK.

The viability of NHEK in co-culture with *S. aureus* was increased by all tested LGG mutants, although slightly less so by the LGG spaCBA mutant. Supernatant of all tested LGG mutants inhibited *S. aureus* growth in a pH-dependent manner. Host TLR receptor activation was reduced in the LGG *spaCBA* mutant and increased in the LGG *welE* mutant compared to wild-type LGG. Inflammatory TLR activation by live *S. aureus* was diminished upon co-incubation with wild-type LGG, LGG *spaCBA* and LGG *welE* mutants.

Overall, inhibition of *S. aureus* NHEK infection by LGG appears to be multifactorial, with both probiotic surface molecules and pH-reducing agents involved. SpaCBA pili were the key adhesive factor of LGG in our experimental set-up, and additional factors involved in probiotic interactions with *S. aureus* are currently being investigated.

P10. A probiotic formulation (*Lactobacillus rhamnosus* & *L. helveticus*) reverses the effects of maternal separation on neural circuits underpinning fear expression and extinction in infant rats

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Early environmental factors, such as exposure to stress or disruption of the gastrointestinal microbiota, are known to increase vulnerability to both physical and mental health problems. Rat pups exposed to early-life maternal separation stress prematurely exhibit an adult-like profile of fear regulation characterized by extended fear retention and relapse-prone extinction (Callaghan & Richardson, 2011, 2012). That is, maternally-separated pups exhibit a predisposition towards persistent fear, one of the hallmarks of clinical anxiety. We have previously shown that a probiotic treatment attenuates these effects of maternal separation on fear regulation, restoring a typical infant phenotype of rapid forgetting and relapse-resistant fear extinction (Cowan *et al.*, 2016). In the current series of experiments, we examined the neural correlates of fear behavior following stress and probiotic treatment. We focused on the medial prefrontal cortex (mPFC), a brain region that typically only becomes incorporated into the fear regulation circuit late in the juvenile period (Kim *et al.*, 2009, 2012). Untreated MS rat pups, but not standard-reared or probiotic-exposed MS pups, exhibited an adult-like pattern of neuronal activation in the mPFC during fear regulation. Specifically, only untreated MS infants displayed elevated levels of pMAPK in the prelimbic region of the mPFC following fear expression and in the infralimbic region following fear inhibition. These results add to the cross-species evidence that early adversity hastens maturation in emotion-related brain circuits. Importantly, the results also demonstrate that precocious neural maturation in stressed infants can be prevented by a probiotic treatment.

P11. Investigating the impact of chronic consumption of inulin blended with arabinoxylan on markers of appetite regulation, in healthy weight men

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Prebiotics such as inulin can beneficially modify the composition of the microbiota. The gut microbiota is increasingly implicated in the aetiology of the obese phenotype, and saccharolytic fermentation in the gut may influence appetite. By blending arabinoxylan with inulin (I+ABX) it was hoped that consumption would lead to significant bifidogenic effects in the colon and positively regulate satiety in healthy weight men. A double-blind, placebo-controlled crossover study was carried out on 20 healthy weight men who consumed either 9.46g I+ABX or 9.23g flavoured maltodextrin consumed in 2 doses daily for 21 days, followed by a 21-day washout, followed by the alternate treatment. Changes to bacterial ecology (FISH-FLOW), SCFA concentration (HPLC), satiety scores (VAS), systemic metabolites (NMR) were assessed and energy intake during an ad libitum meal and postprandial appetite were evaluated both before and after treatment with placebo and I+ABX. There was no change in satiety scores following treatment with I+ABX, however there were significant increases in the abundance of bifidobacteria (P=0.017), *Propionibacterium* (P=0.021), as well as elevated acetate production (P=0.009) and reduced food intake (P=0.030). This complimentary blend may be helpful to those in maintaining a healthy weight.

P12. Correlations between production parameters and the bioactivity of *Lactobacillus reuteri* DSM 17938

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The microbiota of the human gut has been increasingly investigated during the last century. Meanwhile, a variety of diseases have been associated with the composition and function of the gut microbiota. Selected probiotics have been proven as effective treatments able to ameliorate many of these diseases. We study the probiotic bacterium *Lactobacillus reuteri* DSM 17938, having a proven efficacy in relieving colic and acute diarrhea among other diseases and disorders. Most probiotic bacteria are administered in a lyophilized form and it is well known that the production parameters have a large impact on the storage stability. However, how to improve the probiotic capacity, *i.e.* the tolerance to gastric pH and bile as well as the bioactivity, through modifications of the production parameters is largely unknown.

This project is part of a recently initiated larger programme with the aim to increase the knowledge on how to produce lyophilized *L. reuteri* DSM 17938 with optimized probiotic activity. As models for survival in the upper gastrointestinal tract, we expose the bacteria to simulated gastric juice and bile salts. Furthermore, cell models with relevance for host-microbe interactions are used. We have previously shown that *L. reuteri* mitigate the loss of epithelial integrity caused by enterotoxin producing *Escherichia coli* (ETEC). By exposing the intestinal cell lines IPEC-J2 and Caco-2 to ETEC we induce a leaky epithelium. Furthermore, the nociceptive effect of DSM 17938 previously described by Perez-Burgos and colleagues is evaluated in a TRPV-1 receptor Jurkat cell model. We will present the research programme as well as results from the above-mentioned models in a comparison between differently produced batches of *L. reuteri* DSM 17938.

P13. Prediction and assessment of a variety of digested substrates on the growth of health-promoting gut bacteria

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The contribution of the gut microbiota to health and disease is becoming ever more apparent in the last number of years, due to developments in DNA sequencing technology and more well-defined cultivation techniques. This has resulted in the identification of health-promoting bacteria. Until recently, prebiotics, non-digestible food substrates which are selectively utilised by beneficial bacteria, were employed with a view to increasing the growth of well-established health promoting bacteria, namely *Lactobacillus* and *Bifidobacterium*. However, other beneficial bacteria recently revealed may also be targeted to enhance their growth as they establish themselves as the next generation of health-promoting microbes. These include anaerobes such as *Akkermansia muciniphila, Faecalibacterium prausnitzii* and *Eubacterium rectale*. Identification of growth substrates/bioactives through the analysis of genome sequence data can aid in elucidating which substrates may best enhance the growth of these microbes which are often difficult to grow. The phenotypic microbial trait analyser, Traitar, can predict 67 phenotypes based on the genome. This, combined with laboratory-based investigations, will detect the best possible nutrients or combination of nutrients for optimal growth. The behaviour of these cultures will then be assessed through *in vitro* digestion of the bioactive compounds and the implementation of an *in vitro* gut model in order to establish the effects within a gut environment. The results of which may be useful in selectively manipulating the gut for the benefit of the individual through the addition of these compounds in functional foods.

P14. *In vitro* and *in vivo* probiotic potential of *Lactobacillus* spp. for otitis media

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Otitis media (OM) is one of the most important problems of the upper respiratory tract in children. The dysfunction of the Eustachian tube is mostly caused by physical obstruction or by infections with pathogens such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*. Recent microbiome studies indicate that lactic acid bacteria can be endogenous members of the nasopharyngeal niche. Interestingly, an inverse correlation has been observed between the occurrence of lactobacilli and pathogens. The objective of this study was to investigate whether *Lactobacillus* species, among which well-documented probiotic strains, can have direct antimicrobial effects against the main OM pathogens.

All *Lactobacillus* strains tested showed antimicrobial activity against especially *M. catarrhalis*. Several also demonstrated activity against *H. influenzae* and *S. pneumoniae*. Screening of active molecules in the spent culture supernatant (SCS) of these strains pointed towards molecules that were heat-stable, non-proteinaceous and pH-dependent. Subsequently, a link between the concentration of lactic acid in the SCS and the antimicrobial activity was observed. SEM pictures suggest membrane damage by lactic acid but also points to other active metabolites in the SCS. In addition, the immunomodulating effect of selected *Lactobacillus* strains was further investigated to explore how this could counteract inflammation caused by the main OM pathogens. Furthermore, an intervention study was started in which children with OM were administered a mixture of *L. rhamnosus* GG and *Bifidobacterium lactis* BB-12. The nasopharyngeal microbiome of the children and the concentrations of pathogens/probiotics is monitored and compared with a control group.

P15. Study on the relationship between structural properties of xylooligosaccharides and their prebiotic activity

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Although prebiotics are already commercially available, being fructooligosaccharides (FOS), galactooligosaccharides (GOS) and inulin derivatives the most consolidated in the market, there still is a broad range of studies about the use of novel prebiotics. Among them, Xylooligosaccharide (XOS) is getting attention as an emerging prebiotic due to its better characteristics when compared to commercial prebiotics, and it's already commercialized in a few countries. Enzymatic hydrolysis with endoxylanase enzyme of previously alkali-extracted xylan is the most common method employed to produce XOS. This method yields mostly deacetylated xylobiose and xylotriose. However, the influence of XOS's structural properties (*i.e.*, degree of polymerization – DP and degree of acetylation – DA) on its prebiotic activity is still unclear. In this context, auto-hydrolysis, an alternative method based on xylan hydrolysis in acidic medium, was used with the aid of a statistical design of experiment to create a library of XOS fractions with varying structural properties (DA and DP). Then, the influence of these properties on XOS's prebiotic activity was investigated by *in vitro* assays. In this presentation, we will discuss how a better understanding of the relationship between structural properties of XOS and its prebiotic activity can be used to tailor the process conditions to produce XOS with specific DP and DA, and consequently, with enhanced prebiotic activity.

P16. Defensin-like bacteriocins, a previously undiscovered group of class II bacteriocins

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Bacteriocins are small ribosomally synthetised antimicrobial peptides which can have a narrow or broad spectrum of inhibition. They are natural by-products of fermentation and microbial production can be considered a probiotic trait. Bacteriocins also have applications in food preservation and potential for clinical use as a possible alternative to antibiotics. *Actinomyces* spp. are common commensals and opportunistic pathogens of the GI tract frequently isolated from the oral cavity. Their bacteriociniogenic potential has previously been undetermined. A pan-genomic in silico approach paired with a functional screen of an *Actinomyces* isolate of sheep faeces identified a novel bacteriocin which was not identified by bacteriocin detection software, BAGEL. The bacteriocin-like compound was found to have a broad spectrum of inhibition. MALDI-TOF MS, N-terminal amino acid sequencing and genome sequencing were used to identify the bacteriocin and its gene which were previously undescribed. The peptide shares sequence identity and features with the well studied eukaryotic antimicrobial peptides, defensins. Subsequent genome analysis identified 37 coding sequences for homologous peptides within the genus *Actinomyces*, and one within a *Corynebacterium* sp. Following further investigation, bacteriocin production was observed from another *Actinomyces* sp. isolate, the second in a novel class of defensin-like bacteriocins.

P17. Reconciling species taxonomy with public genome data for the *Lactobacillus* Genus Complex

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Thousands of genomes of the *Lactobacillus* Genus Complex (LGC) are currently publicly available and this number is rapidly increasing. To harness the information in this huge dataset, it needs to be properly structured using taxonomy. However, there are currently three problems associated with bacterial taxonomy: genomes are not systematically classified to official species, official species are often inconsistent in their size and some genomes do not fit into any currently known species.

We attempted to solve these problems by constructing a de novo species taxonomy for the LGC and comparing it to the official taxonomy. We downloaded all publicly available LGC genomes from genbank and calculated their pairwise core nucleotide identities (CNIs). We then clustered the genomes into de novo species using a fixed CNI cutoff. Finally, we compared these de novo species to the official LGC species using two methods: (i) the identification of genomes of type strains and (ii) a comparison of extracted 16S sequences to 16S sequences of type strains.

We found that the LGC genomes did not vary along a continuum, but rather could be divided into groups with very high intra-group similarities and low between-group similarities. The size of these groups corresponded roughly to the commonly used bacterial species delimitation cutoff. Clustering with this cutoff resulted in 239 genome clusters that we call de novo species. Comparison to the official taxonomy lead to the suggestion that seven pairs of official species can be joined and one species can be split. Further, we propose that at least eight genome clusters constitute new species. Finally, as a result of our approach, we were able to accurately classify 98 unclassified genomes and reclassify 74 wrongly classified genomes

P18. Potential beneficial attributes of vaginal *Lactobacillus crispatus*

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Lactobacillus crispatus is the most commonly isolated species from the vaginas of healthy women. Producing antimicrobial compounds, including lactic acid and bacteriocins, it maintains a healthy vaginal pH and host environment. The absence of this species is associated with vaginal dysbiosis, a decrease in beneficial bacteria and increase in pathogenic bacteria. This condition can lead to bacterial vaginosis, which affects millions of women annually and can result in adverse pregnancy outcomes which include preterm birth and low-weight infants. The prevalence of *L. crispatus* in healthy women implies it has a specific ability to survive in the vaginal environment, albeit certain strains have superior adherence ability to vaginal epithelial cells and higher rates of colonization. We hypothesize that *Lactobacillus crispatus* vaginal isolates produce compounds that enhance its ability to inhabit the vagina and counter pathogenic bacteria.

Using BAGEL four putative bacteriocins were found within the genomes of the 20 *L. crispatus* strains that we have access to. To confirm bacteriocin production well diffusion assays were conducted. In addition, metabolomics experiments were completed to determine if other relevant metabolites are produced by this species.

We determined that inhibition of the pathogenic bacteria tested varied by strain. Additionally, although organic acid production did not vary by strain, the production of other beneficial metabolites did. The variation seen within the well diffusion assays suggests that the strains each have a difference in ability to inhibit pathogen growth. A strain able to survive vaginal dysbiosis, that has a beneficial metabolic profile, and can inhibit the growth of urogenital pathogens may be a potential probiotic candidate.

P19. Novel bile salt hydrolase from *Lactobacillus gasseri* FR4 and its application as an alternative to antibiotic growth promoters

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Probiotic bacteria are beneficial to the health of poultry animals, thus are used as alternative candidates for antibiotics used as growth promoters (AGPs). However, they also reduce the body weight gain due to innate bile salt hydrolase (BSH) activity. Hence, the addition of a suitable BSH inhibitor along with the probiotic feed can decrease the BSH activity. In this study, a BSH gene (981 bp) encoding 326-amino acids was identified from the genome of *Lactobacillus gasseri* FR4 (LgBSH). The LgBSH-encoding gene was cloned and purified using an *Escherichia coli* BL21 (DE3) expression system, and its molecular weight (37 kDa) was confirmed by SDS–PAGE and a Western blot analysis. LgBSH exhibited greater hydrolysis toward glyco-conjugated bile salts compared to tauro-conjugated bile salts. LgBSH displayed optimal activity at 52°C at a pH of 5.5, and activity was further increased by several reducing agents (DTT), surfactants (Triton X-100 and Tween 80), and organic solvents (isopropanol, butanol, and acetone). Riboflavin and penicillin V, respectively, inhibited LgBSH activity by 98.31 and 97.84%. A homology model of LgBSH was predicted using EfBSH (4WL3) as a template. Molecular docking analysis revealed that the glycocholic acid had lowest binding energy of -8.46 kcal/mol; on the other hand, inhibitors, i.e., riboflavin and penicillin V, had relatively higher binding energies of -6.25 and -7.38 kcal/mol, respectively. Our results suggest that *L. gasseri* FR4 along with riboflavin might be a potential alternative to AGPs for poultry animals.

P20. Surface proteins of three probiotic lactobacilli exhibit strain specific anti-inflammatory effects in TNBS-induced colitis mice

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Probiotic lactobacilli strains, *L. plantarum* MTCC 5690 and *L. fermentum* MTCC 5689 are known to exert protective effects in colitis mice. However the knowledge on its mode of action and the effector molecules mediating the anti-inflammatory effect needs to be identified in order to build a safer and sustainable strategy to combat inflammatory bowel diseases. Here, we investigate the ameliorative effects of total surface proteins derived from these strains along with *L. acidophilus* NCFM in colitis mouse model. The total surface proteins from the three lactobacilli strains were extracted by LiCl method, characterized by SDS-PAGE analysis and quantified by lowry's method. The extracted proteins were then orally gavaged to respective mice groups for an initial 14 days, followed by induction of colitis with TNBS (trinitrobenzenesulphonic acid). The colitis severity was significantly reduced in surface proteins administered mice when evaluated both biochemically and histologically. The inflammation marker, Myeloperoxidase was increased by TNBS but significantly reduced in NCFM treated groups followed by MTCC 5690 and MTCC 5689. Likewise, a similar trend was observed in the histological scores and histology of colon in probiotic surface proteins treated mice groups. Although, no statistically significant decrease was observed in the levels of pro-inflammatory cytokine, TNF-α, the levels of anti-inflammatory cytokine, IL-10 was significantly increased in the treated mice groups. Hence, it is evident from the present study that surface proteins of probiotic test strains exhibit strain specific anti-inflammatory effects in colitis condition, however, the principal effector protein needs to be identified in order to design strategies for the management of the inflammatory diseases.

P21. Modulating the phyllosphere microbiome to improve crop production and crop protection

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The phyllosphere is the surface of plants in contact with the atmosphere. It is occupied by bacteria, fungi and other micro-organisms. This microbial community forms intimate interactions with the host plant and has an influence on plant growth and plant health. Phyllosphere commensals can protect the plant in various ways, exclusion of pathogens trough competition for nutrients and space, production of antibiotic metabolites, triggering the plants immune system or the disruption of biofilms. There is a huge potential in harnessing this microbial community to improve crop production and crop protection while decreasing the negative impacts of pesticides on human health and on the environment. Bacteria with a beneficial effect for the plant, plant probiotics, are being explored in this research through two different approaches, originating either from the phyllosphere or compost teas. Compost teas are watery fermentations (aerobic or anaerobic) and have been used in agriculture for over 4 000 years.

Firstly, 16s rRNA gene amplicon sequencing is used to determine the community structure of the phyllosphere in different agricultural systems. Secondly, bacteria from the phyllosphere and from compost teas are isolated, characterized and screened for potentially beneficial traits such as antipathogenic activity. Finally, these bacteria are applied on plants and their impact on plant physiology and on the resident microbiome is monitored. Preliminary experiments of the application of bacteria from the phyllosphere and from compost teas on plants resulted in deeper insight in community dynamics of the phyllosphere.

P22. Effect of yeast beta-glucan on bile acid signalling and metabolism in healthy and diet-induced obesity mice

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Introduction: Bile acid serves as a signalling molecule and play an important role in development of many diseases, including metabolic syndrome. At the same time, yeast beta-glucan has been shown to provide beneficial health effects. Therefore, we study whether bile acid is associated to the protective effect of yeast beta-glucan against metabolic alteration.

Aim: To evaluate the effect of particulate (P Y-BG) and soluble (S Y-BG) yeast beta-glucan on bile acid synthesis and metabolism in healthy and diet-induced obesity (DIO) mice.

Method: P Y-BG or S Y-BG were given to mice fed with chow or 60% high fat diet. Body weight gain, body fat mass, liver weight, hepatic triglyceride (TG) level and fasting blood glucose were used to evaluate the metabolic beneficial effect. Hepatic bile acid profile and related gene expression in liver and ileum are evaluated.

Results: In healthy mice, P Y-BG upregulated bile acid synthesis as reflected in both gene expression and hepatic bile acid profiling data. For the effect towards obesity, S Y-BG group had slightly higher weight gain and hepatic TG level, while P Y-BG group have slightly reduced fasting blood glucose and liver weight. P Y-BG group also showed a downregulated TNF-a and SREBP1c mRNA expression. For bile acid metabolism, compared to healthy mice, DIO mice showed a higher level of shp and Fgf15 mRNA expression in the ileum, which is reduced by P Y-BG in both healthy and DIO mice.

Conclusion: P Y-BG enhance bile acid synthesis in healthy mice. P Y-BG treated group showed slightly inhibited metabolic syndrome which is associated to inhibition of FXR-shp and FXR-fgf15 pathway.

P23. Dietary supplementation of green tea epigallocatechin gallate with probiotic *Lactobacillus fermentum* acts as second generation synbiotic by modulating cellular immune responses and antioxidant capacity in aging mice

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In present study, we systematically identified and evaluated a synbiotic combination of green tea phytochemical epigallocatechin-gallate (EGCG) and probiotic bacteria for amelioration of immunosenescence and oxidative stress in aged mice. Inhibitory effects of EGCG against different bacterial species were evaluated *in vitro*, followed by analysis to identify potential combination of EGCG and probiotic bacteria against alleviation of oxidative and inflammatory stress *ex vivo*. The best synbiotic combination, vis-à-vis prebiotic and probiotic supplementation alone, was then evaluated in aged Swiss albino mice for modulation of various immunological and anti-oxidative parameters. It was observed that EGCG strongly inhibited the growth of pathogenic microbes as compared to probiotic bacteria. A combination of EGCG with probiotic *Lactobacillus fermentum* (LF) provided evidence of additive effects in the amelioration of oxidative and inflammatory stress-induced cell death. *In vivo* study revealed that combined supplementation of LF and EGCG significantly enhanced neutrophil oxidative index, CD3+ cell numbers and activation status (CD3+CD69+), Th1/Th2 cytokines in splenic supernatants and liver Nrf-2 expression in comparison to treatments with LF or EGCG alone. However, the combined application of EGCG and LF did not simply result in additive or synergistic effects, but instead showed differential effects in relation to individual treatments. Together, these observations suggest that EGCG could be considered as a potential prebiotic that can offer second generation synbiotic health beneficial effects for the alleviation of some of the deleterious aspects of immunosenescence and aging.

P24. Resistant starch promotes equol production and improves Polycystic Ovary Syndrome (PCOS) symptoms in rats treated with soy isoflavones: A gut microbiota perspective

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Polycystic ovary syndrome is the most common endocrine disorder found in the women of their reproductive age. It is currently treated with lifestyle modifications and other medications which have temporary effects. Equol, the microbial metabolite of daidzein has greater estrogenic activity than the isoflavones (ISO), genistein and daidzein. Resistant starch (RS), which has a prebiotic activity and is a dietary fiber, was reported to promote equol production. In our study, we hypothesized that metabolites of soy isoflavones produced by gut microbiota are key effectors on the alleviation of PCOS symptoms. 7 weeks old female SD rats were divided into 5 groups and 4 groups were orally treated with 0.5mg/kg body weight of letrozole for 21 days to induce PCOS symptoms. After that, diet treatment was done for 14 days; C (Control) and P (PCOS) groups: control diet, PS group: 0.05% ISO, PSR group: 0.05% ISO+11% RS and PSA group: 0.05% ISO+antibiotic cocktail. All groups showed a reduced number of cysts compared to the control group but a decreased testosterone concentration was observed only in PSR and PSA groups. Also, an improved menstrual irregularity was only observed in PS and PSR groups. PSR group showed an elevated level of equol compared to other groups. Furthermore, no notable differences were seen in the alpha and beta diversities of PS group compared to C and P groups but in PSR and PSA groups. We observed some genera level significant differences of gut bacteria in PS, PSR and PSA groups when compared to C and P groups. In addition, positive correlations between the three PCOS symptoms and some bacterial families and genera were observed. Collectively, our results suggest that the estrogenic effect of equol could be responsible for the alleviation of PCOS symptoms.

P25. Selected *Lactobacillus* taxa inhibit the hyphal morphogenesis of *Candida albicans* thanks to the chitinase activity of their major peptidoglycan hydrolase

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Candida albicans belongs to the most prevalent fungal pathogens, causing both superficial mucosal candidiasis and life-threatening invasive infections. Probiotic treatments with *Lactobacillus* strains have emerged as *C. albicans* management strategy, but the validation of lactobacilli as probiotics against *Candida* infections in several clinical trials showed variable results. Predicting and understanding the clinical efficacy of *Lactobacillus* strains is hampered by an overall lack of insights into the modes of action. In this study, we aimed to unravel molecular mechanisms underlying the inhibitory effects of lactobacilli on hyphal morphogenesis, the most crucial step in *C. albicans* virulence.

Based on a screening of different human-associated *Lactobacillus* strains, we found that the closely related taxa *L. rhamnosus*, *L. casei* and *L. paracasei* showed stronger activity compared to other *Lactobacillus* species tested. By exploring the activity of purified compounds and mutants of the model strain *L. rhamnosus* GG, we could map the contribution of different molecules (including lactic acid, the galactose-rich exopolysaccharides, the SpaCBA pili, the Llp1 and Llp2 lectins, and major secreted protein 1 (Msp1)) to the anti-hyphal activity. We found that the major peptidoglycan hydrolase Msp1, conserved in the three closely related taxa, is a key effector molecule. We could show that this activity of Msp1 was due to its ability to degrade chitin, the main polymer in the hyphal cell wall of *C. albicans*.

The identification of a *Lactobacillus* specific protein with chitinase-like properties showing anti-hyphal activity will assist in better strain selection and improved application in future clinical trials for *Lactobacillus*-based *Candida*-management strategies.

P26. *Bacillus subtilis* 168: a promising probiotic candidate for the treatment of nephrolithiasis

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The prevalence of nephrolithiasis in North America has risen to approximately 10%, and is associated with significant morbidity. The gut microbiota is known for its role in human health and disease, including in kidney stone formation. Intestinal colonization with *Oxalobacter formigenes* is suggested to protect against formation of calcium oxalate (CaOx) stones by reducing urinary oxalate. In stone patients, probiotic supplementation with oxalate-degrading bacteria has been suggested as a potential preventive therapy, but clinical trials with *O. formigenes* have been limited and inconclusive. We sought to investigate the oxalate-degrading properties of *Bacillus subtilis* 168 (BS168) as a potential probiotic candidate for nephrolithiasis prevention. BS168 was grown in media conditioned with oxalate to validate both survival and substrate utilization. Using an established *Drosophila melanogaster* model of urolithiasis and HEK293/MDCK cell lines we evaluated how stone burden was impacted upon exposure to BS168. Flies were administered BS168 and fed 1% sodium oxalate food (OF); stone burden was assessed with survival curves and quantitation of crystal formation. BS168 preferentially grew in high oxalate. Flies administered BS168 on OF developed fewer fecal crystals compared to flies without bacterial treatment and had increased survival. BS168 was viable in fly faeces up to 5 days after supplementation. These findings suggest that BS168 is a promising human probiotic candidate because it can reduce stone formation through degradation of oxalate in established insect and cell line models. Further studies are now required to determine whether this bacterium can reduce the highly prevalent and morbid condition in humans.

P27. Zinc and the gut microbiota

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Zinc is an essential micronutrient in humans partly due to its requirement in more than 300 metalloenzymes. However, zinc deficiency affects 25% of the world population and can have detrimental effects on the host which include impaired growth, a reduced immune system and weakened brain functions. Although zinc supplementation is used to increase zinc intake, the impact it has on the gut microbiota have yet to be fully explored. We are investigating the effect of zinc on the gut microbiota by using anaerobic batch cultures containing a defined gut model medium with the addition of 77, 192.5 or 770 µM of zinc (equivalent to 10, 25 and 100 mg zinc intake per day). The cultures were inoculated with 0.1% faecal matter from healthy volunteers. The effect of zinc regime on the gut culture growth was determined by NGS-based community profiling (16S rRNA sequencing) and by measuring total bacterial numbers using Flow-FISH. In addition, metabolic end products (e.g. short-chain fatty acids, SCFA) were measured using gas chromatography and metal levels were determined by ICP-OES. This will be the first study examining the link between zinc intake and the impact on the human gut microbiota, although in animal studies shifts in the microbiota profile are caused by zinc supplementation.

P28. Effect of agave fructans on hematological, metabolic parameters and oxidative stress in diabetic mice

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In type 2 diabetes there is a state of oxidative stress with organ damage. It has been suggested that Agave fructans can be efficient as prebiotics and antioxidant. The objective of the present study was to determine the effect of agave fructans on metabolic and biochemical parameters and antioxidant protect in various organs in diabetic db/db mice. Twenty female mice, 10 of strain C57BL/6 and 10 of strain db/db were used. Two control groups were formed (1, healthy and 3, diabetic) and two groups treated with 10% Agave fructans (2, healthy and 4, diabetic) for 8 weeks. The results show that the mice of group 2 and 4 decreased their food intake (12.5 ± 0.6g, 26.7 ± 1g, $p < 0.01$) with respect to groups 1 and 3 (15.6 ± 0.84g, 32.3 ± 1.8g) and the water intake (20.4 ± 0.6mL; 35.6 ± 1mL; $p < 0.01$) with respect to groups 1 and 3 (22.9 ± 1.8mL; 41.8 ± 0.6mL). Group 4 had a lower excretion (139.9 ± 7.1g, $p < 0.01$) compared to its control (178.1 ± 7.1g). In addition, significant changes in hematological parameters were observed due to the effect of fructans in the diabetic group with treatment. On the other hand, fructans significantly decreased the carbonyls content in heart of diabetic mice compared to untreated diabetic mice (11.5 ± 1.2 and 28.4 ± 6 ng of carbonyl / mg of protein respectively, $p < 0.05$), and the amount of carbonyl in kidney of non-diabetic mice compared with non-diabetic mice without treatment (3.3 ± 0.5 and 17.04 ± 4.8 ng / mg of protein, respectively, $p < 0.05$). Agave fructans attenuate the clinical signs of diabetes, polyphagia, polyuria and polydipsia by controlling hyperglycemia and decreasing oxidative damage in the body, suggesting that they may work as an alternative treatment for this condition.

P29. The microbiome in vulvovaginal candidosis during supplementation with lactobacilli with *in vitro* anti-*Candida* effects

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In their lifetime 75% of women will experience at least one episode of vulvovaginal candidosis (VVC). This condition with severe symptoms is normally treated with azoles. However, high recurrence rates and increasing resistance motivates scientists to explore alternative treatments. We formulated a vaginal gel containing three lactobacilli selected based on their *in vitro* ability to inhibit *Candida albicans* growth, hyphae formation and adhesion to vaginal keratinocytes. This gel was then evaluated in 20 patients suffering from acute VVC over four weeks including a 10-day treatment period. The ability of the gel to modulate the microbiome (fungal and bacterial) was assessed through 16S rRNA and internal transcribed spacer (ITS) sequencing and lactobacillary and fungal loads were quantified with quantitative PCR. Two *Candida albicans* variants occurred commonly in our data set. One of these occurred predominantly in samples that showed a dominance of *Lactobacillus iners* in the bacterial community. In 45% of women the *Lactobacillus* treatment improved their symptoms, without the need for rescue medication (3x 200 mg fluconazole). Specific *Lactobacillus* or *Candida* ASVs could not be linked to response to the *Lactobacillus* supplementation, in attempt to stratify responder and non-responder groups. However, there were some indications the rescue medication reduced *Lactobacillus* concentrations, which could be an undesired side effect of the azoles. Our study thus points towards important aspects for future selection of lactobacilli for probiotic use in VVC and indicates azole treatment might unexpectedly influence the vaginal bacterial community.

P30. Magnetic resonance imaging of commensal and pathogenic bacteria

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Magnetic resonance (MR) imaging is a non-invasive imaging modality with superb resolution and penetration depth, ideal for examining the soft tissues of the intestinal mucosa and other sites occupied by microbes. Molecular imaging may provide a useful adjunct to the current ex vivo microbiome analyses and may be improved with contrast agents for long-term contrast enhancement. Here, we examine MR relaxation rates of bacterial isolates *in vitro* in an effort to explore microbial behaviour, growth and dispersion *in vivo* using MRI.

Isolates of various microbes of the human gut microbiota and urinary tract were cultured, then loaded into Ultem wells by centrifugation and mounted in a gelatin phantom to measure MR contrast at 3 Tesla. Images were evaluated using custom MATLAB software. Longitudinal and transverse relaxation rates were measured. Statistical analyses were done with GraphPad Prism 7.03.

Transverse relaxation rates are high with little to no reversible component, providing a basis for bacterial detection amidst host tissues. Relaxivity differs significantly between *E. coli* strains with no consistent difference between strain characteristics or origin. *L. gasseri* 33323 shows drastically higher relaxation rates than other bacterial species examined, suggesting these bacteria would have higher contrast and detectability when compared to host tissues. This is the first report of MR relaxation rates in microbes. *E. coli* provide detectable MR contrast, with relaxivity much higher than those of mammalian cells. Therefore, we propose that the large MR signal in the gut may reflect a large bacterial contribution that has not been previously examined. In the future, MRI may be useful for non-invasively studying microbial behaviour in the gut microbiome or infection.

P31. Biomass production and spray drying of a potential probiotic strain from human breast milk, *Bifidobacterium lactis* INL1

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Breast milk bacteria are involved in the gastrointestinal colonisation of the newborn and the adequate maturation of the gut mucosal immune system. Previously, we have reported the isolation of *Bifidobacterium lactis* INL1 from human breast milk. This strain has shown probiotic potential in *in vitro* and *in vivo* models. Also, *B. lactis* INL1 has demonstrated to be resistant to processes commonly used during the production of probiotic cultures, as concentration, freezing and dehydration (lyophilization, spray drying and alternative technologies), retaining its functional properties. The aim of this work was to optimize the production of a spray dried culture of *Bifidobacterium lactis* INL1, grown on a whey permeate-based culture medium. Biomass production was adjusted in biofermentor with an orthogonal composite central design (variables yeast extract and pH). Next, a strip plot design was used to study the effect of the production (pH 6, 5.5 or pH free) and spray drying (SD) conditions: inlet temperature (IT, 115° or 140°C) and protectants (skim milk (SM), SM+WPC and SM+GOS), on the survival and storage stability (20°C, 5 months) of the spray dried cultures of *B. lactis* INL1. The moisture content of the powders was only affected by IT (< 5% in all cases). Satisfactory viabilities after SD (> 9 log CFU/g) were obtained in all cases. Survival after SD and storage were affected by IT, and interactions between pH and protectant (and time for storage test) were detected. The growth capacity and resistance to spray drying makes *B. lactis* INL1 a promising candidate for developing a probiotic dehydrated culture for foods and supplements.

P32. Design and application of a triple-strain probiotic to improve the health of honey bees

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Persistent global decline in honey bee (*Apis mellifera*) population size over recent years is a well-known societal quandary and multifaceted phenomenon with loss of habitat, infection, and pesticide exposure representing the suspected causal factors. Here, we take a systems level-approach to addressing this issue by developing a three-strain honey bee-specific probiotic containing *Lactobacillus rhamnosus* GR-1, *Lactobacillus plantarum* Lp39, and *Lactobacillus kunkeei* BR-1. The rationale for this is based on previous findings that demonstrate these strains to have pesticide detoxifying properties, immune boosting potential, and an ability to improve host nutrient uptake, respectively. Results will be discussed from several field trials in which the triple-strain combo was supplemented to honey bees under a variety of experimental conditions and tested for its ability to: A) vanquish *Paenibacillus* larvae (a nearly ubiquitous spore-forming bacterial pathogen and causal agent of American Foulbrood – the most serious brood disease of honey bees) from hives as a stand-alone supplement and as an adjunct treatment following guideline-recommended antibiotic administration, B) modulate diversity profiles of the 8-10 core phylotypes associated with nutrient partitioning in the adult honey bee gut microbiota, and C) alter gene expression of Cytochrome P450 enzymes involved in detoxification of environmentally-relevant pesticides. Altogether, this work suggests the triple-strain probiotic can simultaneously address the suspected causal factors implicated in honey bee decline and provides a framework for future investigations on the usage of beneficial bacteria to improve the health and productivity of this ecologically and economically important species.

P33. Polyamines modulate differentially microbial adhesion to human mucus according to age of mucus donor and bacterial strain

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Polyamines (PAs) are primordial polycations mainly known for their essential role in cell growth and proliferation. Their functions also include other critical processes such as biofilm formation and metabolite biosynthesis. The objective of the present study was to evaluate whether exogenous PAs would alter the adhesive properties of *Lactobacillus rhamnosus* GG, *Bifidobacterium animalis* subs. *lactis* Bb12, *Cronobacter sakazakii* ATCC 29544 and *Escherichia coli* TG1.

To extract the intestinal mucus, fecal samples from two age groups of healthy Finnish infants (0-6 month-old, n= 5; 6-12 month-old, n=5) and adult subjects (n=10) were obtained and pulled. The crude mucus was isolated by dual ethanol precipitation, purified and dialyzed. The strains were radioactively labeled, mixed with PAs and added to the polystyrene microtiter plate covered with mucus. The adhesion efficacy was detected using liquid scintillation.

Polyamines significantly ($p < 0.05$) increased Bb12 adhesion in early infant mucus (0-6 months), but spermidine and spermine reduced the adhesion in the oldest infant mucus group (6-12 months). On the other hand, polyamine combinations reduce LGG adhesion during adult life but not in infants ($p < 0.05$), while spermine significantly ($p < 0.05$) reduced *C. sakazakii* adhesion in early infancy (0-6 months). *E. coli* mucus adhesion seems to be independent of exogenous polyamines at any age.

Our data suggest for the first time that exogenous presence of specific PAs and PAs combination may modulate the bacterial adhesion to mucus depending on the bacterial strain and type of mucus. These results suggest that dietary polyamines may modulate probiotic activity and infective capacity in an age-associated manner, but further confirmation of these findings is required.

P34. *Lactobacillus casei* AMBR2 shows potential as probiotic for chronic rhinosinusitis

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Chronic rhinosinusitis (CRS) is a common upper respiratory tract (URT) disease with a major impact on public health. Despite the exact role of bacteria in the disease pathology is still controversial, antibiotics are often prescribed. Current treatment options, including antibiotics, often fail, and there is a high need for alternative treatment strategies. Here, we aimed to analyze the URT microbiota to better understand the difference in potential health-promoting and pathogenic bacteria in CRS patients versus healthy controls, and to develop new treatment strategies based on beneficial bacteria from the URT.

The microbiome of different URT niches was compared for healthy controls (n=100) versus CRS patients (n=225) by Illumina 16S rRNA gene amplicon sequencing. Furthermore, lactic acid bacteria were cultivated from the URT and their potential as probiotics as alternative treatment option for CRS was explored.

Microbiome comparison of the URT showed that some taxa were more associated with CRS, and are thus potential sinus pathobionts. Lactic acid bacteria on the other hand, including *Lactobacillus* species, were more associated with the healthy URT. Several of these potential beneficial strains could be cultured from the healthy URT. One of these isolates, *L. casei* AMBR2, showed interesting potential as URT probiotic due to its oxidative stress-resistance properties, its good adherence capacity to airway cells, its antimicrobial activity against URT pathogens and its immunomodulatory properties. In a preclinical trial in healthy individuals, the safety of this strain in a nasal formulation was tested, as well as the capacity of the strain to colonize the URT. The spray was well-tolerated and was able to temporally colonize the URT after nasal administration.

P35. Transcriptomics reveals novel insight into co-operative interactions between two human gut symbionts, *Ruminococcus bromii* and *Blautia hydrogenotrophica* in co-culture

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The human gut microbiota harbours a dense collection of anaerobic bacterial species whose metabolic interactions determine the stability of the microbial community. Fermentation of dietary resistant starch (RS) is a major energy source for microbial growth in the large intestine and *Ruminococcus bromii* has been described as a 'keystone' species for its ability to degrade RS. Here the potential for metabolic cross-feeding between *R. bromii* and the human colonic acetogen *Blautia hydrogenotrophica* was investigated in co-culture *in vitro* fermentations. *R. bromii* produced formate, ethanol and acetate in approximately equal molar proportions from starch when grown in batch culture. *B. hydrogenotrophica* utilized added formate while growing on glucose in pure culture. Co-culturing of starch-grown *R. bromii* and *B. hydrogenotrophica* diminished formate levels and increased acetate production which could be explained largely by the routing of formate via the Wood Ljungdahl pathway of *B. hydrogenotrophica*. RNA-seq transcriptomic analysis showed that co-culture of these microbes with starch as substrate significantly altered transcriptional profiles of a total of 75 *R. bromii* genes and 420 *B. hydrogenotrophica* genes. There was upregulation of *B. hydrogenotrophica* genes involved in the Wood Ljungdahl pathway and branched chain amino acid fermentation, corresponding with increased formation of branched chain fatty acids in the co-cultures. We propose that formate cross-feeding involving formate-producing species and acetogens is likely to contribute to high rates of acetate production. Transcriptome analysis also suggested competition for the vitamin thiamine and down regulation of dissimilatory sulfate reduction and certain redox proteins in *R. bromii* in the co-cultures.

P36. Optimisation of *Weissella paramesenteroides* for mannitol production in dairy fermentations

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Mannitol is a six-carbon polyol, sugar alcohol, used widely in the pharmaceutical industry as a coating for drugs, as well as a food additive due to its sweet taste and cooling effect. Mannitol is 50-70% as sweet as sucrose with less than half the calories, 1.6kcal/g in comparison to 4.0cal/g. Polyols have two EFSA approved health claims; reduction of the glycaemic response and protection against tooth decay. Mannitol is produced industrially by chemical synthesis or enzymatically, the fermentation process is a less utilised method for purification. While mannitol is used as an additive in a range of industries, very little is known about its production in situ in fermented foods. We screened 261 lactic acid bacteria for mannitol production using HPLC. From this screen 29 strains were positive for mannitol production. *Weisella paramesenteroides* LL-677 was found to be the highest producer and was further investigated for its potential use in dairy fermentations. A number of sugar combinations and concentrations were added to MRS broth to find the optimal combination for mannitol production. In broth supplemented with fructose/glucose, 23.48g/L of mannitol was produced following overnight growth. The results from the initial studies in broth were applied in skim milk fermentations to optimise sugar concentration and fermentation time for mannitol production. The strain will be applied in milk fermentations alone, and as an adjunct to a commercial yogurt starter culture. The pH, mannitol production, and sugar concentrations are being monitored over time. This study aims to investigate if in situ production of mannitol has potential to produce a low calorific, sweetened fermented milk product for the dairy industry.

P37. Assessing the ecological role of yeasts in the human gut

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Background: It is well documented that microorganisms in the gut play an integral role in health and disease. However, the influence of yeasts within the gut microbial ecology have not been as widely studied as bacteria, despite associations with gastrointestinal disorders.

Objective: The objective of this study is to understand the role of yeasts within the healthy and diseased human gut. *Candida albicans* is a gut commensal which can opportunistically flourish as a pathogen and has been linked to gastrointestinal disorders, such as Irritable Bowel Syndrome (IBS) and Inflammatory Bowel Disease (IBD). We hypothesise that higher proportions of yeasts, such as *C. albicans*, will be found in the guts of patients with IBS and IBD. We hope that determining their presence and interactions within the gut that may reveal the cause of the symptoms experienced and will help to develop treatments for these patients.

Design: Urine, faecal and blood samples will be collected from 40 healthy controls, 40 IBS and 40 IBD patients for microbial and metabolic analysis using primarily flow cytometry-fluorescence in situ hybridisation (FC-FISH) and proton nuclear magnetic resonance (1H-NMR) spectroscopy, respectively. Calprotectin levels will be analysed to indicate gastrointestinal inflammation. Statistical analysis will identify yeasts and their metabolites associated with the cohorts of interest.

Proposed analysis: Although yeasts that are already associated with gastrointestinal disorders, such as *C. albicans*, would be expected at levels below 10^4 cfu/g as a gut commensal of healthy subjects, higher levels would be expected in the faecal samples of patients with IBS and IBD. Multivariate statistical analysis will identify associations with IBS and IBD symptoms that can be further investigated to develop potential therapeutics to target these gut microorganisms, alleviate symptoms and improve the quality of lives for patients with IBS and IBD.

P38. The effect of prebiotic oligofructose enriched inulin supplementation on microbiota and protein metabolism in people consuming high protein diets

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Dietary protein levels are increasing worldwide and high protein consumption can be detrimental due to the generation of various toxic metabolites from gut bacterial fermentation. On the contrary, consumption of prebiotic carbohydrates allows specific microbiota changes, which may confer benefits upon host wellbeing and health. A double-blind, crossover, placebo controlled, randomised study in healthy individuals aged 18-60 years old was performed to evaluate the effects of prebiotic use (oligofructose enriched inulin) on gut bacterial proteolysis.

Volunteers were recruited from the Reading local community and 43 people completed the trial. Fasting blood, 24 hour urine and fresh faecal samples were collected at the University of Reading. Gastrointestinal symptoms and defaecation records were taken throughout the trial.

Bacteria were enumerated by fluorescence in situ hybridisation-flow cytometry and 16s sequencing. A significant increase in *Bifidobacterium* and *Anaerostipes* was observed with the addition of prebiotic treatment. Urine, blood plasma and faecal water metabolite changes were monitored by 1H-NMR. There were lower concentrations of aromatic metabolites in urine with prebiotic treatment, however, differences were not significant between the two interventions.

P39. Towards better benchmarking of the female microbiome in a Flemish cohort

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The female reproductive health and safety are steadily gaining more attention from both the general population and the scientific community especially in the global microbiome research field. Detailed analysis of the female microbiome has recently become possible due to cutting-edge developments in microbiome analysis techniques.

Here, we aim to gain detailed insight in the Flemish female microbiome composition and dynamics at sexual contact-related body sites and their link to lifestyle and environmental conditions, which will be relevant in both clinical and forensic contexts. To achieve this, we will establish a novel pipeline, building on the experience of the lab in microbial community sequencing. This innovative approach will include shotgun sequencing, microbial fingerprinting, in-depth taxonomic analyses and classification models. Our preliminary bioinformatics analysis showed the abundant presence of marker *Lactobacillus* taxa (especially *Gardnerella vaginalis*, *Lactobacillus crispatus*, *L. gasseri*, *L. iners* and *L. jensenii*) in vaginal fluid, and more data from volunteer body site and fluid samples is currently being analyzed.

Furthermore, we aim to isolate and characterize probiotic vaginal strains that can offer health benefits in the reproductive niche. The results of this study can contribute to a better understanding of the composition and diversity of a healthy female microbiome in various body sites and fluids, as well as to the evaluation of biological evidence in criminal investigations. For applied science in general, this proves an unmistakable added value with regard to the international state-of-the-art.

P40. Non-invasive longitudinal imaging of live fluorescent probiotic *Lactobacillus rhamnosus* GG in a mouse model of birch pollen-induced allergic asthma

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Live probiotic lactobacilli can have beneficial immunomodulatory properties when administered directly to the airways. In this study, innovative preclinical lung imaging is used to better understand beneficial probiotic-host interactions in the airways during preventive intranasal administration of *L. rhamnosus* GG (LGG) in a mouse model of allergic asthma.

Live intranasal LGG administration at $5x10^8$ CFU/dose and allergic asthma induction with birch pollen extract was performed, and airway inflammation and function were assessed as previously described (Spacova *et al*., Allergy. 2018; doi: 10.1111/all.13502). On different time points mice were analyzed by micro-computed tomography, fluorescence imaging and fibered confocal fluorescence imaging with recombinant fluorescent LGG strains.

Beneficial effects of LGG pretreatment were observed, including reduced allergic airway inflammation (eosinophilia, lung inflammatory cytokines) and airway hyperreactivity. Administration of fluorescent LGG in mice and subsequent imaging parameters were optimized, and lung read-outs reflecting disease- and LGG-treatment progression *in vivo* were imaged in a non-invasive manner.

Thus, beneficial effects of LGG were explored from an immunological and lung imaging perspective in a mouse model of allergic asthma. By applying multimodal airway imaging technology, we can provide *in vivo* longitudinal information on dynamic probiotic-host interactions at a whole-body, whole-organ and cellular level.

P41. Bioengineering a synthetic enzyme for the production of authentic human milk oligosaccharides (HMOS)

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2'-Fucosyllactose (2'FL) is one of most abundant functional oligosaccharides in human breast milk and has been associated with many health benefits. Current methods for production of fucosylated human milk oligosaccharides (HMOs) are largely limited to genetic modification of whole bacteria, which is both expensive and non-specific, with respect to the types of oligosaccharide products obtained or to the use of fucosyltransferases, additionally limited to specificity of the products formed. These approaches are costly and the resulting products lack the structural diversity seen in human HMOs. It has been shown that some β-galactosidases are able to utilise fucose monosaccharides to give rise to fucosylated-oligosaccharides (FucOS), an important component of human milk. Our previous work, using predictive and experimental tools to assess the impact of domain truncations and site-directed mutagenesis on *Bifidobacterium bifidum* β-galactosidase III (BbgIII), which has higher transgalactosylation activity than other galactosidases, led to the development of improved BbgIII enzyme variants that gave galactooligosaccharides (GOS) with high purity mixtures. We have discovered further that two of our isolated, protein engineered β-galactosidase enzymes have the ability to produce FucOS, when provided with lactose and fucose, or GOS and fucose, as substrates. In this regard, to our knowledge, the sole use of a modified b-galactosidase enzyme to produce specific FucOS, as authentic HMOs, has not been shown previously. The overall aim of this project is to use a structure-guided, protein engineering approach to synthesise a number of variant b-galactosidases with the ability to produce specific FucOS, as authentic HMOs for use in infant formula, with health benefits.

P42. Effect of prebiotic Synergy-1 during iron supplementation, haem and iron restriction on the human gut microbiota using continuous culture colonic model systems

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Iron is an essential nutrient that is required by all lifeforms, including bacteria (with very few exceptions) and humans. Iron deficiency is considered the most common form of human malnutrition resulting in iron- deficiency anaemia (IDA) affecting millions of adults and children in the world. Those with IDA suffer from fatigue, poor concentration, weakened immunity, and compromised professional performance– representing a major economic and societal burden. Iron supplementation is the main strategy to increase iron levels during deficiency. However, most of the iron will not be absorbed and will reach the colon where could cause oxidative damage. In addition, iron supplementation may promote potential pathogens providing them with a supply of iron for their growth. In this work, we used four *in vitro* three-stage continuous culture human colonic model system comprised of vessels simulating anatomical regions of the human colon. Effect of iron supplementation ($FeSO_4$ 250µM), haem (77µM) and iron restriction were studied in these models by triplicate. In addition, the prebiotic -Synergy-1 (oligofructose-enriched inulin) was administered in one of the gut models during iron supplementation to investigate if the prebiotic could lead to a beneficial shift in the microbiota helping against the dysbiosis associated with iron supplementation. The effect of iron and prebiotic on the faecal microbiota composition was determined using NGS-based community profiling (16S rRNA sequencing). In addition, metabolic end products such as lactate and short chain fatty acids were quantified using gas chromatography, and metal levels were determined by ICP-OES. Overall, changes were detected under the different conditions and data will be presented at the ISAPP 2019 annual meeting.

P43. *In vitro* enrichment: a method for selection of synergistic probiotic strains

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Synbiotics are prebiotic-probiotic combinations used to modulate the gastrointestinal (GI) microbiome. Synergistic synbiotics consist of prebiotics that specifically stimulate the growth of the cognate probiotic and can provide the strain with a competitive advantage in the GI environment. The goal of this study was to develop an *in vitro* enrichment (IVE) method for selecting putative probiotic strains capable of synergism. Step-wise *in vitro* fecal fermentations with the prebiotic, xylooligosaccharide (XOS) were used to enrich for XOS-utilizers. Portions (1%) were transferred into fresh medium every 24 h for a total of 3 transfers or about 20 generations, and samples were plated onto *Bifidobacterium* selective agar. Isolates were obtained and identified by 16S rRNA sequencing. Quantification of bifidobacteria species was performed by qRT-PCR. In addition, 16S rRNA community sequencing was used for identification of amplicon sequence variants (ASVs) and taxonomic profiling. One of the enriched isolates, *Bifidobacterium longum* subsp. *longum* CR15 was then re-introduced into 20 fermentation vessels, each containing a unique fecal sample and 1% XOS. After 20 generations, the CR15 strain was established in 18 samples. Within XOS driven communities, an ASV that was representative of *B. longum* CR15 was detected along with high concentrations of acetate. Subsequent genome sequencing of CR15 revealed pathways encoding for enzymes involved in XOS utilization. The IVE method is a rational approach for isolating synergistic probiotic strains capable of specific substrate utilization. However, strain establishment may also depend on the host microbiome phenotype.

P44. Isolation and characterization of upper respiratory tract bacteria from healthy children and their characterization as potential probiotics against classic otopathogens

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Middle ear infections are traditionally associated with *Moraxella catarrhalis*, *Streptococcus pneumoniae* or non-typeable *Haemophilus influenzae* (NTHi) which ascend from the upper respiratory tract through the Eustachian tube. As these bacterial species – which are also implicated in other respiratory diseases – are present in the nose and nasopharynx of almost all humans, the question arises under which conditions they are able to cause disease. According to one hypothesis, pathobionts (commensal bacteria with a pathogenic potential in a non-immunocompromised host) cause disease when other bacteria, which usually suppress their pathogenic potential, are decreased in their relative abundance or lost from a niche. Conversely, adding such protective bacteria could potentially prevent middle ear infection or cure chronic or recurrent cases.

To find bacteria beneficial for upper respiratory tract health, the nare and nasopharynx of children without a recent history of middle ear disease were sampled with a main focus on lactic acid bacteria. *Streptococcus*, *Dolosigranulum* and *Leuconostoc* isolates were tested for their ability to inhibit the growth of *M. catarrhalis*, *S. pneumoniae* and NTHi through direct (Spot Assay) and indirect (Well-Diffusion Assay) bacterial interference. The most effective isolates were further tested for their adherence to human macrophages (differentiated THP-1 cells) and respiratory mucosa cells (Calu-3 lung carcinoma cells). The absence of antibiotic resistance genes on transferable elements was assessed through Minimum Inhibitory Concentration (MIC) assays and – for promising isolates – whole genome sequencing.

P45. Effect of administering kefir on the changes in fecal microbiota and symptoms of Inflammatory Bowel Disease: A randomized controlled trial

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Background/ Aims: Kefir is a kind of fermented probiotic dairy product. The objective of this study was to investigate effects of kefir consumption on Inflammatory Bowel Disease patients' micro flora, biochemical parameters, symptoms and also quality of life.

Materials and Methods: The study was performed as a single center, prospective, open-label randomized controlled trial. 48 patients were separated into two groups (28 for treatment, 20 for control). Treatment group consumed kefir, have $2x10^{10}$ cfu/400 ml *Lactobacillus*, control group didn't. Their gaita *Lactobacillus*, *Lactobacillus kefiri* content was quantitated by Real Time-qPCR before and after 28 days.

Results: *Lactobacillus* bacterial load of feces of all subjects were between 10^4–10^9 cfu/g and first and last measurements were statistically significant (p=0.001 in Ulcerative Colitis; p=0.005 in Crohn's Disease). The *Lactobacillus kefiri* bacterial load in the gaita of 17 subjects was measured as between 10^4–10^6 CFU/g. For Crohn's Disease patients, there was a significant decrease in Erythrocyte Sedimentation Rate and C-reactive protein while hemoglobin increased. Last 2 weeks, bloating scores were significantly reduced (p = 0.012), feeling good scores increased (p = 0.032). There was a statistically significant difference between the ulcerative colitis and control group in terms of stool frequency in the first two weeks (p = 0.026), but no statistically significant difference was found in terms of other variables.

Conclusion: According to data from this study, kefir consumption may modulates gut microbiota and regular consumption of kefir may improve patients both symptoms and quality of life in short term.

P46. Fish consumption alters the gut microbiome and metabolome in healthy women: A randomised controlled trial

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Adequate fish consumption is associated with a number of health benefits, such as improved cognition and reduced risk of cardiovascular disease, and is considered a key constituent of a healthy, balanced diet. Adequate fish consumption is defined as consuming at least two portions of fish per week, of which, at least one portion should be oily fish. Diet is considered a major determinant on the composition and metabolic activity of the GM, where undigested food components provide a fermentation substrate for competing microbes resulting in the production of an array of metabolites referred to as the metabolome. Forty nine healthy women who were low habitual fish consumers were recruited and randomly allocated into 5 groups: Control, 1 portion of Sardines per week, 2 portions of Sardines, 1 portion of Tuna and 2 portions of tuna for 8 weeks. Gut microbial composition was assessed by 16S rRNA sequencing and non-targeted GC-MS was employed to analyse the gut metabolome. Consuming fish significantly altered beta diversity but not alpha diversity in a dose response manner. Tuna consumption increased the relative abundance of *Faecalibacterium*, *Lachnoclostridium* and *Lachnospira* while sardine consumption increased *Coprocccus* and *Marvinbryantia* compared to control. Analysis of the metabolome revealed that all fish consuming groups separated from the control in principal component analysis, with two portions of tuna having the greatest effect.