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Poster Abstracts

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Probiotics and Prebiotics: Excellence in Science and Clinical Translation

Topic: Translational Microbiome Studies

Abstract No: 7125

Alteration in the Gastric Microbiota and Its Restoration by Probiotics in Patients with Functional Dyspepsia

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Background and Rationale

Little is known about the gastric microbiota or its role in the pathophysiology of functional dyspepsia (FD).

Objectives: Indicates the purpose of the study

This study was performed to comparatively analyze the gastric fluid (GF) microbiota between FD and healthy controls (HC), and to assess the effect of probiotics on the microbiota.

Methodology: Describe pertinent experimental procedures

Twenty-four Japanese patients with FD who met the Rome III definition and 21 age- and gender-matched HC volunteers were enrolled. The FD patients had been treated with LG21, a probiotic strain. GF was sampled after an overnight fast using a nasogastric tube. Bile acids concentration was determined by ELISA. The V3-V4 region of 16S rRNA gene was amplified using bacterial DNA from the GF, and then about 30,000 high-quality amplicons per sample were grouped into operational taxonomic units for analyses.

Results: Summarize the results of the research

The ratio of GF samples in which the bile acids was detectable was significantly greater in the FD than in the HC groups. In the bacterial composition analysis at the phylum level, the GF microbiota had a *Bacteroidetes*>*Proteobacteria* abundance and an absence of *Acidobacteria* in the FD group, in contrast, the GF microbiota had a *Bacteroidetes*<*Proteobacteria* abundance and the presence of *Acidobacteria* in the HC group. Probiotic therapy in patients with FD shifted the composition of the gastric fluid microbiota to that observed in the HC volunteers.

Conclusions: State the main conclusions

Alteration in the GF microbiota was found in FD patients compared with HC volunteers. Reflux of the small intestinal contents, including bile acids and intestinal bacteria, to the stomach was suggested to induce a bacterial composition change and be involved in the pathophysiology underlying FD. Probiotics appear effective in the treatment of FD through the normalization of gastric microbiota.

Keywords: Functional dyspepsia; Gastric microbiota; LG21 strain

Prebiotic properties of oligosaccharide produced from the spent coffee grounds by mannanase from *Bacillus* sp. GA2(1)

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Background and Rationale

Agricultural and agro-industry wastes such as sugarcane bagasse, straw, soybean grounds, and spent coffee grounds mostly contain lignocellulose as main component which are digested by lignocellulosic enzyme to produce oligosaccharide from cellulose and hemicellulose. These oligosaccharides can use as substrate in various industries such as energy, food, and animal feed industry. In addition, the important property of oligosaccharides is prebiotic substance.

Objectives: Indicates the purpose of the study

The objectives of this research were to analyze type of oligosaccharide from spent coffee grounds (SCG) producing by *Bacillus* sp. GA2(1) mannanase by thin layer chromatography (TLC) and high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). In addition, prebiotic properties of oligosaccharide were evaluated as follow the growth of lactic acid bacteria (LAB).

Methodology: Describe pertinent experimental procedures

Treated-SCGs were digested by mannanase from *Bacillus* sp. GA2(1) to produce the oligosaccharides. The products were analyzed type of sugar by TLC and HPAEC-PAD methods. In addition, the important property of oligosaccharide is prebiotic substance so the growth of *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus plantarum* cultivated with oligosaccharide products were examined compared with control and commercial prebiotics (Fructooligosaccharide; FOS).

Results: Summarize the results of the research

The oligosaccharide products from SCGs digested by mannanase from *Bacillus* sp. GA2(1) are mannose, mannobiose, mannotriose and mannopentaose. The determination of prebiotic properties of oligosaccharide found these products can promote the growth of *L. acidophilus*, *L. casei* and *L. plantarum* as much as the commercial prebiotics (FOS).

Conclusions: State the main conclusions

This study provided early evidence that oligosaccharides from SCGs digested by mannanase from *Bacillus* sp. GA2(1) are prebiotic substance, which can be alternative prebiotic for industry.

Keywords: *Bacillus* sp. GA2(1), Mannooligosaccharide, Prebiotics, Spent coffee grounds

Effects of An Intervention with An Affordable Starter Culture and Training Program for the Local Production of Probiotic Yoghurt in East-Africa

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Background and Rationale

In rural Africa, there is a need for more income diversification through non-farm activities in order to facilitate economic growth. Secondly, the high incidence of bacterial infectious diseases and the presence of toxic contaminants in the food chain demands for improved food safety and improved gut health. Microbial fermentation of food materials can address both the economic and health related needs.

Objectives: Indicates the purpose of the study

The objective of the study is to establish a model to provide impoverished communities in Africa access to *Lactobacillus rhamnosus* GG under the name *Lactobacillus rhamnosus* yoba 2012, world's first generic probiotic strain by means of an intervention with a dried starter culture and an associated training program.

Methodology: Describe pertinent experimental procedures

A locally adapted training program is used to transfer knowledge on the production of fermented milk at small-scale in rural settings in East Africa. Hereto, the affordable and stable starter culture is cultured in 1 L of milk. This fresh starter can be used to ferment 100 liter of milk by using locally available basic equipment and resources.

Results: Summarize the results of the research

Without external financial support 107 communities or small entrepreneurs have been able to start, expand and maintain a business by sustainable production and sales of probiotic yoghurt, with cumulative volumes exceeding 20,000 litres per week with 59% of the production units owned by women. The yoghurt was able to pass international standard of microbial quality and safety. Applied business models and success rate in terms of revenues and profitability varied per region and depended on culture, wealth status, and gender.

Conclusions: State the main conclusions

The intervention of an innovative starter culture for locally produced probiotic yoghurt created additional sources of income and employment for 703 people (64% female), and has the potential to reduce the incidence and severity of diarrhea, alleviate the symptoms of stomach ulcers and reduce the uptake of aflatoxins in the body.

Keywords: Lactobacillus rhamnosus yoba 2012; East-Africa; fermented milk; rural business

Cloning and expression of gene coding for KAC5 from *Lactobacillus reuteri* KUB-AC5 by food grade vector system

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Background and Rationale

Background: *Lactobacillus reuteri* KUB-AC5 isolated from chicken intestine was verified as probiotics which produced antimicrobial peptide (AMP of 4.7 kDa inhibiting various pathogenic bacteria, especially *Salmonella* serovar Enteritidis S003. Gene coding for AMP was cloned into *E. coli* by genomic library resulting in a possible open reading frame consisting of 153 and 630 nucleotides encoding 50 and 209 amino acids, respectively. To find out the gene coding for AMP, sub-cloning technique was further performed.

Objectives: Indicates the purpose of the study

Objective: This study aimed to localize for the gene encoding AMP by food grade vector system using lactobacilli as host cells. The character of an active AMP was also presented.

Methodology: Describe pertinent experimental procedures

Methodology: Gene coding for AMP was sub-cloned and expressed in *Lb. plantarum* TG02 and *Lb. reuteri* KUB-AC5 by food grade vector pSIP609. The positive clone of both bacteria was screened by colony PCR method and inhibition activity determination by well diffusion method using *S. Enteritidis* S003 as an indicator strain.

Results: Summarize the results of the research

Results: Subcloning into *Lb. plantarum* TG02 resulted in an active recombinant clone namely ACLP-C46-F2.1 consisting of 153 nucleotides and exhibiting intracellular inhibition activities of 14.6 mm named KAC5 under induction system of IP-673. It was tolerant at wide pH range of 2-9, high temperature up to 121°C and exhibited activity against both G+ and G- bacteria except lactic acid bacteria. The DNA fragment, I-C46-F2.1, was further subcloned into the wild type *Lb reuteri* KUB-AC5 resulting in the recombinant clone *Lb. reuteri* ACLR-C46-F2.1 exerting high activities of 400 AU/ml without IP-673 inducer. The KAC5 would be developed and applied for food and feed safety in the future.

Conclusions: State the main conclusions

Conclusion: Overexpression of KAC5 from the recombinant *Lb. reuteri* ACLR-C46-F2.1 displayed higher inhibition activities for 1.6 folds comparing to the wild type strain. KAC5 characters were wide inhibition spectrum and tolerance to a wide pH range and high temperature.

Keywords: KAC5, gene coding for KAC5, *Lactobacillus reuteri* KUB-AC5, pSIP609

Identification of antimicrobial substance producing lactic acid bacteria presenting in human milk and its antimicrobial substance characters.

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Background and Rationale

The discovery of new group of antibiotics slowly increase while the number of bacteria resistant to the antibiotics is increasing. Vancomycin resistant Enterococci (VRE) is a strain of Enterococci "superbugs" that have become to be difficult to treat and, occasionally, may be life threatening. Bacteriocin from lactic acid bacteria (LAB) was proposed as an interesting source to control those multiple drug resistance bacteria. Its production has been considered an important trait in the selection of probiotic strains.

Objectives: Indicates the purpose of the study

The objective of this study was to identify bacteriocin producing LAB strain isolated from human milk and to characterize its bacteriocin.

Methodology: Describe pertinent experimental procedures

Antimicrobial activity was determined by spot on lawn method using VRE as indicator strain. Morphology, biochemical characters, peptide profile by MALDI TOF Mass and 16S rRNA gene of the strain was determined. The bacteriocin was purified to homogeneity by Amberlite XAD-16 resin, ion exchange, reverse-phase chromatography and further analysed by MALDI-TOF mass spectrometry for molecular weight determination. Characteristics of bacteriocin were evaluated for the sensitivities to hydrolytic enzymes, the stabilities to temperature and pH including inhibition spectrum.

Results: Summarize the results of the research

Based on the characters of morphology, carbohydrate fermentation, peptide profile and 16S rDNA sequence, the effective LAB strain HM02-04 belonged to *Enterococcus hirae*. Mass spectrometry analyses revealed a molecular mass of bacteriocin was 2.605 kDa. This antimicrobial substance was sensitive to proteases demonstrating its proteinaceous nature namely enterocin HM02-04. It was heat stable up to 121°C and displayed at wide pH range of 3.0 to 9.0. Enterocin HM02-04 displayed a narrow inhibitory spectrum limited to genus *Enterococcus* and some LAB species of *Lactobacillus plantarum*, *Lb. coryniformis* and *Lactococcus lactis*.

Conclusions: State the main conclusions

E. hirae HM02-04 isolated from breast milk produced a thermostable and pH-tolerant bacteriocin including narrow inhibition spectrum, especially, *Enterococcus sp.* and those VRE strains.

Keywords: *Lactic acid bacteria; bacteriocin; Vancomycin resistant Enterococci.*

Strain specific inhibition of *Clostridium difficile* by commercial probiotics *in vitro*.

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Background and Rationale

Evidence of the effectiveness of probiotics in managing *Clostridium difficile* infection is limited which could be reasoned to be the attributes of the probiotics used for the study and lack of *in vitro* studies to streamline potential strains.

Objectives: Indicates the purpose of the study

The aim of the study was to investigate the influence of live cells and metabolic products of commercial probiotics strains (*Lactobacillus acidophilus* LA-5[®], *Bifidobacterium lactis* BB-12[®]) on the growth of *C. difficile* *in vitro*.

Methodology: Describe pertinent experimental procedures

The strains were incubated as pure cultures and then as co-cultures with *C. difficile* in Brain Heart Infusion (BHI) broth supplemented with 0.1% (w/v) L-cysteine hydrochloride and 0.1% (w/v) sodium taurocholate and monitored in an isothermal microcalorimeter. Unmodified, concentrated and pH modified metabolic products produced by the probiotic strains were also co-cultured with *C. difficile* in broth and monitored with the microcalorimeter.

Results: Summarize the results of the research

Characteristic signatures of the pure strains were obtained from the microcalorimeter. *C. difficile* was inhibited by the live cells of the probiotics. Using the unmodified and pH-modified metabolic products produced by the probiotic strains, inhibition was noted to be pH-dependent. Further investigation to determine whether inhibition was solely pH-dependent by use of concentrated neutralized metabolic waste revealed the possibility of non-acidic metabolites contributing to inhibition. However, total inhibition in the microcalorimeter occurred when metabolic products produced from *L. acidophilus* was concentrated by 20 fold and that of *B. lactis* by 5 fold inferring that rate of inhibition differed between the two strains. The results also showed that *C. difficile* growth was maximum when the pH of the medium was between 6.45 and 6.9.

Conclusions: State the main conclusions

In conclusion, the results show that some commercial probiotic strains can inhibit *C. difficile*; however, inhibition and degree of inhibition could be strain specific.

Keywords: *Clostridium difficile*; inhibition; probiotics; microcalorimetry; metabolic products

A practical guide for the use of probiotics in the prevention of antibiotic-associated diarrhea in The Netherlands

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Background and Rationale

The efficacy of probiotics in the prevention of antibiotic-associated diarrhea (AAD) has been proven by several clinical studies. Although individual healthcare professionals may recommend their use alongside antibiotic therapy, no official agreement exists around the prescription of probiotics in The Netherlands.

Objectives: Indicates the purpose of the study

We aimed at providing evidence-based recommendations to clinicians and patients in The Netherlands for the choice of probiotics to prevent AAD.

Methodology: Describe pertinent experimental procedures

To compile the guide, we followed a workflow consisting of four steps: (1) selection of a relevant medical condition and assessment of the role of probiotics (preventive or curative), (2) identification of effective probiotics through a systematic review of relevant clinical studies, (3) compilation of a list of available probiotic products, and (4) recommendation of probiotic products matching effective formulations. The selection of clinical trials to review was based on specific inclusion criteria. We reviewed randomized, double-blind and placebo-controlled studies, including a clear definition of AAD, and during which probiotics were administered for at least the entire duration of antibiotic therapy.

Results: Summarize the results of the research

Through review of thirty-two clinical trials satisfying the inclusion criteria, we identified seven single or multiple-strain probiotic formulations effective for the prevention of AAD. *Lactobacillus rhamnosus* GG was the most effective strain. From a list of all probiotic dairy products and food supplements available in The Netherlands, we selected for recommendation those matching, both in probiotic strain and number of colony-forming units, the effective formulations. We identified eight probiotic products for recommendation, including six food supplements and one dairy product. Two products based on *Lactobacillus rhamnosus* GG received a strong recommendation as their effect was supported by three clinical studies.

Conclusions: State the main conclusions

We developed a practical guide that informs healthcare professionals and patients on the efficacy of probiotic products to prevent AAD and on the availability of such products in The Netherlands.

Keywords: meta-analysis; probiotics; antibiotics; antibiotic-associated diarrhea

Supplementation with prebiotic inulin-type fructans on host metabolism and gut microbiota: the first randomized double-blind placebo-controlled trial in China.

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Background and Rationale

Background and rationale: The gut microbiota has been implicated in host lipid and glucose metabolism. Now recent findings suggest that prebiotic results in bacterial ecosystem shift. Whereas, a comprehensive characterization of this effect is quite inadequate in Chinese Population.

Objectives: Indicates the purpose of the study

Objectives: Here, the objective of the present study was to assess the consequences of supplementation with inulin-type fructans (ITF), fructo-oligosaccharides (FOS) and inulin, on the composition of gut microbiota and human metabolism.

Methodology: Describe pertinent experimental procedures

Methods: A randomized double-blind placebo-controlled study was conducted with 135 healthy adults with FOS and inulin for 4 weeks (15g/d). Blood and fecal sampling, oral glucose tolerance test and anthropometry were performed before and after intervention. 16S rDNA microbiota profiling was applied to assess the composition of gut microbiota. Machine learning with random forest model was adopted to predict the response of OGTT following ITF prebiotics intervention.

Results: Summarize the results of the research

Results: 4-week intake of ITF prebiotics, but not placebo, markedly increased abundance of *Bifidobacterium* with significant effects on overall microbial richness and diversity. Meanwhile, the manipulation of ITF on gut microbiota was also manifested in the decreased abundance of *Roseburia*, *Phascolarctobacterium*, *Ruminococcaceae*, *Ruminococcus*, *Holdemania* and *Lachnospiraceae*. Importantly, we did not observe any differences in host anthropometric, lipid metabolism, blood glucose metabolism, nor insulin sensitivity. In addition, using the baseline data of gut microbiota in random forest model, we built an index to successfully predict the response of OGTT after ITF intervention.

Conclusions: State the main conclusions

Conclusions: 4-week supplementation of ITF indeed led to ecosystem-wide microbiota shifts, but this did not produce significant alteration in host lipid and glucose metabolism. While with the data of initial gut microbiota, we can predict the individualized response of OGTT after ITF intervention, which make potential practical value in nutritional strategy.

Keywords: *Prebiotics, inulin-type fructans, gut microbiota, randomized controlled trial (RCT), machine learning prediction model.*

Milk Microbiota Core During First 8 Months of Lactation in Healthy Mothers from China

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Background and Rationale

Due to the differences in microbiota development between breastfed and formula-fed children and the higher risk for some diseases in formula-fed children, supplementation of formula with probiotics is an important field of research. However, breast milk is a dynamic fluid which is difficult to mimic. The composition changes along lactation and a complex microbiome is composed of hundreds of different bacteria, whose contribution to infant development has not been elucidated yet.

Objectives: Indicates the purpose of the study

Our aim was to characterize milk microbiota changes during lactation in healthy mothers from China.

Methodology: Describe pertinent experimental procedures

Longitudinal milk microbiota composition and diversity from 12 healthy mothers was analyzed by 16S gene sequencing.

Results: Summarize the results of the research

Higher interindividual microbiota variation was observed among mothers and within mothers at different time points. Colostrum microbiota was dominated by *Staphylococcus*, *Acinetobacter* and *Streptococcus*; early milk and mature by *Acinetobacter*, *Pseudomonas* and Enterobacteriaceae; and late milk by *Acinetobacter*, *Stenotrophomonas* and Enterobacteriaceae. In addition, significant changes ($p < 0.05$) were found along lactation with an increase in Gammaproteobacteria group and a reduction in Firmicutes, Bacilli, *Staphylococcus* and *Streptococcus*. No differences in microbial diversity and richness during lactation were found.

A stable microbial core from birth to 8 months of age was found. Nine genera were present at all the time points: *Acinetobacter*, *Lactobacillus*, *Pseudomonas*, *Propionibacterium*, *Rothia*, *Sphingomonas*, *Staphylococcus*, *Spirosoma* and *Streptococcus*, with changes in the abundance depending of the time point.

Conclusions: State the main conclusions

Despite the high inter-individual variability, there is a milk microbiota core during lactation. The potential biological effect of milk microbiota core needs be identified in order to understand their impact on infant health. Such a role includes the development of infant gastrointestinal tract and immune system, leading to the development of novel infant formulas supplemented with probiotics.

Keywords: Microbiota; breast milk; lactation; milk microbial core

A synbiotic mixture of scGOS/lcFOS and *Bifidobacterium breve* M-16V is able to restore the delayed colonization by *Bifidobacterium* in C-section delivered infants

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Background and Rationale

C-section born infants have a compromised gut microbiota at birth. Epidemiological data have indicated associations between C-section and the development of immune and metabolic disorders. Nutritional modulation of the gut microbiota may aid to reduce the disease risk associated with C-section.

Objectives: Indicates the purpose of the study

The objective of this study was to determine the effect of a specific synbiotic mixture in restoring the delayed colonization by *Bifidobacterium* in C-section delivered infants.

Methodology: Describe pertinent experimental procedures

In a multi-country double-blind, controlled study, 153 infants born by elective C-section were randomised to receive (1) an infant formula supplemented with short-chain galacto-oligosaccharide/ long-chain fructo-oligosaccharides (scGOS/lcFOS) and *B. breve* M-16V, or (2) a formula supplemented with scGOS/lcFOS, or (3) a control formula from birth until age 4 months. Thirty infants born vaginally were studied in parallel. Stool samples were collected to determine the effect of the intervention at day 3, day 5, week 4, week 8, week 12, week 16, and week 22 (6 weeks post-intervention). The proportion of bifidobacteria, different *Bifidobacterium* species including the probiotic strain were determined with molecular tools. pH and SCFA were also measured in the stool samples.

Results: Summarize the results of the research

We confirmed the delayed colonization by *Bifidobacterium* in C-section delivered infants. The synbiotic supplementation resulted in a higher proportion of *Bifidobacterium* from day 3/5 ($P<0.0001$) till week 8 ($P=0.041$) and a reduction of Enterobacteriaceae from day 3/5 ($P=0.002$) till week 12 ($P=0.016$) compared to the control group. This was accompanied with higher acetate and a lower fecal pH. In the synbiotic group, *B. breve* M-16V was still detected in 38.7 % of the infants at week 22.

Conclusions: State the main conclusions

An infant formula supplemented with scGOS/lcFOS and *B. breve* M-16V supported the early modulation of *Bifidobacterium* in C-section born infants that was associated with the emulation of the gut physiological environment observed in vaginally delivered infants.

Keywords: Synbiotic, C-section, Microbiota, early life

Compromised Gut Microbiota At Birth

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Background and Rationale

A compromised gut microbiota in early life has been associated with the development of immune and metabolic disorders.

Objectives: Indicates the purpose of the study

The objective of this study was to determine the effect of mode of delivery and intrapartum antibiotic prophylaxis (IAP) on the maturation of the infant gut microbiota.

Methodology: Describe pertinent experimental procedures

Three groups of healthy term-born infants were recruited from the National University Hospital (Singapore): (1) Infants born vaginally (VD, n=21); (2) Infants born vaginally whose mothers received IAP (IAP, n=22); and (3) Infants born by C-section (CS, n=25).

Stool samples (n=246 samples) were collected at birth, day 7 and, 1 and 3 months. The composition of the gut microbiota was determined by 16S rRNA sequencing.

Results: Summarize the results of the research

A delayed colonization by *Bifidobacterium* was observed in IAP and CS but not in VD born infants. This was statistically significant at day 7 (VD vs IAP, p=0.020; VD vs CS, p=0.003) and 1 month (VD vs IAP, p=0.003; VD vs CS, p=0.015). In contrast to infants born vaginally (with and without IAP exposure), CS born infants featured a delayed colonization by *Bacteroides* from day 7 (CS vs VD, p<0.0001; CS vs IAP, p=0.007) till 3 months (CS vs VD, p=0.003; CS vs IAP, p=0.007). The relative abundance of Enterobacteriaceae was higher in IAP and CS compared to VD born infants at day 7 (IAP vs VD, p=0.007 and CS vs VD, p=0.037) and 1 month (IAP vs VD, p=0.031 and CS vs VD, p=0.049). A negative correlation between *Bifidobacterium* and Enterobacteriaceae was observed at day 7 and 1 month. The species diversity was higher in VD compared to IAP and CS at day 7 (p=0.007 and p=0.001).

Conclusions: State the main conclusions

Intrapartum Antibiotic Prophylaxis and C-section impair the early microbial colonization and this may represent a risk factor for the development of immune/ metabolic disorders.

Keywords: Microbiome, Gut Microbiota, Intrapartum antibiotic prophylaxis, Cesarean section Delivery

Antibiotics-perturbed Microbiome and Its Effects in Alzheimer's Disease Pathogenesis: A Potential Future for the Probiotic (*Akkermansia Muciniphila*) Pre-clinical Studies in This Model.

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Background and Rationale

Recent evidence suggests an involvement of the microbiota-gut-brain axis in Alzheimer's disease (AD). Specific bacteria i.e. *Akkermansia Muciniphila* showed promising results in obesity and type 2 diabetes (T2DM) by reducing endotoxin and inflammation. Obesity is now considered a primary risk factor for the development of T2DM, and in turn, T2DM is a risk factor for the pathogenesis of AD. As these conditions share several anomalies such as impaired glucose metabolism and insulin signaling, low-grade chronic inflammation and oxidative stress, we hypothesize that *A. Muciniphila* could also impact AD pathogenesis.

Objectives: Indicates the purpose of the study

To investigate the influence of microbiome in the AD, we used antibiotics in AD transgenic mice. We plan to investigate the role of *A. Muciniphila* in our proposed model.

Methodology: Describe pertinent experimental procedures

Alzheimer's transgenic mice (APP/PS1) were gavaged with antibiotics during post-natal (p14-p22) and followed with 1:50 diluted antibiotics (drinking water) until the age of 7 weeks or 3 months. Gut, brain, feces and serum samples were collected.

Results: Summarize the results of the research

Antibiotics (Abx) T_x resulted in significantly lower Abeta pathology in the cortex at 7 weeks (Abeta burden; control: 0.40±0.01, Abx: 0.06±0.001) and 3 months (control: 1.42±0.20, Abx: 0.61±0.07) as well as in the hippocampus at the 3 months (control: 0.50±0.07, Abx: 0.14±0.02). The Abx group also showed altered brain resident macrophages (microglia characteristics) and serum cytokine profile suggesting changes in inflammation. To correlate this data with the microbiome, we observed significant changes in the microbiome profile with Abx at the age of 3 months (p22 and 7 weeks of age: under evaluation). Among the significantly different taxa, *Akkermansia Muciniphila* showed higher abundance (also highest magnitude of changes among all other significant taxa) in Abx.

Conclusions: State the main conclusions

Our data shows antibiotics-induced higher *A. Muciniphila* is associated with lower Alzheimer's pathology. Results from *A. Muciniphila* probiotics in this model will help us to understand its role in AD.

Comparative genomic and phenotypic analysis of the vaginal probiotic *Lactobacillus rhamnosus* GR-1

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Background and Rationale

Lactobacillus represents a versatile bacterial genus, which can adapt to a wide variety of ecological niches, including human body sites such as the intestinal and urogenital tract. While the genome sequences of various gastrointestinal and dairy probiotic *Lactobacillus* strains have been published since 2004, the vaginal strains are lagging behind. Nevertheless, probiotic application holds greater promise in the vagina, because of the dominance of lactobacilli in the niches under health conditions.

Objectives: Indicates the purpose of the study

In this study, the complete genome sequence of the vaginal probiotic *L. rhamnosus* GR-1 was determined and compared to other *L. rhamnosus* strains at genomic and phenotypic level. The strain LGR-1 was originally isolated from a female urethra and was assessed with *L. rhamnosus* GG from a feces sample of a healthy male, and *L. rhamnosus* LC705 from a dairy product.

Methodology: Describe pertinent experimental procedures

First, the genome of LGR-1 was determined, followed by calculating the pan-genome of all publicly available *L. rhamnosus* strains and constructing a high-resolution phylogenetic tree. Subsequently, the phenotypic differences between LGR-1, LGG, and LC705 were evaluated by performing various experiments, such as carbohydrate utilization, adhesion to epithelial cells, detection of pili and EPS molecules, as well as different stress survival assays.

Results: Summarize the results of the research

A key difference is the absence in GR-1 and LC705 of the *spaCBA* locus required for pili-mediated intestinal epithelial adhesion. Also, the LGR-1 genome contains a unique cluster for EPS production, which is postulated to synthesize glucose-rich, rhamnose-lacking exopolysaccharide molecules that are different from the galactose-rich EPS of LGG. Compared to LGG, LGR-1 was also genetically predicted and experimentally shown to better metabolize lactose and maltose, and to better withstand oxidative stress, which is of relevance in the vagina

Conclusions: State the main conclusions

Ultimately, this study could thus provide a molecular framework for the selection of the optimal probiotic strain for each targeted niche and condition

Keywords: probiotics, adaptation, vaginal niche, genome comparison

Bifidogenic effects of a unique synbiotic mixture (scGOS/lcFOS and *Bifidobacterium breve* M-16V) in healthy infants

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Background and Rationale

Human milk from healthy mothers is considered the optimal nutrition for infants and contains about 10⁴ viable bacteria. It is one of the bacterial sources to seed the infant's gut which is normally dominated by infant-type *Bifidobacterium* species such as *B. breve*. Bacteria like bifidobacteria play a key role in gut health and immune maturation during early life. However, not all infants are dominated by bifidobacteria and some are even devoid of them. Infants born by C-section or with antibiotics administration during early life have compromised microbiome development. Synbiotics containing *Bifidobacterium* species can be used to increase the level of bifidobacteria in the infant's gut.

Objectives: Indicates the purpose of the study

The aim of the study was to evaluate the effects of two different doses of synbiotics on bifidogenic effects in healthy infants.

Methodology: Describe pertinent experimental procedures

An exploratory, multi-centre randomised, double-blind, controlled study was conducted in Thailand in 290 healthy infants aged from 2-5 months. Infants, who were exclusively formula-fed for at least two weeks were recruited. After 2 weeks run-in period, infants were then randomized to receive either control product or one of the investigational products (containing 0.8g/100 ml scGOS/lcFOS with *B. breve* M-16V at a dose of either 1x 10⁴ cfu/ml (Syn4) or 1x10⁶ cfu/ml (Syn6)). Exclusively breast-fed infants were included as a reference.

Results: Summarize the results of the research

Syn4 and Syn6 significantly increased not only the total bifidobacteria proportion but also the prevalence and genomic copy numbers of *B. breve* and *B. breve* M-16V in healthy infants when compared with control group.

Conclusions: State the main conclusions

An infant-type *Bifidobacterium*, *B. breve* M-16V combined with scGOS/lcFOS (9:1) at a level close to the level of bacteria in human milk, increased infant type *Bifidobacterium* species in infants. This relatively low dose of viable bacteria may be a suitable approach to support the normal development of the gut microbiome in healthy infants during early life.

Keywords: infant; bifidobacterium; gut; microbiota; development;

Competition in a multi-species probiotic liquid suspension

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Background and Rationale

There is little information about the stability of multi-species probiotic products. A commercially available probiotic aqueous-based suspension, P1 which contains *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus* and *Enterococcus faecium* was tested using an isothermal microcalorimeter, an instrument which can monitor real-time metabolism of microorganisms.

Objectives: Indicates the purpose of the study

The goal of the study was to detect the relative growth of the species of product P1 to determine whether inhibition occurs amongst the species.

Methodology: Describe pertinent experimental procedures

The component probiotic species of P1 and the product were each inoculated into Brain Heart Infusion (BHI) broth or MRS broth (Oxoid, Basingstoke, UK) supplemented with 0.05% w/v L-cysteine hydrochloride (BHlc; MRSc) (in 3 mL calorimetric glass ampoules) to give pure cultures of densities of 10^6 CFU/mL and monitored in the microcalorimeter. The species were also inoculated into the medium to give a mixed culture of the individual species at concentrations of 10^6 CFU/mL of each. Cell free supernatant (CFS) produced by each species was also tested against the producing species and the other species by inoculating 10^6 CFU/mL of the species in broth with the CFS, monitoring in the microcalorimeter and enumerating the species at the end of incubation.

Results: Summarize the results of the research

The growth profiles in the microcalorimeter were characteristic and unique to each species while the growth profile of P1 was most similar to that of *L. plantarum*, suggesting this is the dominant organism in mixed-culture. The species showed mutual inhibition however *L. plantarum* was found to be the most effective species at inhibiting *L. rhamnosus*. Conversely, *L. rhamnosus* was the most effective at limiting the growth of *L. plantarum*. Both *L. plantarum* and *L. rhamnosus* were inhibitory toward *L. acidophilus* and *E. faecium*. *E. faecium* was the least inhibitory towards all the other species.

Conclusions: State the main conclusions

The study shows that some probiotic mixtures may not be stable as a product.

Keywords: probiotic; multi-strain; interspecies inhibition;

The effect of non-ionic detergent on the growth of *Faecalibacterium prausnitzii* in vitro

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Background and Rationale

Faecalibacterium prausnitzii is considered as a potential probiotic because of its anti-inflammatory properties. Its delayed colonization in childhood is correlated with development of auto-immune diseases, such as allergy and type 1 diabetes. Recently, hygiene hypothesis is believed to affect the infant gut microbiota composition. Parents tend to clean their baby frequently nowadays, which may cause an overexposure to non-ionic detergent. Previous findings show that *F. prausnitzii* often co-occurs with *Bacteroides thetaiotaomicron*. Therefore, we hypothesize that an overuse of detergent could affect the colonization of *F. prausnitzii* and that *B. thetaiotaomicron* could help to overcome this effect.

Objectives: Indicates the purpose of the study

To investigate if the detergent could affect the colonization of *F. prausnitzii* and the interaction between different species and to explain the mechanism by discussing the integrity and function of bacteria cell membrane.

Methodology: Describe pertinent experimental procedures

The experiments were performed by growing *F. prausnitzii* and *B. thetaiotaomicron* separately and together in YCFAG medium (with and without acetate) with different concentrations (0 to 120 µg/ml) of Triton X-100 for 48 hours. Samples hereof were analyzed with fluorescence in situ hybridization (FISH) to enumerate the subpopulations. Microbial fuel cell (MFC) and transmission electron microscopy (TEM) were employed to investigate the cell membrane integrity and function.

Results: Summarize the results of the research

We found that the growth of *F. prausnitzii* was inhibited by Triton X-100. FISH counting results showed *B. thetaiotaomicron* stimulated the growth of *F. prausnitzii* independent of the presence of Triton X-100 and acetate. TEM and MFC results showed the thickness of bacteria cell envelope and the current produced by *F. prausnitzii* were affected.

Conclusions: State the main conclusions

Our study shows Triton X-100 inhibits the growth of *F. prausnitzii* maybe because it destroys the integrity and function of cell membrane, the mechanism still needs to be investigated further. *B. thetaiotaomicron* could help to overcome this effect.

Keywords: *Faecalibacterium prausnitzii*; *Bacteroides thetaiotaomicron*; Detergent; Cell membrane; Microbial fuel cell

Timing of first probiotic exposure during infancy and the risk of celiac disease in genetically at risk children: TEDDY study

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Background and Rationale

The preventive effect of probiotics on celiac disease remains unresolved.

Objectives: Indicates the purpose of the study

The objective of this study was to examine the association between timing of first probiotic exposure by the age of 1 year and risk of celiac disease.

Methodology: Describe pertinent experimental procedures

Newborns at genetic risk for celiac disease selected from general population followed in The Environmental Determinants of Diabetes in the Young (TEDDY) study were annually screened for tissue transglutaminase autoantibodies (tTGA). For the purposes of this study, celiac disease was defined as having a Marsh score >1 or having a persistently tTGA level ≥ 100 U if a biopsy was not performed. Use of probiotics was monitored using questionnaires and diaries. Time-to-event analysis examined the association between timing of first probiotic exposure and the risk of celiac disease adjusting for potential confounders.

Results: Summarize the results of the research

Among 6520 TEDDY participants screened for tTGA, 455 (7%) were diagnosed with celiac disease at the median age of 4.3 (IQR 3.2-6.2) years. Exposure to probiotics in the first year of life was reported for 1460 children, but it did not affect the risk of celiac disease (HR=1.10; 95%CI 0.85, 1.41; p=0.48). The time of first probiotic exposure did not predict celiac disease among children exposed before 12 weeks of age (HR=0.93; 95% CI 0.86, 1.01, p=0.07). However, among those exposed after 12 weeks, the risk increased 4% (HR=1.04; 95%CI 1.02, 1.07; p=0.002) for every week of probiotic delay.

Conclusions: State the main conclusions

Overall, exposure to probiotics in infancy does not appear to protect from celiac disease. However, timing to first probiotic exposure may be important and its association with celiac disease needs to be further explored.

Keywords: probiotics; infant diet; celiac disease

Effect of vitamins B2 and C on the development of mucositis

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Background and Rationale

Chemotherapy-induced mucositis is a severe side-effect of anti-cancer treatment. This inflammatory disorder induces alterations in the composition of the gut microbiota, including a decrease in anaerobic bacteria. Redox active compounds, such as vitamins B2 and C, were shown to reduce inflammation in the gut and to enhance the growth of anaerobic bacteria.

Objectives: Indicates the purpose of the study

We aim at investigating the effects vitamins B2 and C on the development of mucositis and on the composition of the gut microbiota in the methotrexate (MTX)-induced mucositis rat model.

Methodology: Describe pertinent experimental procedures

MTX-induced mucositis rats were daily gavaged with vitamins B2 and C, alone or in conjugation, and MTX (45 mg/Kg) or saline solution (control) was administrated via intravenous injection. The severity of mucositis was determined by food intake, body weight and plasma citrulline and the gut microbiota composition was characterized by 16S rRNA sequencing. A combination of different concentrations of vitamins was added to bacterial cultures isolated from rats and their growth assessed.

Results: Summarize the results of the research

Vitamin C supplementation (250mg/Kg/day) in rats attenuated the severity of mucositis reflected by changes in body weight, food intake and plasma citrulline. A dose-dependent effect of vitamin B2 was found, as higher doses (60mg/Kg/day) have a negative impact on mucositis whereas lower doses (10 mg/Kg/day) have no effect. Anaerobic bacteria *Blautia coccooides* and *Roseburia intestinalis* significantly grew better in the presence of oxygen, when a combination of 0.5 mM or 1 mM of both vitamins is added to the culture.

Conclusions: State the main conclusions

Vitamin C ameliorates mucositis in our rat model. Vitamins B2 and C enhance the growth of anaerobic bacteria under oxidative stress.

Keywords: Mucositis, cancer treatment, methotrexate, vitamins, prebiotics

The effect of prebiotic oligofructose enriched inulin supplementation on microbiota, protein metabolism and gastrointestinal (GI) symptoms in people consuming high protein diets

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Background and Rationale

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.

Objectives: Indicates the purpose of the study

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.

Methodology: Describe pertinent experimental procedures

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.

Results: Summarize the results of the research

Bacteria were enumerated by fluorescence *in situ* hybridisation-flow cytometry. A significant increase in bifidobacteria was observed with the addition of prebiotic treatment ($p < 0.0001$). Urine, blood plasma and faecal water metabolite changes were monitored by ^1H -NMR. There were lower concentrations of aromatic metabolites in urine and lower concentrations of lipids in blood plasma with prebiotic treatment, however, differences were not significant between the two interventions. Stool frequency was significantly ($p < 0.01$) higher with prebiotic supplementation comparing to the placebo group and stool consistency had a trend towards softness as based on the Bristol scale ($p = 0.06$). Mild symptoms of bloating and flatulence were reported with the prebiotic, however these were tolerable. Total bacteria and bifidobacteria changes during interventions were significantly correlated with stool frequency ($p \leq 0.05$).

Conclusions: State the main conclusions

In conclusion, favourable bacterial and metabolic changes with ITF supplementation were found. ITF could exert health benefit improve health and wellbeing for high protein consumers, especially who also consume low fibre diet.

In vitro enrichment: a novel method for formulating synergistic synbiotics

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Background and Rationale

One strategy to modulate the gastrointestinal microbiota and improve gut and systemic health is by consumption of probiotics, prebiotics, or synbiotics. The latter can be formulated in one of two ways. Complementary synbiotics are comprised of combinations of probiotics and prebiotics, with each component functioning independently. In contrast, synergistic synbiotics contain probiotic strains that consume and are stimulated by the cognate prebiotic. Synergistic synbiotics may, therefore, have an ecological advantage by enhancing the ability of the probiotic to compete in the gastrointestinal tract.

Objectives: Indicates the purpose of the study

The goal of this study was to develop a rational method for selecting putative strains that would have synergistic properties.

Methodology: Describe pertinent experimental procedures

Our approach was to use stepwise *in vitro* fermentations to enrich for strains able to use the prebiotic, xylooligosaccharide (XOS). The *in vitro* enrichment (IVE) method was started by addition of a fecal slurry to fermentation vessels containing XOS. Every 24 hours, portions were transferred into fresh medium, until about 20 generations had been achieved. Samples were plated on *Bifidobacterium* selection agar, and XOS-fermenting isolates were obtained and identified by 16S rRNA sequencing. Genus- and species-specific real-time (RT)-PCR was used to quantify bifidobacteria.

Results: Summarize the results of the research

One isolate, *Bifidobacterium longum* subsp. *longum* CR15 was re-introduced into nine fermentation vessels, each containing XOS and different fecal samples. After approximately 20 generations, the CR15 strain was recovered in six vessels and was the dominant *Bifidobacterium* strain in three. Genome sequencing of *B. longum* CR15 revealed the presence of enzymes belonging to two different glycosyl hydrolases families crucial for XOS utilization.

Conclusions: State the main conclusions

In conclusion, the IVE method is a quick, convenient method for isolating potential synergistic probiotic strains capable of fermenting XOS or other specific prebiotic substrates. These *in vitro* results also suggest that the resident microbiota still has a profound influence on the establishment of particular strains, even when synergistic synbiotics are used.

Keywords: in vitro; synbiotic; xylooligosaccharide

Lactobacillus rhamnosus GG consumption characteristically modulate gut microbial composition of healthy Japanese subjects. -a randomized, double-blind, placebo-controlled study

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Background and Rationale

Lactobacillus rhamnosus GG (LGG) is one of the well-researched probiotics in the world. This bacterium can promote digestive health, alleviate various physiological/or clinic disorders such as atopic eczema and gastroenterological diseases, and might reverse dysbiosis to restore gut mucosal homeostasis. However, the benefits of LGG to the healthy subjects remain largely unclear.

Objectives: Indicates the purpose of the study

This study was to evaluate how LGG could affect intestinal environment and gut microbiota composition in healthy Japanese subjects.

Methodology: Describe pertinent experimental procedures

Lyophilized LGG was orally administrated to total 89 of healthy Japanese subjects with the dosage of 1.4×10^{10} CFU/day in a randomized, double-blind, placebo-controlled trail. Before and after 4 weeks intervention, the faecal were collected from the tested subjects, and fecal microbiota was profiled using sequencing of 16S rRNA genes of faecal DNA.

Results: Summarize the results of the research

Phylum *Proteobacteria* were significantly decreased ($P=0.01$) and tend to decrease family *Enterobacteriaceae* ($P=0.06$) after administration of LGG. Significant decreases in genus *Clostridium* (family *Clostridiaceae*) ($P=0.04$) and increase in *Bacillus* ($P=0.01$) were also found after intervention. However, no significant changes were observed in the placebo group. Interestingly, the same tendency was also found in female subject treated with LGG ($n=34$), the abundance of family *Enterobacteriaceae* were decreased ($P=0.02$) and genus *Bacillus* and *Lactobacillus* were increased significantly ($P=0.01$, $P=0.05$), while in male, genus *Turicibacter* and *Roseburia* were increased in man ($n=11$) after LGG consumption.

Conclusions: State the main conclusions

These results indicate LGG might benefit to healthy subjects though modifying intestinal microbes characteristically. These findings suggest the prospective studies to investigate the LGG–microbiome interaction in human health and disease.

Keywords: Gut microbiota, Probiotics, Healthy subjects, Japanese, lactobacilli, LGG

Resistant Starch Ameliorates Advanced Glycation Endproduct-Induced Albuminuria in A Mouse Model Of Type 2 Diabetes

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Background and Rationale

Excessive intake of dietary advanced glycation endproducts (AGEs) contributes to chronic renal injury. Recent research implicates gut dysbiosis in the progression of diabetic nephropathy, however, the role of dietary AGEs in gut dysbiosis and renal injury remains unexplored.

Objectives: Indicates the purpose of the study

This study investigated whether excess consumption of dietary AGEs cause gut dysbiosis, exacerbating renal injury in a mouse model of type 2 diabetes. A secondary aim was to elucidate whether resistant starch (RS) supplementation is protective against diabetic nephropathy via altering gut homeostasis.

Methodology: Describe pertinent experimental procedures

Six week old diabetic mice (db/db) and age-matched non-diabetic control mice (db/m) were randomised (n=12/group) to receive a low AGE (unbaked rodent chow) or a high AGE diet (baked at 160°C for 1 hour), with or without 25% RS for 10 weeks. All diets were isocaloric. 24-hour urine was collected for the assessment of albuminuria. Intestinal permeability was assessed *in vivo* by the clearance of FITC-labelled dextran. Cecal contents were collected and the V3-V4 region of the bacterial 16S rRNA gene were sequenced.

Results: Summarize the results of the research

The high AGE diet exacerbated albuminuria in db/db mice, and this was attenuated by RS. Db/db mice had increased gut permeability compared to db/m mice. Furthermore, the high AGE diet increased gut permeability of db/db mice, an effect not observed in RS-fed db/db mice. In db/db mice, a high AGE diet was associated with an increase in the Firmicutes/Bacteroidetes (F/B) ratio, which was ameliorated by supplementation with RS.

Conclusions: State the main conclusions

A high AGE diet led to increased intestinal permeability, an increase in F/B ratio, and worsening albuminuria in db/db mice. RS was protective against high AGE induced albuminuria in db/db mice. These preliminary studies support the notion that dietary AGEs contribute to renal disease via alterations in gut homeostasis and suggest a potential role for RS as a renoprotective agent.

Keywords: diabetes, resistant starch, kidney, gut microbiota, glycation

Gut Microbiota in Pregnant Women: Connection with Obese Status and Inflammatory Biomarkers.

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Background and Rationale

Obesity has been linked with dysbiosis and low grade inflammation. In pregnancy is associated with increased risk of childhood obesity and other diseases. To study the microbiota composition during pregnancy and how it is affected by pregestational BMI (pre-BMI) and the inflammatory status of the mothers can be the first step to elucidate new probiotic interventions towards a healthier microbial ecosystem.

Objectives: Indicates the purpose of the study

Our aim was to study microbiota composition in mothers at third trimester of pregnancy, and to identify inflammatory biomarkers-microbiota interactions and their potential role in assessing overweight or obese status.

Methodology: Describe pertinent experimental procedures

Fifty-four pregnant women were classified according to pre-BMI as normoweight, overweight or obese. At third trimester, blood and faecal samples were collected and serum inflammatory biomarkers (hs_CRP, haptoglobin and suPAR) and gut microbiota composition (16S gene sequencing) were assessed. Bioinformatics and statistical analysis were used to analyze all data.

Results: Summarize the results of the research

Higher Firmicutes levels and a higher ratio Firmicutes/Bacteroidetes were observed in the obese group and high hs_CRP and haptoglobin levels correlated with decreased microbiota diversity (Shannon index). All the inflammatory markers were positively associated with pre-BMI but only haptoglobin was significantly higher in overweight and obese mothers. A core of positive and negative correlations between pre-BMI, biomarkers and specific bacterial groups were found. Whereas hs_CRP and haptoglobin values were correlated with specific microbiota components such as *Ruminococcus gnavus* and *Faecalibacterium*, no significant correlations were found for suPAR.

Conclusions: State the main conclusions

Microbiota composition at third trimester was affected by pre-BMI and the inflammatory status of the mothers. Haptoglobin and hs_CRP reflected overweight and related microbiota components but haptoglobin was a better biomarker for microbiota associated overweight. suPAR was not related to deviated microbiota profiles. Understanding the composition and specific alterations of microbiota throughout pregnancy and finding biomarkers that reflect these changes, is of great value for opening new research avenues and probiotic therapeutic approaches.

Keywords: obesity; pregnancy; inflammatory biomarkers; microbiota; third trimester

Genomic Insights Into Host Adaptation Trends of Probiotic Species, *Lactobacillus Reuteri*: Transforming Probiotic to Super-Probiotic

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Background and Rationale

The disadvantage of current probiotic strains is their inability of long-term colonization and biofilm formation in the human gut. Hence, they are needed to be taken continuously and the host is also devoid of metabolites, requiring a longer stay in the gut. *Lactobacillus reuteri* is a successful probiotic, demonstrates noteworthy host adaptation. The underlying factors defining this peculiar host adaptation is still a mystery. This study focuses on deciphering factors and gene clusters underpinning human adaptation of *L. reuteri*. The revealed gene clusters can be utilized to synthesize customized probiotic strains by genome editing of existing ones. The customized probiotic strains will adapt to human gut, enabling long-term colonization and hence, transforming to super-probiotics.

Objectives: Indicates the purpose of the study

Understanding underlying mechanisms, gene clusters and mobilomes defining human adaptation of *Lactobacillus reuteri*.

Methodology: Describe pertinent experimental procedures

In-house whole genome sequencing and comparative analysis of a novel taxonomic outlier strain, *Lactobacillus* spp. M31 and *L. reuteri* strains belonging to different lineages

Results: Summarize the results of the research

pdu-cbi-cob-hem (vitamin B12 and reuterin biosynthesis) cluster, conserved in human lineage is ancestral and hence, is crucial for its human adaptation. Other lineage-specific clusters, *urease*, *secA2-secY2* (adhesion) and *levansucrase* (biofilm formation), are lost by human lineage. Most of the genes, present in other lineages are lost by human-adapted lineage. A load of mobilomes was seen and Insertion sequence (IS200) expansion was observed in the human lineage. Apart from revealing an inter-kingdom host jump of *L. reuteri*, the study also provided insights into adaptation of *L. reuteri* to other vertebrates' lineages.

Conclusions: State the main conclusions

The present study has allowed understanding the exceptional success of *L. reuteri* from the level of species to strain. The study pinpoints genes and factors crucial for host adaptation, which will be invaluable in rationale development of probiotics for human and animal health.

Keywords: Super-probiotic; *Lactobacillus reuteri*; Host Adaptation; Colonization

Alterations of Gut Microbiota Associated with Distinct Allergic Phenotypes: Big Data from An Asian Longitudinal Birth Cohort Study

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Background and Rationale

Allergic diseases usually originate in early life. The interaction between gut microbiota and host immune cells shapes the immune development and affects the manifestations of allergic phenotypes. Whether gut microbiota change is different among distinct allergic phenotypes has never been elucidated in a longitudinal birth cohort.

Objectives: Indicates the purpose of the study

We aimed to determine the difference of gut microbial colonization in distinct allergic diseases in infants age 9-12 months who participated in the longitudinal birth cohort.

Methodology: Describe pertinent experimental procedures

A longitudinal population-based birth cohort study was conducted in Bangkok, Thailand. Diagnosis of allergic diseases was confirmed by allergists. The gut microbiome from stool of allergic infants and matched healthy controls (1:1) were analyzed using 16s amplicon sequencing method.

Results: Summarize the results of the research

Of the 336 subjects, the incidence of allergic diseases was 12.5%. Three atopic phenotypes including atopic dermatitis (AD) 58.8%, food allergy (FA) 17.7% and subjects who have both AD and FA (AD/FA) 23.5% were identified. Shannon index revealed that microbial diversity of allergic infants was not significantly different compared to controls. However, the diversity index of FA samples was the lowest at 2.08 whereas the others were in range of 2.32-2.38. Microbial compositions between four groups were mostly similar. Interestingly, the amount of family *Erysipelotrichaceae* in AD and AD/FA groups was significantly higher than controls ($p < 0.05$). *Erysipelotrichaceae* was suggested to correlate with inflammation in human. The relative abundance of *Bacteroidaceae* and *Enterobacteriaceae* in FA and AD/FA group was slightly higher than controls, while *Bifidobacteriaceae* was oppositely presented.

Conclusions: State the main conclusions

The change of gut microbiota of infants related to allergic diseases. Different allergic phenotypes have impact on shaping of microbiome in infant. This finding serves for further study in longitudinal birth cohort. Understanding the dynamic of microbial colonization patterns in allergic diseases will lead to discovering a promising clinical target for allergy prevention and treatment.

Keywords: birth cohort; gut microbiota; food allergy; atopic dermatitis; South East Asia

Understanding the cultivable autochthonous bacteria from human gut

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Background and Rationale

'Gut microbiota' refers to the ecosystem of microorganisms that have adapted to live on intestinal mucosal surface or within gut lumen (WGO Handbook of gut microbes, 2014). Since microbial composition varies along the length of gastrointestinal tract, use of tissue samples provides better expression of autochthonous bacterial population that have colonised and survived in the human gut for long periods of time. Studying their metabolic abilities and surface characteristics will help in understanding their adaptations for survival in human gut.

Objectives: Indicates the purpose of the study

To exploit gut microbes for health benefits by isolating and characterizing the bacterial species inhabiting our gut.

Methodology: Describe pertinent experimental procedures

Biopsy samples were collected from three different regions of colon (terminal ileum, caecum and recto-sigmoid) from healthy volunteers. They were homogenized, enriched and plated to obtain cultivable gut bacteria. Representative isolates were identified by 16S rRNA sequencing and were studied for their acid tolerance ability. Further studies on their surface characteristics and metabolic abilities like oxalate degradation are underway.

Results: Summarize the results of the research

Around 40 isolates were obtained from ileum and caecum, which were grouped into 9 clusters based on morphology.

Key observations include

- i. Enrichment helps in increasing the number of surviving bacteria.
- ii. Anaerobic enrichment gives higher colony number.
- iii. Striking difference observed in colony numbers obtained from ileum under aerobic and anaerobic enrichment - surviving numbers were highly reduced in aerobic enrichment.
- iv. Colony diversity, however, does not vary significantly
 - Among the three regions – ileum, caecum, and recto-sigmoid.
 - Between aerobic and anaerobic enrichments.
- v. They were able to survive from pH 3.0. Poor growth was observed in pH 2.0.

Conclusions: State the main conclusions

The organism *Weissella confusa* was isolated from caecal tissue of two different patients. This organism may be a predominant facultative anaerobe in the caecum. However further validation in this direction is required. Ileal isolates gave a better diversity of bacteria including *Lactobacillus*, *Enterococcus* and *Pediococcus*.

Keywords: Microbiota; biopsy; autochthonous; probiotic

Autoinducer-2 Quorum Signaling in Probiotics: A Mechanism of Gut Microbiome Modulation

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Background and Rationale

Bacterial symbioses are essential in the human host. Especially, gut microbiota is reported that closely related to physiological traits and diseases. Therefore, the gut microbiota modulation by probiotics as bacterial therapeutics are being focused, and many of studies are supporting the concept. However, the mechanism of gut microbiota modulation by probiotics administration is not revealed obviously.

Bacterial quorum sensing is common events in the bacterial community. In particular, LuxS-mediated autoinducer-2 (AI-2) signaling system is found in a wide variety of Gram-positive and Gram-negative bacteria, including some of probiotics lactic acid bacteria species. It has been reported to regulate corresponding gene expression of adaptation and resistance to the environment. Furthermore, recent studies suggested that AI-2 activity can assist to restore the balance of microbiota in antibiotic-induced dysbiosis and the response of the epithelial innate immune system.

Objectives: Indicates the purpose of the study

Therefore, we expect that AI-2 signaling status may a cause for the modulation of the gut microbiome.

Methodology: Describe pertinent experimental procedures

The bacterial community of each sample was analyzed using qRT-PCR, Illumina Mi-Seq through the 16S rRNA gene. Mouse host and bacterial gene expression were profiled by Illumina Hi-Seq 2000 RNA sequencing, and its untargeted metabolites were analyzed by GC-TOF-MS. ILC3 cells changes observed by BD Aria FACS system

Results: Summarize the results of the research

AI-2 molecules changed the composition ratio of *Firmicutes/Bacteroidetes* compared to the control. Mouse gut metabolites were also partially modulated by AI-2 and QSI treatment in the organic acids, nucleic acids, and amino acids. But the correlation between metabolite production and mRNA expression was not observed.

Conclusions: State the main conclusions

Although the AI-2 signaling in gut bacteria are still insufficiently understood, the results indicate that the AI-2 signaling property of probiotics may a key mechanism cause the gut microbiome modulation.

Gut microbiota of healthy young Thai children consuming synbiotics supplemented formula

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Background and Rationale

Young children (1-3 years old) undergo a progressive transition of diet beginning with introduction of weaning food. Diet plays a role to provide macro & micro-nutrients required for their growth and have an impact on microbiota composition. The gut microbiota of young children is presumed to be adult-like. However, there is limited knowledge and reports of microbiota composition at this age especially in Asian countries.

Objectives: Indicates the purpose of the study

To understand the influence of young child formula supplemented with synbiotics short-chain galacto-oligosaccharides (scGOS) and long-chain fructo-oligosaccharides (lcFOS) with *Bifidobacterium breve* M-16V on the gut microbiota of 1-3 years old Thai young children.

Methodology: Describe pertinent experimental procedures

From a randomized, double-blind, placebo-controlled, multi-centre, 12 week clinical study in Thailand, fecal samples were collected from subjects at baseline, week 6 and week 12. The intervention consisted of young child formula (YCF) supplemented with specific mixture of prebiotics (1g/100mL scGOS/lcFOS 9:1 ratio) and 1.8×10^7 CFU/g of *Bifidobacterium breve* M-16V (n=65), or control formula (n=64). Quantitative real-time PCR (qPCR) and 454 pyrosequencing of V3-V5 16S rRNA gene were performed to provide insights into the microbiota community over time.

Results: Summarize the results of the research

This study confirmed a high prevalence of the *Bifidobacterium* genus (20.7% average) in young children at all time points regardless of intervention – composing of *B. breve*, *B. longum*, *B. bifidum*, *B. catenulatum* and *B. adolescentis*. We also demonstrated a bifidogenic effect in the intervention group, and observed that the synbiotics supplementation also did not diminish endogenous *B. breve*. 16S rRNA sequencing revealed that toddler microbiota is not only dominated by *Firmicutes* but also *Actinobacteria* which was presumed to dwindle in childhood.

Conclusions: State the main conclusions

YCF supplemented with a synbiotics mixture significantly increased fecal bifidobacteria level in healthy young children aged 1-3 years. However, the influence of bifidobacteria on other beneficial bacteria in this microbial ecosystem has to be further investigated.

Keywords: microbiota; synbiotics; bifidobacteria; young children

A machine learning model basing on initial gut microbiome data for predicting changes of bifidobacterium after prebiotics consumption

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Background and Rationale

Short-term prebiotics intervention effect in Intestinal flora base on high-throughput sequencing has not been set forth. Personal effect linking to initial gut microbiome after prebiotics consumption still unclear.

Objectives: Indicates the purpose of the study

The aim of the study was to investigate the effects of 9 days prebiotics supplementation on gut microbiota structure and function, and then to establish a machine learning model based on initial gut microbiota for predicting the variation of *Bifidobacterium* after prebiotic intake, which might provide a guidance to personalized diet.

Methodology: Describe pertinent experimental procedures

35 healthy subjects consumed FOS or GOS for 9 days (16g per day) in a randomized double-blind self-controlled study. 16S rRNA gene high-throughput sequencing was performed to investigate the change of gut microbiota after prebiotics intake. PICRUSt was used to infer differences between the functional modules of the bacterial communities. Random forest model based on initial gut microbiota was used to identify the change of *Bifidobacterium* after 5 days prebiotic intake and then built a continuous index to predict the change of *Bifidobacterium*.

Results: Summarize the results of the research

Feces samples analysis with QIIME revealed that both FOS and GOS supplement decrease α -diversity. The continuous index could successful predict the change of *Bifidobacterium* ($R=0.45$, $p=0.01$). Prediction was accurate in a validation model ($R=0.62$, $p=0.01$).

Conclusions: State the main conclusions

Short-term prebiotics intervention could significantly decrease α diversity of intestinal flora. A machine learning model based on initial gut microbiota could accurately predict the change of *Bifidobacterium*. This method might provide reference for personalized nutrition intervention and precisely modulating intestinal flora.

Keywords: prebiotic, microbiota, personalized diet, machine learning, bifidobacterium

Assessment of potential prebiotic activity of pineapple by-products (peels and stems) extracts and maintenance of bioavailability through *in vitro* gastrointestinal tract system

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Background and Rationale

Pineapple (Ananas comosus Merr.) is the third most important tropical fruit in the world production and one of the most processed fruit, but is the most important generating residues. These residues contain high content of bioactive compounds, but generally not directly available and for that reason is necessary to extract and characterize the feasible bioactive compounds.

Objectives: Indicates the purpose of the study

In previous work, was demonstrate the ability of pineapple extracts as prebiotic ingredients. Therefore, the focus of this research work was to perform an extended study on the prebiotic activity of pineapple by-products (peels and stems) extracts to explore the potential of the development of a new functional ingredient.

Methodology: Describe pertinent experimental procedures

Frozen pineapple vy-products (peels and stems) were submitted to a milling and pressing processes, creating a pineapple juice and solid semi-dried extract. The soluble fraction was submitted to an extraction of the pineapple enzymes (Bromelain) and the resulting soluble fraction was assessed.

Results: Summarize the results of the research

*An initial screening was performed using six different probiotic strains from two different genera, *Lactobacillus* sp. and *Bifidobacterium* sp. All of the microorganisms showed a positive growth towards the pineapple extracts, with an exception of *Lactobacillus acidophilus*. The showed activity was in range of those promote by the positive control (frutoooligosaccharide).*

The extracts were studied to determine compounds profile by HPLC, establishing and understanding the relationship between structure and activity. Through HPLC analysis was showed two major peaks of oligosaccharides comprising MW of 2000 and 600 Da and identified as galactomannans and two monosaccharides, glucose and fructose, which explains the general high prebiotic activity. Finally, the extracts were submitted to gastrointestinal tract in order to evaluate the bioavailability of prebiotic activity after gastrointestinal conditions.

Conclusions: State the main conclusions

At this research work was possible to evaluate the pineapple by-products extracts as a prebiotic ingredient and its potential maintenance through gastrointestinal conditions.

Keywords: Pineapple byproducts; bioactive compounds; oligossacharides; galactomannans; prebiotic ingredient

The Impact of LGG Probiotic Supplement on Cognition in Middle-Age and Older Adults: Pilot Study

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Background and Rationale

Studies show that composition of the gut microbiome is associated with cognitive function in healthy adults and that probiotic supplementation is associated with better cognitive function in animal models and persons with HIV-1. Such findings raise the possibility that supplementation with *Lactobacillus rhamnosus* GG (LGG) may also improve cognitive function in healthy middle-age and older adults. We hypothesized that individuals who received LGG would show improvements in cognitive function.

Objectives: Indicates the purpose of the study

We examined whether providing LGG probiotic supplementation in middle- and older-adults improves cognitive functioning.

Methodology: Describe pertinent experimental procedures

Twenty healthy middle- and older-adults ($M_{\text{age}} = 64.54$; 47.5% male) were recruited from the local community. Exclusion criteria included history of/current neurological disorder or significant gastrointestinal illness or surgery. Participants were instructed to ingest 2 capsules each day for 90 days. Cognitive function was assessed using NIH Toolbox, known to have minimal practice effects. An age-corrected composite score of cognitive functioning was created through performance on the following tests: Picture Sequence Memory Test, Dimensional Change Card Sort Test, Flanker Inhibitory Control and Attention Test, List Sorting Working Memory Test, and Pattern Comparison Processing Speed Test. Repeated Measures ANOVA determined changes in scores over time.

Results: Summarize the results of the research

Repeated measures ANOVA showed that age-corrected composite cognition t-score significantly improved from baseline to follow-up in persons taking LGG [$F(1,19) = 30.22$, $p < .01$; $M_{\text{baseline}} = 99.38 \pm 16.41$, $M_{\text{followup}} = 107.13 \pm 16.88$]. Follow-up analyses to identify the specific subtests revealed that much of this effect was driven by improvements in Pattern Comparison Processing Speed Test [$F(1,19) = 9.07$, $p < .05$; $M_{\text{baseline}} = 94.45 \pm 18.32$, $M_{\text{followup}} = 104.45 \pm 20.21$].

Conclusions: State the main conclusions

This pilot study suggests that LGG supplementation may have cognitive benefits, particularly concerning attention and processing speed, for neurologically-healthy middle-age and older adults. Future studies should clarify this possibility through a placebo-controlled comparison and examine possible mechanisms, especially composition of the gut microbiome and indices of glycemic control and inflammation.

Keywords: *Lactobacillus GG; cognition*

The Human GallBiome, At The Interface of Hepatic Health and Disease

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Background and Rationale

While more than one million cholecystectomies (gallbladder removal surgeries) are performed throughout Europe each year, the bacterial communities associated with the human gallbladder and its disease states remain unknown. Studies that characterize the effects of cholecystectomies on the gut microbiome are indeed lacking.

Objectives: Indicates the purpose of the study

Without the ability to regulate bile entering the duodenum during food intake, it is expected that gallbladder removal will lead to downstream changes in the intestinal population. Once the gallbladder microbiome has been elucidated, the impact of its removal on the gut microbiome will be assessed, with the objective of assessing the potential for a new probiotics to restore the gut microbiome in patients who can no longer emulsify dietary fats.

Methodology: Describe pertinent experimental procedures

Here, the microbial composition of human bile, gallbladder mucosa, and biopsies of surgically removed healthy gallbladders (adherent and non-adherent microbiota) are being investigated using molecular and cultivation based techniques.

Results: Summarize the results of the research

The profiles are being compared to samples of a second cohort undergoing emergency cholecystectomies, in order to identify possible biomarkers for gallbladder disease. Stool samples (collected during the recovery period) have also been collected for community composition, metabolomics, bile, fat and energy content. Clear differences in bile and gallbladder metabolomics were observed in the pilot data set, and a number of novel species have been recovered.

Conclusions: State the main conclusions

Ultimately, GallBiome will form the basis for establishing relationships between the gallbladder microbiota, gut microbiota, and human health with a view to informing future development of diagnostics and therapeutics. Lessons learned from this study may serve as a basis for suggesting probiotic use for patients following their surgery in order to recuperate properly. Characterization of the core gallbladder microbiome has important biological and medical implications with potential to lower the risk and incidence of cholelithiasis and the negative effects of cholecystectomies.

Targeting gut microbiota in obesity: Protective effects of selected probiotic strains

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Background and Rationale

The incidence of chronic pathologies notably obesity, has been rising dramatically during the last decades and are important public health concerns. Obesity is associated with a chronic, low-grade inflammatory state that is crucial in the development of insulin resistance and type 2 diabetes. The gut microbiota is involved in maintaining human health and is critical as homeostatic regulator of host energy metabolism and immune responses. Accumulating evidence over the past decade has linked the development of obesity and related metabolic syndrome to gut microbiota dysbiosis.

Objectives: Indicates the purpose of the study

The potential use of probiotics therefore gained attention, although results were sometimes conflicting. Probiotics can exert their protective properties through multiple mechanisms, for instance by maintaining and restoring the gut barrier function, by regulating the mucosal immunity and the entero-endocrine function. Our project was to evaluate the protective effects of several probiotic strains using different *in vitro* models.

Methodology: Describe pertinent experimental procedures

We selected strains with the capacity to induce the production of anti-inflammatory cytokines, to restore the epithelial barrier (*in vitro* gut permeability model) and to favor the secretion of gut peptides (entero-endocrine STC-1 cell line). Selected strains were then evaluated in a murine model of diet-induced obesity.

Results: Summarize the results of the research

Some strains induced a significant limitation of body weight gain and an improvement of metabolic and immune parameters, including insulin resistance, fatty liver, dyslipidemia and inflammation. Depending on strains, protective effects were associated with an increase in short chain fatty acids, particularly butyrate and propionate, a restoration of microbiota dysbiosis and also with hypothalamic modifications of leptin and leptin receptor gene expression.

Conclusions: State the main conclusions

This work provides crucial clues for the selection of strains or mixture with the best potential for the development of more efficient therapeutic approaches in the management of obesity and also brings substantial insights into how the host-microbial interaction govern protective effects

Keywords: Probiotics, obesity, microbiota, inflammation, SCFA , epithelial barrier, enteroendocrine peptides

Occurrence of lactobacilli with probiotic potential in Argentinian breast milk.

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Background and Rationale

Differences in environmental and gut microbiota are factors that favor the exploration of locally sourced probiotics. Such probiotics target the particular populations to improve their gut health. This could lead to local developments and activities which may pave the way to their application in social programs. Such probiotics are particularly beneficial for people in developing countries, who generally lack access to affordable commercial probiotics and are more often exposed enteric infections.

Objectives: Indicates the purpose of the study

To explore the occurrence in breast milk samples collected in the city of Santa Fe (Argentina) of lactobacilli with functional and technological potential to become locally sourced new probiotics.

Methodology: Describe pertinent experimental procedures

104 mothers donated 161 breast milk samples. Lactobacilli were isolated using MRS agar, preliminary identified by MALDI-TOF and then the identity was confirmed by partial 16S RNA sequencing. Hydrophobicity was determined (hexadecane and xylene partition). Strains were freeze-dried in 10% lactose or 10% polydextrose and survival was assessed after accelerated (37°C, 4 weeks) or regular (5° and 25°C, 12 months) storage. Strains were co-cultured with murine RAW 264.7 macrophages for screening the capacity to induce the anti-inflammatory cytokine IL-10.

Results: Summarize the results of the research

14 LAB isolates were obtained and identified as *L. plantarum* (7 isolates), *L. gasseri* (3), *L. fermentum* (2), *L. rhamnosus* (1) and *L. gallinarum* (1). Hydrophobicity ranged from 7.4 and 95.9%. *L. gasseri* 70a, 70b and 73a were the strains with higher hydrophobicity and capacity to induce IL-10 production by macrophages. Very low correlation between survival at 37°C/1 month and 5°/12 months was observed. Better correlation at 25°C was observed for lactose ($R^2 = 0.77$) compared to polydextrose ($R^2 = 0.65$). Less than 1 log order of cell death was observed in polydextrose for *L. gasseri* 70a, 70b and 73a after 12 months of storage at 5°C.

Conclusions: State the main conclusions

Three *L. gasseri* strains were isolated, which displayed promising functional and technological properties for further studies on their health promoting properties, to be used as locally sourced probiotic strains.

Keywords: *lactobacilli, breast milk, freeze-drying, IL-10, probiotics*

Application of isomaltooligosaccharide from rice starch in diet formulas for chronic kidney disease's patient

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Background and Rationale

Chronic kidney disease (CKD) is one of prevalence disease that impact to health care system in the world including Thailand. CKD's patients have to control on diets particular limit in protein and minerals. In addition, they have high expenses for hemodialysis and payment for diet formula. Diet formula is mostly imported and unaffordable for long-term treatment of CKD's patient in Thailand.

Objectives: Indicates the purpose of the study

This research aims to develop the complete diet formula to be alternative a current commercial product for CKD's patient. The raw material for this study is rice starch that producing in Thailand. Isomaltooligosaccharide (IMO) is enzymatically synthesized from rice starch then it is used as main ingredient in diet formula for CKD's patient.

Methodology: Describe pertinent experimental procedures

IMO was enzymatically synthesized using 3 steps including liquefaction with Kleistase E5NC, saccharification with b-amylase F and transglucosylation with Transglucosidase L. The chemical composition and sugar contents of rice IMO was analyzed by HPAEC-PAD. Formulation of diet bar and powder for CKD's patient were developed according to recommended nutritional requirement.

Results: Summarize the results of the research

Chemical compositions of IMO consisted of carbohydrate as major component (97.05%). The energy value of IMO was 3.91 kcal/g which lower than regular carbohydrate. Sugar contents of IMO were 20.74% isomaltose, isomaltotriose and panose, 55.89% of glucose and 26.29% of total IMO. The IMO was used as major component in diet formula.

Conclusions: State the main conclusions

Isomaltooligosaccharide was achieved producing from rice starch. Powder and diet bar were successfully developed and met nutritional requirements for CKD's patient. The products developed was higher acceptable than a commercial product.

Keywords: Chronic kidney disease; Isomaltooligosaccharide; Rice starch

Enhancement of the anti-inflammatory activity of *Lactobacillus plantarum* strains by optimization of their culture condition.

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Background and Rationale

Background and Rationale: Recent reports suggest that culture conditions of lactic acid bacteria (LAB) affect their immunomodulatory properties. The anti-inflammatory activity of LAB is deduced to be an essential mechanism of their anti-metabolic disorder effects. Studies on the health-promoting effects of LAB are numerous, but few have comprehensively investigated the relationship between LAB functionality and their culture conditions.

Objectives: Indicates the purpose of the study

Objectives: In the present study, we verified the effect of differences in culture conditions on *Lactobacillus plantarum* OLL2712 (OLL2712) functionality; this strain exhibits anti-inflammatory activity and preventive effects against metabolic disorders.

Methodology: Describe pertinent experimental procedures

Methodology: We measured cytokine productions in murine immune cells treated with OLL2712 cells prepared under various culture conditions. We also verified the preventive effects of OLL2712 cells at different culture conditions against inflammation and metabolic disorders in obese and diabetic mice.

Results: Summarize the results of the research

Results: The results showed that IL-10-inducing activity of OLL2712 cells differed dramatically between OLL2712 groups at different culture phases, and under different culture medium components, temperatures, and neutralizing pH. We demonstrated several culture conditions of OLL2712 for higher anti-inflammatory activity. In particular, exponential phase cells had much more IL-10-inducing activity than stationary phase cells, and adding decaglycerol mono-oleic acid ester further improved the IL-10-inducing activity. Similar phenomena were observed in another strain of *L. plantarum*. These differences in IL-10-inducing activity were linked to differences in the toll-like receptor (TLR) 2 stimulation activity. We also demonstrated functional differences by culture phases *in vivo*; OLL2712 cells at exponential phase had more anti-inflammatory activity and anti-metabolic disorder effects on obese and diabetic mice than those at stationary phase.

Conclusions: State the main conclusions

Conclusions: We confirmed that the functionality of OLL2712 cells differed by their culture conditions *in vitro* and *in vivo*. Our results suggest that culture conditions of LAB affect the anti-inflammatory activity, and optimization of culture conditions is quite important for their health-promoting functionality.

Keywords: culture phase, interleukin-10, *Lactobacillus plantarum*, metabolic disorder, mono-oleic acid ester

Intranasal *Lactobacillus rhamnosus* GG ameliorates airway hyperreactivity and allergic airway inflammation in mice.

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Background and Rationale

Probiotic bacteria have long shown potential for the attenuation of allergy-related conditions. Their intranasal administration represents a promising alternative delivery method in the context of airway inflammatory diseases.

Objectives: Indicates the purpose of the study

The goal of this project was to explore the potential of preventive intranasal probiotic instillations using the model probiotic strains *Lactobacillus rhamnosus* GG and *L. rhamnosus* GR-1 in an experimental allergic asthma model in mice.

Methodology: Describe pertinent experimental procedures

L. rhamnosus GG and *L. rhamnosus* GR-1 were intranasally administered in mice for two weeks. Intranasal colonization and translocation of the bacteria was assessed by plating nasal mucosa, fecal and lymph node samples on selective medium. Fluorescent strains of *L. rhamnosus* were used to study their binding to murine airway epithelial and immune cells *in vitro*. After the two-week *L. rhamnosus* treatment, allergic asthma was induced in mice with intranasal instillations of birch pollen extract, and effects of the treatment were analyzed based on antibody concentrations in blood, inflammatory cell counts in the lungs, lung Th2 cytokine levels and airway hyperreactivity.

Results: Summarize the results of the research

Intranasal *L. rhamnosus* administration was demonstrated to be innocuous and transient colonization of the nasal passages was observed. *L. rhamnosus* GG demonstrated superior binding to airway epithelial and immune cells *in vitro* compared to *L. rhamnosus* GR-1. In the murine asthma model, *L. rhamnosus* GG instillations also significantly reduced airway hyperreactivity and allergic airway inflammation, reflected in less pronounced lung eosinophilia and decreased Th2 cytokine levels. Compared to *L. rhamnosus* GG, intranasal *L. rhamnosus* GR-1 had rather a neutral effect on allergic airway inflammation, although a tendency towards attenuated airway hyperreactivity was also observed.

Conclusions: State the main conclusions

Intranasal instillation of *L. rhamnosus* GG is a feasible administration approach in the treatment of airway allergic disease. It results in amelioration of the main hallmarks of allergic asthma in our mouse model, although validation in human clinical trials is still warranted.

Keywords: *intranasal; lactobacillus; allergy; asthma; LGG*

Lessons learnt from the first Medicines Control Council approved probiotics trial in South Africa

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Background and Rationale

Bacterial vaginosis (BV) increases acquisition of sexually-transmitted infections (STI), including HIV, and adverse reproductive outcomes. The current standard of care for BV, antibiotics, are not effective long-term, with 6-month recurrence rates of 50%. There is thus an urgent need for durable treatment of BV. Biotherapeutics, including adjunctive probiotics, may improve efficacy and durability. No randomized trial comparing antibiotic treatment to probiotics as an adjunct to antibiotics has been performed in South Africa.

Objectives: Indicates the purpose of the study

A single-blind, randomized controlled trial enrolling BV positive, STI negative South African women with vaginal discharge was initiated to compare standard of care (metronidazole, n=20) to a combination of metronidazole and a commercially-available probiotic for vaginal health sold in South Africa (n=30).

Methodology: Describe pertinent experimental procedures

The primary endpoint is BV status at one month. Secondary endpoints are durability of treatment, changes in genital inflammatory milieu, microbiome and presence of the probiotic bacteria in the vagina over six months after enrolment.

Results: Summarize the results of the research

This is the first probiotics trial in South Africa that was approved by the regulatory authorities, the Medicines Control Council (MCC), thus laying the groundwork for the local regulatory landscape. Intense education of doctors, nurses and participants about probiotics has facilitated the recruitment. Enrollment and follow-up are ongoing although preliminary results show that screening based on vaginal symptoms alone has high screen-failure rate, suggesting the use of BV rapid tests in pre-screening. This study describes the regulatory framework by which probiotics are regulated within South Africa and the hurdles that need to be overcome to enable testing of products not currently approved by the MCC.

Conclusions: State the main conclusions

Well-designed, clinical trials are needed in South Africa to assess the efficacy of adjunctive biotherapeutics for BV treatment. Vaginal discharge poorly predicts BV. The regulatory landscape for probiotics in South Africa is evolving and communication between scientists, clinical staff, participants and regulatory authorities are needed.

Keywords: live biotherapeutics, vaginal health, Medicines Control Council, randomised clinical trial

Considering antibiotic resistance profiles of *Lactobacillus* strains isolated from the female genital tract of African women, relevant to their development as probiotic strains

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Background and Rationale

Antibiotic resistance is a threat to global health. The antibiotic resistance profiles of *Lactobacillus* spp., a significant component of microbiota at various body sites, are of major interest.

Objectives: Indicates the purpose of the study

The susceptibility of potentially probiotic *Lactobacillus* strains isolated from female genital tracts and commercial vaginal probiotics, used to treat bacterial vaginosis (BV), to metronidazole and clindamycin, the standard treatment for BV, as well as to a selection of inhibitors of protein-, cell wall-, membrane- and DNA synthesis commonly used in South Africa was determined.

Methodology: Describe pertinent experimental procedures

The susceptibility of *Lactobacillus* strains isolated from probiotics for vaginal health (*L. reuteri* [n=3], *L. rhamnosus* [n=8], *L. acidophilus* [n=3]) and from cervico-vaginal fluid from healthy South African women (*L. crispatus* [n=10], *L. gasseri* [n=9], *L. jensenii* [n=18], *L. vaginalis* [n=8], *L. mucosae* [n=12]) to metronidazole and clindamycin was determined using the disc diffusion method. The antibiotic susceptibility profile was determined for a subset of 20 selected strains to 21 antibiotics: protein synthesis inhibitors (chloramphenicol, linezolid, tetracycline, tigecycline, clindamycin, erythromycin, streptomycin, gentamycin, quinupristin/dalfipristin), cell wall/membrane inhibitors (penicillin, vancomycin, nitrofurantoin, oxacillin, ampicillin, daptomycin), DNA synthesis inhibitors (moxifloxacin, ciprofloxacin, levofloxacin) using Sensititre™ plates.

Results: Summarize the results of the research

While all *Lactobacillus* isolates were resistant to metronidazole, the clinical *L. vaginalis* strains were the most susceptible to clindamycin, followed by *L. jensenii* (clinical), *L. mucosae* (clinical), *L. reuteri* (probiotic), *L. rhamnosus* (probiotic), *L. crispatus* (clinical), *L. acidophilus* (probiotic), and lastly *L. gasseri* (clinical). The strains isolated from commercial probiotics were the least susceptible to the 21 antibiotics tested while clinical *L. gasseri* isolates showed the most susceptible patterns.

Conclusions: State the main conclusions

These antibiotic susceptibility patterns for common probiotic and clinical *Lactobacillus* isolates highlight the need to determine the resistance mechanisms that may influence their utility in probiotic BV treatment. Specific antibiotic resistance determinants carried on mobile genetic elements constitute a potential reservoir of drug resistance for genital tract pathogens.

Keywords: Antibiotic susceptibility profiles, clinical and probiotic *Lactobacillus* strains, safety of potential probiotic strains

Efficiency of b–glucan extract from *Auricularia auricula-judae* and *Schizophyllum commune* in Thailand on the recovery of cell lines treated with anticancer drug and cytotoxicity in cell lines

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Background and Rationale

Anti-cancer drug, cisplatin is a types of cancer treatments that are well-known and effective. However, the cancer treatment with anticancer drugs results in side effects such as nausea and vomit, causes of nervous system, kidney and bone marrow, and affect eating and nutritional status of patients. b-glucan, a prebiotic derived from mushrooms, plays an important role in health as it enhances or improves the body's immune system including cancer patients.

Objectives: Indicates the purpose of the study

This research aims to evaluate the cytotoxicity of b-glucan extract from two edible mushrooms in Thailand on normal kidney cell lines and the recovery of cell lines treated with cisplatin. Two edible mushrooms, *Auricularia auricula-judae* and *Schizophyllum commune*, in Thailand were selected to extract the b-glucan and then they are used in the experiments.

Methodology: Describe pertinent experimental procedures

b-glucan from *A. auricula-judae* and *S. commune* were extracted at high pressure and temperature of 130 °C for 20 min. b-glucan extracts were purified by purified by ultrafiltration membrane and then freeze dry. The molecular mass of b-glucan extracts was analyzed by High Performance Size Exclusion Chromatography (HPSEC). The efficiency of b-glucan extracts on the recovery of cell lines treated with anticancer drug and cytotoxicity in HEK 293 and LLC-RK1 cell lines were studied.

Results: Summarize the results of the research

b-glucan in extracted sample from *A. auricula-judae* and *S. commune* were 24.50±1.30 %w/w and 25.54±0.55 %w/w while commercial b-glucan from commercial yeast was 46.21±0.37 %w/w. For the cytotoxicity, the cytotoxicity on HEK 293 and LLC-RK1 cell lines increased with increase in concentration of samples but b-glucan could not recover the cisplatin drug treated cell lines.

Conclusions: State the main conclusions

b-glucan extracts from *A. auricula-judae* and *S. commune* has less cytotoxic with kidney normal cell lines. However, they still cannot recovery the damaged cells, they might should be a supplement or ingredient.

Keywords: b-glucan, mushroom, cytotoxicity

Levothyroxine improves thyroid dysfunction closely related with gut microbiota and its metabolites

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Background and Rationale

Many gut problems can suppress thyroid function and conversely low thyroid function induce gut inflammation and enlargement of gut permeability. Thus trillions of commensal bacteria in gut exerts essential roles in keeping homeostasis may including thyroid hormones.

Objectives: Indicates the purpose of the study

The present study aimed to investigate the relationship between hypothyroidism and gut microbiota and mine the beneficial bacterial species as a novel therapy for ameliorating thyroid problems and related complications.

Methodology: Describe pertinent experimental procedures

Hypothyroidism was induced by propylthiouracil (PTU, 0.1% in drinking water) for 4 weeks in rats. Meanwhile administration group was given levothyroxine (200 ug/kg, i.p.) injection once a day for last 2 weeks. Fecal 16S rDNAs were sequenced by Next-generation Sequencing (Ion Torrent) for analyzing gut microbiota.

Results: Summarize the results of the research

Hypothyroidism model was established successfully as evidenced by notably low serum T4 and high serum TSH in PTU treatment group. Levothyroxine treatment significantly ameliorated the change of serum T4 and TSH hormones. Interestingly, PTU markedly reduced the endotoxin level both in fecal and serum as compared to other groups in accordance with reduction of gram-negative bacteria abundance and elevation of intestinal alkaline phosphatase (a major defense regulated by thyroid hormone against lipopolysaccharide) concentration. Besides, it was noticeably found that a holistic profiling change of gut microbiota between PTU induced hypothyroidism group and the other groups, but not gram-positive bacteria. In detail, PTU induced hypothyroidism evidently increased the *Bacteroides acidifaciens*, *Clostridiales*, *Rc4-4* and decreased the *Dorea*. Whereas, levothyroxine distinctly restored these bacteria following the recovery of thyroid function.

Conclusions: State the main conclusions

Taken together, these results demonstrated that levothyroxine improves PTU induced hypothyroidism through maintaining stability of endotoxin and alkaline phosphatase intimately associated with gut microbiota alteration.

Keywords: Hypothyroidism; Levothyroxine; PTU; gut microbiota; endotoxin; ALP

The Association between Thyroid Function and Commensal Microbiota

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Background and Rationale

Recently the roles of gut microbiota on metabolic disease are widely verified. Thyroid hormones are vital factor regarding regulation of energy homeostasis and metabolic processes. However, the relationship between gut microbiota and thyroid function influenced on host metabolism is rarely investigated.

Objectives: Indicates the purpose of the study

To determine whether and how gut microbiota associated with thyroid function.

Methodology: Describe pertinent experimental procedures

In this study metagenomics analysis was performed through 16S rRNA gene sequencing utilizing hyperthyroidism and hypothyroidism rat models which were induced by inducers (T4 and propylthiouracil) or thyroidectomy.

Results: Summarize the results of the research

Our results showed that all the thyroid dysfunction models were definitely established and gut microbiota composition was obviously different according to the different thyroid functional status. These alterations may be used to hunt a potential mechanism(s) for regulating thyroid hormone metabolism. Besides, the abundance of S24_7, Prevotella and Prevotellaceae were evidently affected in hypothyroidism status and especially, Prevotellaceae was intensely correlated with thyroid hormone variation.

Conclusions: State the main conclusions

We demonstrated that the interaction of thyroid hormones and gut microbiota did exist and it suppose that modulation of those bacteria may improves thyroid function. Moreover, this is a new perspective study concerning relationship between thyroid dysfunction and gut microbiota using NGS-based microbial sequencing technology.

Keywords: Thyroid dysfunction; Thyroidectomy; Propylthiouracil; Gut microbiota; Metagenomics

Dietary 1-Kestose Improves the Cecal Microbiota Composition in Association with a Remarkable Increase in the Cecal Butyrate Content in Rats

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Background and Rationale

Functional food ingredients, including prebiotics, have been ardently developed for improving composition of gut microbiota. Fructooligosaccharides (FOS), including fructans, are the well researched and commercialized prebiotics. However, few studies have been conducted on the physiological effects of each component of FOS as prebiotics. 1-Kestose, a component of FOS, is composed of one glucose and two fructose molecules, and former studies suggested that the tri-saccharide is a key prebiotic component in short-chain FOS.

Objectives: Indicates the purpose of the study

In the present study, we examined the somatic effects of 1-kestose and its responsible mechanisms.

Methodology: Describe pertinent experimental procedures

We used 40 male Sprague-Dawley rats aged 8 weeks, and the rats were randomly allocated to five groups (n = 8 per group): control (0%), and 0.5-5.0% 1-kestose diet groups. To examine the physiological effect of 1-kestose, short-chain fatty acids, cecal microbiota and blood components (cholesterol, triglyceride, insulin and plasma glucose) were determined.

Results: Summarize the results of the research

We found that dietary 1-kestose induced cecal hypertrophy and alterations in the cecal microbiota composition, including a marked increase in the cell number of *Bifidobacterium* spp: approximately 7,000-fold increase in *Bifidobacterium* spp by feeding of 5% 1-kestose diet. These alterations were associated with significant increases in acetate and lactate, and a marked increase in butyrate in cecal contents. Furthermore, dietary 1-kestose induced a significant decrease in serum insulin concentration in rats fed 2.5-5% 1-kestose diet.

Conclusions: State the main conclusions

These results suggest a potential of 1-kestose to be a prebiotic for improving the metabolism regulated by insulin.

Keywords: 1-kestose, prebiotics, microbiota, butyrate, insulin resistance

The effects of 1-Kestose on intestinal microbiota in dog

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Background and Rationale

1-kestose (kestose) is an important prebiotic short-chain fructooligosaccharide (FOS) composed of one glucose and two fructose molecules. Previous intervention and *in vivo* studies demonstrated that kestose is a promising prebiotic for humans and others.

Objectives: Indicates the purpose of the study

The objective of this study was to study the effects of kestose on intestinal microbiota in dogs.

Methodology: Describe pertinent experimental procedures

Five adult beagle dogs were fed 2 g/head of kestose, once daily for 56 days. The dogs were also given the regular diet from day 0 until day 84 (day 57-84, washing period). Fresh fecal samples collected at day 0, day 28, day 56 and day 84 were subjected to 16S rRNA gene metagenomic analysis and quantitative real-time PCR analysis. Fecal concentration of short-chain fatty acids (SCFA; acetate, propionate, butyrate and lactate) were also determined.

Results: Summarize the results of the research

Metagenomic analysis revealed that the predominant intestinal microbiota in dogs were the genus *Lactobacillus*. The composition of *Bifidobacterium* increased at day 28 and maintained at the high level by the end of the study period. Quantitative real-time PCR analysis revealed that the cell number of *Bifidobacterium* increased at day 28, while that of *Clostridium perfringens* fell below the detection limit. Moreover, measurement of SCFA showed significant increase of butyrate in fecal contents, while specific butyrate producers did not significantly increase.

Conclusions: State the main conclusions

Our findings suggest that oral administration of kestose increases beneficial microbes in gut and decreases possible enteric pathogens in dogs. Gut butyrate level, which is crucial for health of several animals, can be increased in dogs by administration of kestose.

Keywords: 1-Kestose ; prebiotics ; dog ; intestinal microbiota

Lactic acid bacteria regulates blood sugar by metabolizing carbohydrates and regulating the gene expression of intestinal cells.

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Background and Rationale

In recent years, due to diet and lifestyle changes, metabolic syndrome had become more and more common disease. In which, high blood glucose may cause diabetes. Diabetes mellitus type 2 (also known as type 2 diabetes) is a long-term metabolic disorder that is characterized by high blood sugar, insulin resistance, and relative lack of insulin. T2DM may be associated with some complications, including cardiovascular disease, stroke, blindness, kidney failure or Alzheimer's disease. Rates of T2DM have increased markedly since 1960 in parallel with obesity. As of 2015 there were approximately 392 million people diagnosed with the disease compared to around 30 million in 1985. Typically it begins in middle or older age, although rates of T2DM are increasing in young people. In addition, some probiotics effectively ameliorated T2DM by improving hyperglycemia, glucose tolerance. The antidiabetic effect of *Lactobacillus* is increasingly recognized worldwide.

Objectives: Indicates the purpose of the study

It was known that lactic acid bacteria had the functions of maintaining the intestinal environment, anti-inflammation, anti-allergy, and we want to find out the special strain of lactic acid bacteria who could consumed sugar efficiently in gut.

Methodology: Describe pertinent experimental procedures

There were 25 strains of lactic acid bacteria was evaluated by the ability of metabolizing carbohydrates in MRS broth with 2% and 6% monosaccharide. These 4×10^8 lactic acid bacteria were co-cultured with 4×10^5 Caco-2 cells for 20 hours. We measured the gene expression of the glucose transporter gene and the sodium glucose co-transporter gene on different lactic acid bacteria and Caco-2 cell. In the animal experiment, the db/db mice were feeded by lactic acid bacteria for a month to observe the change of blood sugar.

Results: Summarize the results of the research

The in vitro test, we screened few strains lactic acid bacteria by the ability of metabolizing carbohydrates from 25 strains, and 4 strains showed the best metabolic ability, including *Lactobacillus reuteri* GL-104, *Lactobacillus salivarius* AP-32, *Lactobacillus acidophilus* TYCA06 and *Lactobacillus gasseri* MH-68. The intestinal cells co-culture experiment showed the 4 strains of *Lactobacillus* had different regulatory effects on the glucose transporter (GLUT) gene but also the sodium glucose co-transporter (SGLT) gene, respectively.

Conclusions: State the main conclusions

However, the *Lactobacillus reuteri* GL-104 and *Lactobacillus salivarius* AP-32 strains were superior in sugars metabolizing, and the regulation of intestinal cell glucose transporter gene expression was different from other strains. We suggested that GL-104 and AP-32 strains have the potential of regulating blood sugar and could apply on diabetes treatment in the future.

Keywords: Type II diabetes, Lactobacillus reuteri, glucose transporter gene

Characterization of purine degrading lactic acid bacteria and evaluation of the serum uric acid lowering effect in hyperuricemic rats.

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Background and Rationale

Uric acid is the final product of purine metabolism in liver, and it was excreted with urine by the kidney. When the uric acid was excessive in the body, it caused high serum uric acid (hyperuricemia). In the evolution, humans and some primates lose of uricase gene (allantoin) that play the important role of uric acid metabolism, and the refined food culture promoted the prevalence rate of hyperuricemia more dramatic. Hyperuricemia was caused by abnormally high level of uric acid in the blood which results in the gout symptom. It had also been recognized as a risk factor for chronic kidney disease. Lactic acid bacteria, such as lactobacilli, produce lactic acid as a major metabolic end product of carbohydrate fermentation. Oral intake of *Lactobacillus* may have beneficial effects for the host, by, for example, activating immune responses. but few reports have investigated the effect of *Lactobacillus* ingestion on hyperuricemia.

Objectives: Indicates the purpose of the study

Hyperuricemia usually was cured by treated with drug, for example allopurinol, but drug treatment usually caused strong side effects. Therefore, we want to explore whether Hyperuricemia could be treated by supplementing specific lactic acid bacteria.

Methodology: Describe pertinent experimental procedures

Inosine and guanosine were cultured with lactic acid bacteria. The cultured solution were filtered and injected into a HPLC device to analysis the concentration of inosine and guanosine. These purine degrading lactic acid bacteria isolated by HPLC methods were further to use in animal test.

Results: Summarize the results of the research

The *in vitro* results, we isolated four *Lactobacillus* spp. (*L. fermentum*, *L. acidophilus*, *L. reuteri* and *L. plantarum*) that had the ability to degrade inosine and guanosine. Moreover, the *L. reuteri* and *L. fermentum* not only had the high purine nucleosides degrading ability, they also could decrease the serum uric acid level in hyperuricemia animal model.

Conclusions: State the main conclusions

In our study, we found out two strains of lactic acid bacteria which could degrade purine nucleosides and reduce the concentration of uric acid in mice serum. The finding suggest that, Hyperuricemia could be treated by supplementing these lactic acid bacteria.

Keywords: hyperuricemia, *L. reuteri*, *L. fermentum*, uric acid

Selection of vaginal *Lactobacillus* strains for the development of a tailor-made South African probiotic for vaginal health

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Background and Rationale

Bacterial vaginosis (BV) is highly prevalent globally and increases acquisition of sexually transmitted infections and adverse pregnancy outcomes. Standard treatment with antibiotics has high recurrence; and while adjunctive probiotics improve efficacy and durability, the species contained in commercial probiotics for vaginal health in South Africa are not genital tract commensals.

Objectives: Indicates the purpose of the study

We isolated *Lactobacillus* strains from healthy South African women and selected strains exhibiting the best probiotic characteristics for the development of a tailor-made South African probiotic for vaginal health.

Methodology: Describe pertinent experimental procedures

Lactobacillus spp. were isolated from cervico-vaginal fluid (61 strains) of healthy women. Growth kinetics at varying pHs (pH 3.5 – 6.0), ability to change culture pH, production of D- and L-lactic acid and H₂O₂, influence on growth of BV-associated bacteria (six clinical *Gardnerella vaginalis* and *Prevotella bivia* strains), inflammatory cytokine profiles and effect on cervical cell viability *in vitro* were measured. Strains were scored based on their performance regarding desirable probiotic characteristics.

Results: Summarize the results of the research

Healthy South African women were colonized with *L. crispatus*, *L. jensenii*, *L. gasseri*, *L. mucosae* and *L. vaginalis*. *L. crispatus* strains grew better at extremes of pH and lowered pH more effectively (pH 3.7 vs. pH > 4.0) than other vaginal strains. The production of D- and L-lactic acid and H₂O₂ varied between species and strains. Culture supernatants from clinical strains inhibited clinical *G. vaginalis* and *P. bivia* growth strain-specific. *L. crispatus* isolates did not increase inflammation, and some strains even downregulated the inflammatory responses. The majority of clinical *Lactobacillus* strains did not decrease cell viability *in vitro*.

Conclusions: State the main conclusions

Commercial probiotics do not contain common female genital tracts commensals. *L. crispatus* strains had a better probiotic profile than the other species, although this was highly strain-specific. The ten *Lactobacillus* strains that scored the highest included three *L. crispatus*, each two *L. jensenii*, *L. vaginalis* and *L. gasseri* and one *L. mucosae* strain.

Keywords: probiotics for vaginal health, tailor-made, South Africa, bacterial vaginosis

Impact of commercial inactivated yeast derivatives on the growth of *Lactobacillus rhamnosus* HN001 and other probiotic bacteria in milk

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Background and Rationale

Fermented dairy products are popular carriers of probiotics. Nevertheless, the low levels of free amino acids and assimilable peptides, coupled with the weak proteolytic activity of certain probiotic bacteria strains, impede the growth of these microorganisms in milk. Previously, inactivated yeast derivatives (IYDs) used in winemaking have been shown to stimulate the growth of oenological lactic acid bacteria. Therefore, it is hypothesised that IYDs could be utilised to enhance the growth of probiotic bacteria during milk fermentation.

Objectives: Indicates the purpose of the study

The objective of this study was to evaluate the effect of IYD supplementation on the growth of probiotic bacteria in milk.

Methodology: Describe pertinent experimental procedures

UHT skim milk was supplemented with 3 g/L of commercial IYD (OptiRed[®], OptiWhite[®] and Noblesse[®]) and fermented with *Lactobacillus rhamnosus* HN001 at 37 °C for 24 h. The growth of the probiotic was evaluated by enumerating its viable cell count and measuring the pH of the milk prior to and after fermentation. Milk fermentation was also carried out with *L. acidophilus* NCFM, *L. paracasei* LPC-37, *B. animalis* subsp. *lactis* HN019 and *B. longum* BB46. Chemical analyses of the milks and IYDs were performed using HPLC.

Results: Summarize the results of the research

Significantly higher *L. rhamnosus* counts and lower pH were observed in milks supplemented with IYD as compared to the control after fermentation. OptiWhite[®] was the most effective IYD tested, and its growth-stimulating effect also extended to other probiotic lactobacilli and bifidobacteria. IYD supplementation increased the free amino acid content of the milk by 3.3 to 3.7-fold, as well as introduced mannooligosaccharides and β -glucans. In addition, glutathione was detected in OptiWhite[®] and Noblesse[®], which could have contributed to improved viability of probiotic bacteria in milk.

Conclusions: State the main conclusions

Based on the findings of this study, IYDs could potentially be applied to foods fermented with probiotic bacteria to increase the viable cell count of the microorganisms and shorten fermentation time.

Keywords: Probiotics; Dairy; Fermentation; Inactivated yeast derivatives

Lactic acid bacteria growth promotion of xylooligosaccharides derived from alkali-pretreated and acid-debranched rice husk

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Background and Rationale

Rice husk is the rice milling industrial by-product gained in a huge amount. It is rich in lignocelluloses, previously found at 22% of cellulose content and 23% of hemicellulose content. These lignocellulosic materials have been used widely for oligosaccharide productions.

Objectives: Indicates the purpose of the study

In this study, we investigated the effects of alkali pretreatment and acid debranching on changing of arabinoxylan and arabinose/xylose ratio including the ability of commercial xylanases to produce xylooligosaccharides rice husk. The lactic acid bacteria growth promotion of the oligosaccharides was also observed.

Methodology: Describe pertinent experimental procedures

Firstly, rice husk was treated with 2% NaOH and diluted formic acid. The total sugar contents of the alkali-pretreated and acid- debranched rice husk was measured. At the same time, the xylose, arabinose, glucose, galactose and mannose content, the arabinose/xylose ratio (A/X) and arabinoxylan contents were also determined by HPLC analysis. Then, the commercial xylanases were used to produce oligosaccharides (pH 6, 50°C, 4 h). Thin Layer Chromatography and High Performance Anion Exchange chromatography - Pulsed Amperometric Detection were performed to confirm the oligosaccharides profile of the products. The hydrolysate was used as a single carbon source for evaluation of the growth promotion effect on lactic acid bacteria.

Results: Summarize the results of the research

The yields of alkali-pretreated rice husk were decreased to 68.7%, while the total sugar content was increased from 19.9% to 28.0%. The total sugar content of acid-debranched rice husk was also increased up to 4%. Besides, the alkali pretreatment decreased A/X ratio from 0.14 to 0.13 whereas the arabinoxylan content was increased from 11.5% to 18.6%. Distinguishably, xylobiose was produced by using commercial endoxylanases. The hydrolysate could promote the growth of Lactobacilli and Bifidobacteria.

Conclusions: State the main conclusions

This study has proved the possibility of using rice husk as a source of oligosaccharide production.

Keywords: Rice husk; Alkali pretreatment; Acid debranching; Oligosaccharide; Lactic acid bacteria

An investigation of the prebiotic potential of Irish Seaweeds

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Background and Rationale

Seaweed-derived dietary fibre, including alginates, fucoidans and laminarins from brown seaweeds such as *Fucus serratus*, and agarans, xylans and carrageenans from red seaweeds such as *Chondrus crispus*, may provide prebiotic substrates for the human gut microbiota.

Objectives: Indicates the purpose of the study

This study investigated the prebiotic potential of a *Fucus serratus* polysaccharide extract (FSE) and a *Chondrus crispus* depolymerised polysaccharide extract (CCE), using in-vitro batch culture fermentations.

Methodology: Describe pertinent experimental procedures

Anaerobic, pH, and temperature controlled fermentation vessels were inoculated with a pooled faecal slurry (20% v/v), containing either FSE, CCE, FOS (positive control) or cellulose (negative control) as the sole carbon source (1% w/v, n=3). Samples were taken at 0, 5, 10, 24, 36 and 48h for culture based enumeration of *Bifidobacterium* and *Lactobacillus*, SCFA analysis, and 16S rRNA sequencing.

Results: Summarize the results of the research

FSE significantly increased propionate, acetate, and total SCFA production by 2.3-fold, 1.4-fold, and 1.5-fold, respectively after 24h (p<0.05), with no significant changes in butyrate production. The proportion of propionate production was also significantly increased (24% FSE vs. 15% cellulose control). 16S rRNA sequencing revealed that FSE had no effect upon *Bifidobacterium* and *Lactobacillus* abundance after 24h, however, the propionate producing genus *Parabacteroides* and the family Veillonellaceae were significantly increased.

CCE had no impact upon acetate and butyrate production, but significantly increased propionate and total SCFA production after 24h (p<0.05). 16S rRNA sequencing revealed no notable impact on the relative abundance of *Bifidobacterium* and *Lactobacillus* after 24h compared with the cellulose control, and the Christensenellaceae family were reduced. A significant increase in Bacteroidales family S24-7 was observed relative to FOS.

Conclusions: State the main conclusions

These data indicate that polysaccharides extracted from *Fucus serratus* and *Chondrus crispus* may favourably modulate gut microbial composition and metabolic activity, with *Fucus serratus* having more profound effects, but extract characterisation and in-vivo evidence with health-related end points is required to validate seaweeds as a novel source of prebiotics.

Keywords: Microbiota; Prebiotics; Seaweed; Dietary Fibre; Functional Foods

Gum acacia enrichment culture to extract probiotic microorganisms

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Background and Rationale

Background and rational: Aging is associated with decreases in the potentially beneficial bacteria such as bifidobacteria. It is though that through bifidobacteria metabolite, the production of organic acids may effect on the modulation of the gut microbiota. Gum acacia (GA) showed a potential prebiotic effect in previous work and increase the beneficial bacteria. These results imply that the GA is able to impact on the negative changes associated with ageing.

Objectives: Indicates the purpose of the study

Objectives:The aim of this study was to determine key utilisers of GA and isolate putative probiotics.

Methodology: Describe pertinent experimental procedures

Methodology: Using GA enrichment culture experiment for 14 days from three healthy elderly people faeces then investigate the antimicrobial activity of the isolated probioitcs *in vitro* using agar spot test and well diffusion assay via antipathogenic activities against pathogenic strains of *Escherichia coli*, *Salmonella* Typhimurium and *Enterococcus faecalis*.

Results: Summarize the results of the research

Results: The probiotics from three volunteers were able to produce active compounds on solid media with antimicrobial activity. These were also confirmed when cell-free culture supernatants (CFCS) from the putative probiotics were used in agar well diffusion assay. These experiments were able to confirm the capacity of potential probiotics to inhibit selected pathogens. One of the main inhibitory mechanisms may result from the production of organic acids from utilising the GA and consequent lower of culture pH. These observations could lead to the end products of GA metabolism.

Conclusions: State the main conclusions

Conclusion: The potential probiotics show a remarkable antimicrobial activity, this is relevant to elderly population to decrease infections caused by pathogens bacteria.

Keywords: Gum acacia, enrichment culture, probiotics

Assessment of prebiotic potential of beta-glucans and fatty acids to promote diversity of obese and diabetic human gut microbiota *in vitro*.

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Background and Rationale

Obesity is a global health concern and increases the risk of a number of inflammatory associated diseases including type 2 diabetes. Accumulating evidence suggests that the gut microbiota plays a major role in the progression of such diseases through a number of mechanisms. Gut microbiota diversity can modulate immune function and inflammation, and loss of diversity has been observed in type 2 diabetic patients. Specific food ingredients, such as prebiotics, can promote gut microbiota diversity and offer a tool for promoting health and well-being.

Objectives: Indicates the purpose of the study

The aims of this study were to identify potential prebiotics, novel dietary fibres and fatty acids, that can promote gut microbiota diversity in obese and diabetic patients.

Methodology: Describe pertinent experimental procedures

Obese patients were recruited, separated into three groups (obese non-diabetic, obese pre-diabetic and obese diabetic) based on fasting blood glucose and HbA1c levels, and faecal material was collected from the patients. *In vitro* faecal fermentation experiments were carried out using an anaerobic, pH controlled 24 vessel fermentation system (MicroMatrix) to assess the prebiotic potential of beta-glucans isolated from different food sources in combination and alone with the fatty acids, palmitic, oleic acid and fish oil to promote diversity using microbiota from the three cohorts. Microbiota composition and diversity were assessed by 16S rDNA next generation sequencing (Miseq). Effects on the metabolic activity were assessed by measuring SCFA concentrations by GC-FID.

Results: Summarize the results of the research

Beta-glucans increased alpha diversity (Shannon & Simpson indices) and SCFA production, while dietary fatty acids induced significant changes in the beta diversity (Weighted UNIFRAC, Bray-Curtis) in obese human faecal bacterial populations.

Conclusions: State the main conclusions

The results from this study show that both dietary fibres and fatty acids and lipids can modulate the composition and metabolic activity of the gut microbiota and highlight their potential as novel prebiotics to combat T2D.

Keywords: Prebiotics; Gut Microbiome; Dietary fibre; Fatty acids; Functional foods

Effects of Pea Protein Hydrolysates on the Growth of Probiotics

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Background and Rationale

Several important physiological functions of probiotics have been revealed, such as modulating intestinal microflora, antioxidant activity, immunomodulatory properties, etc. Only in the case to ensure that a certain count of viable probiotics can play these probiotic effects in the human body. Bioactive peptides can promote the growth of lactic acid bacteria, improve yogurt fermentation acid and the quality of yogurt sensory. Pea protein have the function of relieving gastrointestinal product gas and promoting the growth of probiotic in the intestine, which can be improved by enzymatic hydrolysis.

Objectives: Indicates the purpose of the study

The effects of adding pea protein hydrolysates (PPH) on the growth of 17 common probiotics were studied in this paper.

Methodology: Describe pertinent experimental procedures

We got the results by comparing the density of bacterial, the viable count and the pH of the fermentation broth of these probiotics in the MRS medium containing PPH or in the MRS medium without PPH.

Results: Summarize the results of the research

It was found that the addition of 4 mg/mL PPH significantly stimulated the growth of *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacterium*, the cell density increased by 10.78% - 49.34%, the viable counts increased by 1-3 grade of viable counts, the pH value of the fermentation broth was significantly decreased. However, the addition of PPH had no effect on the growth of *Lactobacillus plantarum*, *Lactobacillus rhamnosus* and *Streptococcus thermophilus*.

Conclusions: State the main conclusions

PPH can promote the growth of lactic acid bacteria, improve yogurt fermentation acid and the quality of yogurt sensory.

In vitro assessment of the effects of Human Milk Oligosaccharides on the microbiota in Irritable Bowel Syndrome

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Background and Rationale

Irritable Bowel Syndrome (IBS) affects 1 in 5 people at some point in their life, this has huge implications on not only patients but the economy. The syndrome is characterised by pain, irregular bowel movements and general discomfort making IBS difficult to diagnose and treat. However, there is now strong evidence implicating the gut microbiome in the onset and alleviation of many gastrointestinal diseases.

Objectives: Indicates the purpose of the study

The objective of the present study was to assess the potential for using Human Milk Oligosaccharides (HMO) as a new prebiotic to manipulate the microbiome and their functionality to alleviate symptoms and signs of IBS.

Methodology: Describe pertinent experimental procedures

Batch culture fermentation models were used to assess 6 individual HMOs in IBS (n=4) and healthy donors (n=2). Batch culture models were aseptically inoculated with a basal culture medium in the presence of the HMOs with the temperature kept constant (37°C) and the pH regulated (6.8). These models were run for 48 hours and samples were taken at 0, 8, 24 and 48 hours from each culture. The samples were then analysed using a number of different techniques; flow cytometry coupled with fluorescent in-situ hybridization (FISH) for microbial composition and NMR spectroscopy for metabolite profiling, as well as gas chromatography (GC) for short chain fatty acids (SCFA) analysis.

Results: Summarize the results of the research

There is a clear change in microbial composition when introducing HMOs into the culture in both healthy and IBS subjects throughout the fermentation compared to not. Furthermore, there is a significant difference between SCFAs in healthy compared to IBS subject samples. After performing multivariate statistics on the NMR data, preliminary modelling revealed a number of differences between the metabolic profiles.

Conclusions: State the main conclusions

This *in vitro* study provided fundamental evidence that HMOs powerfully, specifically modulate the microbiota, influencing metabolites, and could be a useful tool for the dietary management of IBS.

Keywords: Prebiotic; IBS; HMOs; Microbiome; Metabonomics

Predominant Gut *Lactobacillus .Mmurinus* Strain Mediates the Anti-inflammaging Effects in Calorie Restricted Mice

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Background and Rationale

Calorie restriction (CR), which exerts a potent anti-inflammaging effect, has been demonstrated to induce dramatic changes in the gut microbiota. It remains a question whether the modulated gut microbiota contributes to the attenuation of inflammation by CR and which members of the microbial community are the key mediators.

Results: Summarize the results of the research

Here, we report that a unique *Lactobacillus*-predominated microbial community was rapidly attained in mice within 2 weeks of CR, which decreased the levels of circulating microbial antigens and systemic inflammatory markers such as tumour necrosis factor- α (TNF- α). *Lactobacillus murinus* CR147, an isolate in the most abundant operational taxonomic unit (OTU) enriched by CR, downregulated interleukin-8 production in tumor necrosis factor- α (TNF- α)-stimulated Caco-2 cells and significantly increased the lifespan and the brood size of the nematode *Caenorhabditis elegans*. In gnotobiotic mice colonized with the gut microbiota from old mice, this strain decreased their intestinal permeability and serum endotoxin load, consequently attenuating the inflammation induced by the old microbiota.

Conclusions: State the main conclusions

Our study demonstrated that a strain of *Lactobacillus murinus* was promoted in CR mice and causatively contributed to the attenuation of ageing-associated inflammation.

Keywords: Calorie restriction, gut microbiota, chronic inflammation, lifespan, *Lactobacillus murinus*

Application of isomaltooligosaccharide from rice starch in diet formulas for chronic kidney disease's patient

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Background and Rationale

Chronic kidney disease (CKD) is an enormous public health issue worldwide, and it is a health burden with a high economic cost to health systems in Thailand. Individuals with CKD have to limit the intake of certain nutrients especially protein and minerals to decrease the accumulation of unexcreted metabolic products. Imported commercial diet formulas for CKD patients are available; however, their prices are severely unaffordable to most Thai CKD's patient for long-term consumption.

Objectives: Indicates the purpose of the study

This research aims to develop the complete diet formula which could be used as a substitute for high-cost imported commercial diet formula.

Methodology: Describe pertinent experimental procedures

In this study, rice starch that producing in Thailand was used as a raw material. The main ingredient in this diet formula contains Isomaltooligosaccharide (IMO). Enzymatic synthesis of IMO from rice starch was performed by the steps of: (a) liquefaction with Kleistase E5NC; (b) saccharification with b-amylase F and (c) transglucosylation with Transglucosidase L. The chemical composition and sugar contents of rice IMO were analyzed by high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD).

Results: Summarize the results of the research

Chemical compositions of IMO consisted of carbohydrate as major component (97.05%). The energy value of IMO was 3.91 kcal/g which lower than regular carbohydrate. Sugar contents of IMO were 20.74% isomaltose, isomaltotriose and panose, 55.89% of glucose and 26.29% of total IMO.

Conclusions: State the main conclusions

IMO was achieved producing from rice starch. The complete diet formula was successfully developed and sufficient to meet the nutrient requirement for CKD's patients. Therefore, this complete diet formula would be one alternative for CKD patients to reduce expenses.

Keywords: chronic kidney disease, functional food, dietary supplement, isomaltooligosaccharide, rice

Role of human-residential bifidobacteria in the degradation of food-derived opioid peptides

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Background and Rationale

Food-derived opioid peptides are known as bioactive peptides that can interact with opioid receptors and possess beneficial health effects. However, recent epidemiological evidence has identified some food-derived opioid peptides as potential risk factors for human diseases such as mental disorders, celiac disease, and sudden infant death syndrome. Bifidobacteria, which are the major member of human gut microbiota, particularly in infant gut, have increasingly been shown to exert positive health benefits to host. Bifidobacteria naturally inhabit a range of ecological niches and can be classified into two major groups; bifidobacterial species of human origins as human-residential bifidobacteria (HRB) while other species which are the natural inhabitants of animals or environment as non-HRB.

Objectives: Indicates the purpose of the study

Here we evaluate the potential role of bifidobacteria in the degradation of food-derived opioid peptides.

Methodology: Describe pertinent experimental procedures

We tested the ability of 18 bifidobacterial strains, representing nine infant-type human-residential bifidobacteria (HRB), four adult-type HRB, and five non-HRB, to degrade human milk- and bovine milk-derived casomorphin-7, and wheat gluten-derived gliadorphin-7. Dipeptidyl peptidase (DPP) activity of bifidobacteria was determined using fluorescence assay and the final contents of opioid peptides upon incubation were quantified using LC-MS/MS.

Results: Summarize the results of the research

Our findings reveal an origin-dependent degradative capability of bifidobacteria in food-derived opioid peptides whereby infant-type HRB strains showed a relatively higher DPP activity than non-HRB strains. For instance, strains of *Bifidobacterium longum* subsp. *infantis* and *B. bifidum* effectively hydrolysed all three opioid peptides. We further evaluated the degradative capabilities of 29 strains of *B. bifidum* and selected the strains with higher potential in degrading opioid peptides.

Conclusions: State the main conclusions

These results suggest the possible role of infant-type HRB in infant gut to eliminate food-derived opioid peptides, protect against chronic diseases and contribute to infant health development. Selected strains of *B. bifidum* with high degradative capabilities could be used as novel probiotic interventions to aid in host metabolism and improve human health.

Keywords: Human-residential bifidobacteria; food-derive opioid peptides; potential role; degradative capability

Probiotic Properties and Cellular Antioxidant Activity of *Lactobacillus plantarum* MA2 Isolated from Tibetan Kefir Grains

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Background and Rationale

In recent years, probiotic lactic acid bacteria have received increasing attention because of their long history of safe use and their potential therapeutic benefits for human health. Though abundant researches were conducted to prove the excellent antioxidant effects of LAB on the aspects such as reducing oxidative damage, increasing the free radical scavenging rate, improving the activities of SOD and GSHPx, the systematic elucidation of the antioxidative mechanism of LAB is still in need.

Objectives: Indicates the purpose of the study

Based on the preliminary research which showed MA2 with promising antioxidant potentials. In this study further investigation was pursued to evaluate the MA2 antioxidative effect with the CAA method.

Methodology: Describe pertinent experimental procedures

CAA assay was applied to test the antioxidant capacity.

Results: Summarize the results of the research

The results indicated that MA2 had high antioxidant potential. The cell-free extract of the logarithmic phase was the most active, Fermentation supernatant has antioxidant activities. We found that the relative mRNA levels of the eight antioxidant-related genes (including cat, Gpx, gshR1~4, nox, npx) in MA2 in the logarithmic phase were higher than those in the stationary phase. These values may be attributed to the fact that the bacterial growth reached the stationary phase, MA2 exhibited a high antioxidant activity in the logarithmic phase. The CAA method was successively applied to evaluate the antioxidant capacity of MA2.

Conclusions: State the main conclusions

MA2 exhibited a high antioxidant activity in the logarithmic phase. The CAA method was successively applied to evaluate the antioxidant capacity of MA2. As a natural antioxidant.

Keywords: Lactobacillus plantarum MA2, Antioxidative effects, Cellular antioxidant activity (CAA)

Effect of Domestication on the Intestinal Microbiome of Asturcon Horses.

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Background and Rationale

The Asturcon horse is an old horse breed belonging to the *Celtic ponies* group. Domestic horses are more sensitive to gastrointestinal diseases than wild/feral animals, and the Asturcon breed is no exception. In comparison with modern horse breeds, the Asturcon provides a model in which wild and domesticated populations co-exist over a uniform genetic background. The ancient microbiota may constitute a microbial reference for risk reduction in domestic horses as well as a source for the isolation of specific strains or combinations aimed at enhancing horses' health.

Objectives: Indicates the purpose of the study

Our aim was to compare the microbiome of wild and domestic Asturcons and isolate strains as potential horse probiotics.

Methodology: Describe pertinent experimental procedures

Fresh fecal samples from nine Asturcon mares (three wild and 6 domesticated) were collected. Samples were plated in MRS for isolation of lactobacilli and submitted to microbiome analyses and SCFA determination.

Results: Summarize the results of the research

Wild-horses' showed several microbiota differences, from kingdom to species level, when compared with domesticated animals. The wild Asturcons presented higher levels of *Puccinia striiformis*, *Penicillium sclerotiorum*, *Lawsonia intracellularis*, *Eubacterium halii*, *Bacteroides salanitronis*, *Christensenella masilensis*, *Treponema azotonutricium*, *Lachnospirillum phytofermentans*, *Streptococcus anginosus*, *Streptococcus suis*, *Lactobacillus plantarum*, *Staphylococcus aureus* and *Campylobacter*. Domesticated horses presented higher levels of different rhizosphere and plant-symbiotic microorganisms, including *Sphingomonas taxi*, *Sphingomonas panacis*, *Dietzia timorensis*, *Stenotomonas rhizophila*, *Pseudomonas rhizosphaerae*, *Bradyrhizobium*, *Agrobacterium tumefaciens*, *Fusarium graminearum*, *Rhodococcus fascians*, *Tilletia indicum*, *Actinomyces naeslundii*, *Methylobacterium zatmanii* and *Clostridium cellulovorans*. The *Lactobacillus* strains isolated belonged to the species *Lactobacillus equi*, *Lactobacillus equigenerei* or *Lactobacillus hayakitensis*.

Wild animals presented, in general, higher levels of bacteriophages than their domestic counterparts, specially of lactobacillus-phages, and higher levels of SCFA.

Conclusions: State the main conclusions

Our results underline the impact of domestication on the horse microbiome. The isolates of *L. equi*, *L. equigenerei* and *L. hayakitensis* obtained here constitute a potential source for the development of probiotics for horses.

Keywords: Asturcon horse, microbiota, feral, domestication, *Lactobacillus*

Gut Microbiota Mediates Islet Cell Injury Induced by Low-dose DSS

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Background and Rationale

Emerging evidence showed that microbes may be involved in immune response and antigen presentation during induction and continued persistence. Our study found that low-dose dextran sulfate sodium (DSS) can induce type 1 diabetes (T1D) in mice, but the mechanism is not clear.

Objectives: Indicates the purpose of the study

Trying to clarify the mechanism by which gut microbiota mediates islet injury in T1D.

Methodology: Describe pertinent experimental procedures

Low-dose DSS was used as an oral treatment for C57BL male mice for 3 months. Drinking water, diet and body weight were measured every 3 days and daily faeces were also collected. V3-V4 region in 16s rRNA were sequenced to analyze the structure of gut microbiota. We also tested blood lipids, liver fat, blood glucose, blood insulin and injury of islet. Then we use broad spectrum antibiotics (ABX) to deplete commensal gut microbiota and the effects of low-dose DSS on glycometabolism were observed.

Results: Summarize the results of the research

The mice with low-dose DSS treatment showed a frequent urination, increased thirst and increased hunger phenomenons, without increasing net energy intake since the experiment started. After 8 weeks with low-dose DSS, the mice had abnormal glucometabolism, impaired pancreas, and insufficient insulin secretion. The structure of the gut microbiota also showed significantly shift during the DSS treatment. Moreover, DSS did not induce abnormal glycometabolism in the mice with broad spectrum antibiotic treated.

Conclusions: State the main conclusions

The study suggested that gut microbiota mediates islet cell injury induced by low-dose DSS.

Keywords: DSS; T1D; gut microbiota

Identification of key proteins and pathways in cadmium tolerance of *Lactobacillus plantarum* strains by proteomic analysis

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Background and Rationale

Our previous study confirmed the protective potential of *Lactobacillus plantarum* (*L. plantarum*) strains in alleviation of cadmium (Cd) toxicity *in vivo* and demonstrated that the observed protection largely depended on the tolerance of the strains to Cd-induced stress. It was also observed that there were significant intra-species differences in Cd tolerance of *L. plantarum* strains.

Objectives: Indicates the purpose of the study

The objective of the present study was to investigate the Cd tolerance mechanisms of *L. plantarum* strains based comparative proteomics.

Methodology: Describe pertinent experimental procedures

The Cd-tolerance related key proteins and pathways within the *L. plantarum* species were investigated by using iTRAQ based proteomic approach. *L. plantarum* strains CCFM8610 (strongly resistant to Cd) and CCFM191 (sensitive to Cd) were selected for comparative proteomic analysis. The proteomic results were further confirmed by RT-qPCR and by the measurement of several biological properties of the bacterial cells in response to Cd exposure.

Results: Summarize the results of the research

Of the total 1415 identified proteins, 206 were differentially expressed for the comparison of natural proteomic profiles of CCFM8610 and CCFM191, 27 were differently regulated in CCFM8610 after Cd exposure, and 111 were changed in CCFM191 in response to Cd stress. Both strains showed physiological alterations in energy metabolism, purine and pyrimidine metabolism, global stress responses, lipid and amino acid metabolism, metal binding properties, cell wall biosynthesis and transporters in response to Cd exposure.

Conclusions: State the main conclusions

We propose that the underlying mechanism of the exceptional Cd tolerance of CCFM8610 may be attributed to the following: (a) a specific energy-conservation survival mode; (b) mild induction of its cellular defense and repair system; (c) an enhanced biosynthesis of hydrophobic amino acids in response to Cd; (d) inherent superior Cd binding ability and effective cell wall biosynthesis ability; (e) a tight regulation on ion transport; (f) several key proteins, including prophage P2b protein 18, CadA, mntA and Ip_3327.

Keywords: Probiotics; Cadmium; Heavy metals; *Lactobacillus plantarum*; Tolerance

Effects of fructo-oligosaccharides on the intestinal micro-ecology of constipation induced by loperamide in BALB/c mice

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Background and Rationale

Constipation is one of the most common gastrointestinal complaints worldwide.

Objectives: Indicates the purpose of the study

The aim of this study was to evaluate the effects of fructo-oligosaccharide (FOS) at dosages of 0.8, 4 g/d/kg bw and 8 g/d/kg bw on the composition and activity of the microbiota in the gut of mice with constipation induced by loperamide.

Methodology: Describe pertinent experimental procedures

BALB/c mice were given FOS by gavage once per day for 8 days. Changes in intestinal flora and metabolic activities were measured to evaluate the effects of the FOS.

Results: Summarize the results of the research

FOS increased the concentrations of acetic, propionic, butyric acids and the total acid. Acetic and butyric acids were found to have the most significant effects on constipation. The gut microbiotas were dominated by Firmicutes, Bacteroidetes and Actinobacteria. At the genus level, FOS treatment increased the relative abundance of *Lactobacillus* and *Bifidobacterium*. The relative abundance of *Odoribacter*, *Alistipes* and *Bacteroides* in faeces decreased in the FOS-fed mice groups.

Conclusions: State the main conclusions

In conclusion, our results demonstrate that FOS, administered as a dietary supplement, modulate the composition of gut microbiota and increase the concentration of short-chain fatty acids in the faeces of mice with constipation.

Keywords: Fructo-oligosaccharides; constipation; SCFAs; gut microbiota

Preliminary study evaluating presence and interaction of SCFAs in the lungs

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Background and Rationale

Asthma is a chronic inflammatory disease affecting over 235 million people in the world, with an increasing prevalence. Previous research has demonstrated that the lung microbial community in asthmatics differs from healthy people. Short-Chain Fatty Acids (SCFAs) are major gut microbial fermentation products and recent preliminary work has demonstrated anti-inflammatory effect of SCFAs in asthma-induced rodent models. The mechanisms behind this remain unclear.

Objectives: Indicates the purpose of the study

The objective of the research is to investigate the presence of SCFAs in the lungs and the effect on lung epithelium and cytokine profile.

Methodology: Describe pertinent experimental procedures

Sputum and bronchial alveolar lavage (BAL) samples were obtained from healthy and asthmatic patients to investigate the presence of SCFAs using targeted gas chromatography coupled to a mass spectrometer (GC-MS). Human lung epithelial cell-line was utilized to study the effect of SCFAs on inflammation using a pro-inflammatory cocktail mix. The epithelial cells were pre-treated with different doses of acetate and propionate, followed by treatment of the cocktail mix, to investigate cytokine response.

Results: Summarize the results of the research

In preliminary data, SCFAs were detected in sputum and BAL samples in μM concentrations in both healthy and asthmatic patients with a slight increase in sputum samples. Treating the lung epithelial cells with propionate and inducing inflammation, demonstrated a significant decrease in IL-6 compared to control cells with no added propionate.

Conclusions: State the main conclusions

This investigation presents preliminary evidence suggesting the presence of gut microbial derived products, SCFAs, in the lungs and the possible interaction with the epithelium and immune response. However, more information is needed to elucidate the presence of SCFAs in the lungs and the mechanism with the lung epithelium.

Keywords: Asthma; SCFAs; lung epithelium; lung microbiome

Traditional sour cabbage – an ancient probiotic food from Wallachian Plain

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Background and Rationale

Sour cabbage is considered to be part of the Romanian traditional meal, especially in the cold season. Consumed slightly prepared or as a side-dish, its production is currently divided between small home-made quantities and large industrial production batches. Considered having “miraculous” benefits for GI tract, both the sour cabbage and the brine resulting from the process are often consumed as a universal treatment – from “healing” simple GI disorders to treating symptoms of irritable colon.

Objectives: Indicates the purpose of the study

The study aimed to identify the microbiota involved in the natural fermentation process and to prove the probiotic character of this traditional product.

Methodology: Describe pertinent experimental procedures

The experiment focused on the natural fermentation of cabbage (*Brassica oleracea* var. *capitata* f. *alba* – hybrid Buzaiana) cultivated in Wallachian Plain, in a sour cabbage production plant. The microbiota of the product was analyzed in each of the four fermentation stages. The fermentation was made traditionally, without using any other ingredients but salt. Samples of brine and cabbage were analyzed in each fermentation step for TC, enterobacteriaceae, *Leuconostoc*, *Lactobacillus*, and the lactobacilli found were identified using API50CH strips.

Results: Summarize the results of the research

The first stage is characterized by the decrease of the TC value, due to respiration of the plant material and the consumption of oxygen by facultative anaerobic enterobacteria. In the second stage, the characteristic microorganism is *Leuconostoc mesenteroides*. The third stage was a homofermentation. Both in third and fourth stages colonies of *Lactobacillus plantarum* and *Lactobacillus brevis* were identified at a total maximum number of lactobacilli of 5×10^5 CFU/ml

Conclusions: State the main conclusions

Even if two species of probiotic lactobacilli were found during this preliminary experiments, more identifications work needs to be performed. The study of the fermentation substrate lead also to the presumption that one of the saccharides extracted from the fresh cabbage has an important role in the development of lactobacilli in the brine.

Technology for a beverage fermentated with local Romanian sugary kefir strains using honey as a fermentation substrate

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Background and Rationale

The use of sugary kefir grains is well known in the romanian culture, especially since the communist era, when people did not have access to sweetened drinks. There are several studies of sugary kefir granules on miscellaneous fermentation substrates, but not on honey, and also, the Romanian sugary kefir granules have not been studied yet.

Objectives: Indicates the purpose of the study

The object of the present study is to find a hydromel-like drink with probiotic properties, how well will the sugary kefir grains will adapt on this kind of substrate and what will the microbiota of the sugary kefir grains will evolve.

Methodology: Describe pertinent experimental procedures

A dilluted solution of honey and water (12%, 14%, and 16% concentration) was used as a substrate for the sugary kefir granules. This solutions was fermentated at 20-25°C for 48 h, using two separate methods: one with the direct inoculation of the sugary kefir granules in the substrate, and the other one with a secondary inoculum (prepared by inoculation of sugary kefir granules on a nutrient substrate, incubation for 48 h at 20-25°C and the sieving of the granules). Standard cultural ISO methods were used for the determination of the microbiota existing in the beverage.

Results: Summarize the results of the research

The results obtained encouraged the development of new experiment in the field. Both direct innoculation and the use of secondary inoculum returned good results for low honey concentration (12%). The living microbiota of the beverage obtained consisted of two yeast strains, several lactobacilli strains and even bifidobacteria. For the identification of lactobacilli, API50CH strips were used, resulting 3 known and 1 unknown Lactobacillus strains.

Conclusions: State the main conclusions

Due to the confirmed existence of *Lactobacillus* and *Bifidobacterium* strains, we can conclude that the product presents certainly probiotic valences. Further studies were initiated for improving the viability of the probiotic strains and for identifying, with certainty, all the strains in the resulted beverage.

Safety assessment of *Lactobacillus crispatus* JDM502 based on whole genome sequencing

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Background and Rationale

As most lactobacilli are non-pathogenic, it is difficult to identify inherent strain properties relevant to safety concerns. The information from conventional experimental methods is restricted to the phenotype. While using whole genome sequencing, some latent safety risks such as antibiotics resistance, virulence factors and harmful metabolites can be revealed.

Objectives: Indicates the purpose of the study

The objective of this study was to assess the safety of *Lactobacillus crispatus* JDM 502, a probiotic strain isolated by our group, providing some recommendations for the further application.

Methodology: Describe pertinent experimental procedures

The whole genome of strain JDM502 was sequenced and the subsequent genes were annotated. Virulence, putative adverse metabolites, and antibiotic resistance related genes, together with genes involved with the genomic stability were identified using BLAST searches. The minimum inhibitory concentration (MIC) of 16 antibiotics to JDM502 were evaluated by E-test. Meanwhile, acute toxicity experiments were performed with C57BL/6 mice which were administered with 10^8 cfu, 10^9 cfu, 10^{10} cfu *L. crispatus* JDM502 in 200 μ L PBS and 200 μ L vehicle, respectively, for continuous 7 days. One month later, all the mice were sacrificed, and the pathological injury was measured.

Results: Summarize the results of the research

The genome of strain JDM502 includes many antibiotic resistance genes, but none of which is at risk of transferring. Most of the 222 virulence related genes are generally safe. Nevertheless, there are 10 genes encoding autolysin, hemolysin B, and invasion associated protein. This strain is non-pathogenic in C57BL/6 mice.

Conclusions: State the main conclusions

L. crispatus JDM502 is safe and non-pathogenic in C57BL/6 mice. However, there are still potential risks according to the genomic information. When using as probiotics, strain JDM502 is not recommended for those with leaky gut or immune deficiency.

Keywords: probiotics, safety, *Lactobacillus crispatus*, genome sequencing

The Present of Prenatal Bacterial Microbiome in Uterine Cavity

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Background and Rationale

It was widely thought that during a healthy pregnancy, the fetus developed within a sterile environment. The recent evidence of microbiome in amniotic fluid, utero and placenta suggested that fetal-microbial contact and microbial colonization of the fetal organism possibly began before birth. But the knowledge of the precise timing and origin of maternal intrauterine microbial colonization during pregnancy is still controversy.

Objectives: Indicates the purpose of the study

To reveal if the microbiota presents in uterine cavity that a fetus develops with.

Methodology: Describe pertinent experimental procedures

The microbiome of 10 endometrium, 98 decidual tissues and 64 amniotic fluid were taken from reproductive-age Chinese women. DNA extraction was followed by pyrosequencing V4 region of 16S rDNA gene to evaluate the microbiome present in the environment that fetus develops with. The richness and evenness of microbiome profiles in different groups were assessed by Shannon index and Chao1 index. Microbiome structure was assessed by β -diversity analysis. Viable biomass in amniotic fluid and placenta were assessed by conventional bacterial culture.

Results: Summarize the results of the research

Although the results showed microbiome present in all groups with low abundance, low richness and low diversity, amniotic fluid showed a trend of significant increase in diversity compared with decidual tissues. Analysis of the community structure of endometrium, decidual tissues and amniotic fluid also demonstrated significantly separate clustering of the amniotic fluid from decidual tissues and endometrium. When we cultured samples from amniotic fluid and placenta, 538 out of 1834 placenta samples showed culture positive, whereas all of 50 amniotic fluid samples showed culture negative.

Conclusions: State the main conclusions

Our results suggested that uterine cavity that fetus develops with harbored low abundance and low diversity microbiome. However, amniotic fluid showed a significant increasing diversity of microbiome compared with decidual tissues. Culture-based investigation of amniotic fluid and placenta confirmed the presence of cultivable bacteria in placenta but not in amniotic fluid.

Keywords: uterine cavity microbiome, 16S rDNA gene,

Lactobacillus plantarum ZS2058 produces conjugated linoleic acid to ameliorate colitis

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Background and Rationale

CLA has a strong ability to regulate immunity, and this effect has been demonstrated in a great variety of inflammation-related disorders. Lactobacillus plantarum ZS2058 is an efficient producer of conjugated linoleic acid (CLA), in which the mechanism for CLA production was fully characterized.

Objectives: Indicates the purpose of the study

The aim of the research is to investigate whether CLA producers could show protective effects on colitis through CLA production.

Methodology: Describe pertinent experimental procedures

Different CLA producers and non-CLA producers were selected. An acute colitis mice model was induced with dextran sodium sulfate (DSS). The mice were treated with different bacteria, CLA, or vehicle. After euthanasia, the inflammatory indexes such as disease activity index (DAI), colon length, colonic histopathologic damage and MPO activity, were investigated. As well as inflammatory cytokines, integrity of colonic mucus layer and CLA concentration were analyzed.

Results: Summarize the results of the research

Compared to non-CLA producers, *L. plantarum* ZS2058 significantly alleviated DAI, colon shortening and histological damage. Additionally, *L. plantarum* ZS2058 could significantly regulate inflammatory cytokine expression in colonic tissue (such as TNF- α , IL-1 β , and IL-6, IL-10) as well as protects the colonic mucous layer and epithelium structure. All those results were significantly correlated with CLA production ability. Additionally, colonic CLA concentrations were significantly increased in response to CLA producers treatments, which indicates that CLA producers prevents colitis via generating CLA locally. To further investigation, the key role of CLA on anti-inflammation, the genetic determinates for CLA production in *L. plantarum* ZS2058 were knocked-out. Neither intermediates nor CLA was detected in the knock-out mutants. Compared to the wild type, all the mutants practically lost the protective effects on colitis. Meanwhile, those mutants could recover the ability to convert linoleic acid to CLA as well as anti-inflammation when the corresponding gene was completed.

Conclusions: State the main conclusions

Those current results indicates that lactic acid bacteria with CLA production ability could alleviate colitis via CLA generation.

Keywords: Lactobacillus plantarum; conjugated linoleic acid; colitis; amelioration

Effects of fructo-oligosaccharides on the intestinal micro-ecology of constipation induced by loperamide in BALB/c mice

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Background and Rationale

Constipation is one of the most common gastrointestinal complaints worldwide.

Objectives: Indicates the purpose of the study

The aim of this study was to evaluate the effects of fructo-oligosaccharide (FOS) at dosages of 0.8, 4 g/d/kg bw and 8 g/d/kg bw on the composition and activity of the microbiota in the gut of mice with constipation induced by loperamide.

Methodology: Describe pertinent experimental procedures

BALB/c mice were given FOS by gavage once per day for 8 days. Changes in intestinal flora and metabolic activities were measured to evaluate the effects of the FOS.

Results: Summarize the results of the research

FOS increased the concentrations of acetic, propionic, butyric acids and the total acid. Acetic and butyric acids were found to have the most significant effects on constipation. The gut microbiotas were dominated by Firmicutes, Bacteroidetes and Actinobacteria. At the genus level, FOS treatment increased the relative abundance of *Lactobacillus* and *Bifidobacterium*. The relative abundance of *Odoribacter*, *Alistipes* and *Bacteroides* in faeces decreased in the FOS-fed mice groups.

Conclusions: State the main conclusions

In conclusion, our results demonstrate that FOS, administered as a dietary supplement, modulate the composition of gut microbiota and increase the concentration of short-chain fatty acids in the faeces of mice with constipation.

Keywords: Fructo-oligosaccharides; constipation; SCFAs; gut microbiota

Bioconversion of Ginsenoside Rb1 to Rd using β -Glucosidase from *Leuconostoc mesenteroides* EFEL 15

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Background and Rationale

Ginsenoside Rd is a protopanaxadiol type ginsenoside having tetracyclic terpene sapogenin structure and it is known to exert diverse health promoting activities such as wound healing, immune modulation, anti-obesity, and managed mitochondrial function.

Objectives: Indicates the purpose of the study

We isolated a lactic acid bacterium from Kimchi showing high β -glucosidase activity and constructed a microbial conversion process to produce ginsenoside Rd from ginsenoside Rb1 by using the isolate.

Methodology: Describe pertinent experimental procedures

Lactic acid bacteria were isolated from Kimchi and their β -glucosidase activities were tested. Whole cells and lysed cells were used as the crude enzyme in the bioconversion reactions. The lysed cells were prepared by sonication and the bioconversion of Rb1 to ginsenoside Rd was carried out at 30°C for 72 hrs. The strain was identified by phenotypic and genotypic analyses.

Results: Summarize the results of the research

As result, *Leuconostoc mesenteroides* EFEL 15 strain was isolated as a potential strain. When the whole cells and lysed cells were reacted with ginsenoside Rb1 in an optimized condition, ginsenoside Rd was produced after 24 hrs. TLC and HPLC analyses showed that the enzyme extract and whole cells hydrolyzed 20-C- β -(1 \rightarrow 6)-glucoside linkage of Rb1 compound.

Conclusions: State the main conclusions

Leuconostoc mesenteroides EFEL 15 strain can be used for overproduction of ginsenoside Rd in the ginseng-derived fermented food.

Keywords: Bioconversion, Ginsenoside Rb1, Ginsenoside Rd, β -Glucosidase, Leuconostoc mesenteroides

Isolation of immunomodulatory probiotic strain to be used in Kimchi

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Background and Rationale

The demand for probiotics is increasing due to beneficial health effects of probiotics. As in the case of the yogurt products, it is necessary to develop a new probiotic used for the fermentation of Kimchi products.

Objectives: Indicates the purpose of the study

The purpose of this study was to isolate industrially useful and safe probiotic candidates for Kimchi.

Methodology: Describe pertinent experimental procedures

Using various lactic acid bacteria (LAB) isolated from Kimchi and human feces, their survival rates in the gastrointestinal tract were measured by reacting in the simulated conditions and their adhesion capacities on the human colon cells were tested. For the selected strains, TNF- α , IL-6, and IL-12 inducing activities were measured using mouse peritoneal macrophages to test an immunomodulatory activity.

Results: Summarize the results of the research

Among various LAB tested in acid and bile salt resistance, 11 strains were isolated and they were *Lactobacillus* spp. (9), and *Leuconostoc* spp. (2). When cytokine inducing activities were tested, *L. mesenteroides* EFEL 15 showed the highest values in the secretion of TNF- α , IL-6, and IL-12.

Conclusions: State the main conclusions

L. mesenteroides EFEL 15 can be used as an immunomodulating LAB strain for Kimchi product.

Keywords: Lactic acid bacteria(LAB), Probiotics, Kimchi, Immunomodulatory, L. mesenteroides

Double-Stranded RNA Derived from Lactic Acid Bacteria Augments Th1 Immunity via Interferon- β from Human Dendritic Cells.

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Background and Rationale

Lactic acid bacteria (LAB) are one of the major commensal species of small intestine, and are known to modulate immunity. We previously reported that double-stranded RNA (dsRNA) in LAB triggered interferon- β (IFN- β) production by murine dendritic cells (DCs) via endosomal Toll-like receptor 3, which exerted anti-viral and anti-inflammatory effect.

Objectives: Indicates the purpose of the study

The objective of the present study was to clarify the molecular mechanisms for immunomodulatory effects of LAB focusing on IL-12 and IFN- β production by human DCs.

Methodology: Describe pertinent experimental procedures

We isolated naïve CD4⁺ T cells, BDCA1⁺ DCs (mDC1) and CD14⁺ monocytes from PBMCs. Monocyte-derived DCs (moDCs) were prepared by culturing CD14⁺ monocytes in the presence of IL-4 and GM-CSF. We stimulated these cells with heat-killed LAB and assessed mRNA expression, cytokine production and cell differentiation.

Results: Summarize the results of the research

The secretion of IL-12 from PBMCs provoked by several strains of LAB was abrogated, when LAB were treated with RNase A to deplete both dsRNA and single-stranded RNA (ssRNA). In most strains of LAB, this inhibitory effect was null when the digestion was restricted to ssRNA. IL-12 secretion from PBMCs induced by a strain of LAB, *Pediococcus acidilactici* strain K15, was attenuated by the neutralizing IFN- β monoclonal antibody, indicating dsRNA of LAB primarily triggered the IFN- β -IL-12 pathway. These results were completely reproducible with moDCs. Moreover, the induction of IL-12 by K15 from moDC was abolished by the inhibition of endosomal acidification, confirming the critical role of endosomal digestion process of LAB. By using co-culture system of naïve CD4⁺ T cells and mDC1 from PBMCs, we revealed that mDC1 stimulated with K15 induced IFN- γ -producing T cells.

Conclusions: State the main conclusions

This study indicates that human DCs activated by LAB enhance Th1 immunity, which depends on IFN- β secretion by DCs in response to bacterial dsRNA.

Keywords: LAB; Interferon- β ; dsRNA

Polyphasic microbial analysis of *Laphet*, Myanmar fermented tea leaves

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Background and Rationale

Laphet is a Myanmar traditional fermented tea leaves which has a long eating habit among Myanmar people. It is a steamed tea leaves undergone anaerobic fermentation. Processing of *Laphet* is still traditional and analysis of microbial diversity in processed *Laphet* is necessary to understand the role of microorganisms during fermentation.

Objectives: Indicates the purpose of the study

This study was carried out to analyze the diversity of microorganisms in *Laphet* samples.

Methodology: Describe pertinent experimental procedures

Laphet samples were collected from different parts of Myanmar. Different selective media were used to isolate microorganisms and ARDRA-PCR was used to group the microbial isolates at species level. Identification of microorganisms was carried out according to their morphology and biochemical characteristics. Illumina miseq system and PCR-DGGE were also carried out to analyze the microbial diversity of *Laphet* samples.

Results: Summarize the results of the research

The pH of *Laphet* samples was very diverse ranged from 4.6 to 7.11 revealing the presence of different microorganisms depending on products. The total microbial count was in the range of 10^6 - 10^8 cfu/g of *Laphet* samples. Yeast and bacteria including lactic acid bacteria growth were found in all samples.

Conclusions: State the main conclusions

Yeast and lactic acid bacteria were found as dominant microorganisms in processed *Laphet* samples. Based on this result, starter culture development will be performed for high quality *Laphet* production.

Keywords: Laphet, fermented tea leaves, yeast, lactic acid bacteria

A High-Throughput Sequencing Method to Assess the Structure and Composition of Gut *Bifidobacterium*

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Background and Rationale

The next-generation high-throughput sequencing techniques have introduced a new way to assess the gut's microbial diversity on the basis of 16S rRNA gene-based microbiota analysis. However, the precise appraisal of the biodiversity of *Bifidobacterium* species within the gut remains a challenging task because of the limited resolving power of the 16S rRNA gene in different species. The groEL gene, a protein-coding gene, evolves quickly and thus is useful for differentiating bifidobacteria.

Objectives: Indicates the purpose of the study

The objective of the present study was to develop a new method to assess the structure and composition of gut bifidobacterium.

Methodology: Describe pertinent experimental procedures

To identify *Bifidobacterium* species, a region of 487 or 496 base pairs (bp) located at positions 1066 to 1552 (*B. animalis*) or 1561 (*B. breve*) of the complete groEL gene of ca. 1600 bp was chosen as discriminative target sites. Degenerate primers BifgroEL-F (5-TCC GAT TAC GAY CGY GAG AAG CT-3)/Bif-groEL-R (5-CSG CYT CGG TSG TCA GGAACA G-3) for the genus *Bifidobacterium* were manually designed according to multiple sequence alignment. The detection sensitivity and accuracy of the primer set Bif-groEL-F/Bif-groEL-R were evaluated employing known DNA amounts, ranging from 0.01 to 40 ng, of the artificial sample from 10 different bifidobacterial taxa. Sequence reads were processed with the QIIME package version 1.9.1.

Results: Summarize the results of the research

The novel designed primer set can specifically differentiate *Bifidobacterium* species from non-bifidobacteria, and as low as 10⁴ cells of *Bifidobacterium* species can be detected using the novel designed primer set on the basis of Illumina Miseq high-throughput sequencing.

Conclusions: State the main conclusions

We developed a novel protocol to assess the diversity of gut *Bifidobacterium* species through high-throughput sequencing technologies using groEL gene as a discriminative marker.

Keywords: *Bifidobacterium*; groEL; species level; biodiversity; MiSeq high-throughput sequencing

The protective effects of probiotic against heavy metal toxicity

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Background and Rationale

Heavy metal (such as lead and cadmium) pollution is one of the oldest environmental problems and remains a serious health concern today. Recently, Probiotics have been reported to play a role against heavy metal toxicity.

Objectives: Indicates the purpose of the study

The goal of our study was to evaluate the effects of probiotic against heavy metal toxicity, and to understand the underlying protective mechanism.

Methodology: Describe pertinent experimental procedures

In vitro studies were conducted to screen strains with good heavy metal tolerant and binding abilities. Acute and chronic heavy metal exposure animal models were further established to understand the in vivo protection of probiotics and the related mechanism

Results: Summarize the results of the research

Some specific probiotics such as *Lactobacillus plantarum* CCFM8610 and CCFM8661 were screened out for their good heavy metal binding ability, tolerance, antioxidative capacity and acid and bile salt tolerance. These strains could effectively decrease the mortality of mice, increase the heavy metal levels in the feces, reduce hepatic and renal metal accumulation, alleviate tissue oxidative stress, and ameliorate tissue histopathological changes. The mechanism can be attributed to metal sequestration, gut barrier protection, immune regulation and oxidative stress alleviation

Conclusions: State the main conclusions

Our studies suggested that probiotics can be considered a new dietary therapeutic strategy against heavy metal toxicity.

Keywords: Probiotics; Heavy metal; Gut barrier; Oxidative stress; Dietary supplements

Total polyphenol, total flavonoid contents, and antioxidant activity of *Laphet*, Myanmar fermented tea leaves

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Background and Rationale

Laphet is a Myanmar traditional fermented tea leaves which has a long eating habit among Myanmar people. Although *Laphet* has been widely consumed in Myanmar, scientific study of *Laphet* has not been done enough.

Objectives: Indicates the purpose of the study

This study was carried out to investigate total polyphenol, total flavonoid contents, and antioxidant activity of *Laphets* collected from different Myanmar markets.

Methodology: Describe pertinent experimental procedures

Twenty four *Laphet* samples were collected from different parts of Myanmar and total polyphenol content, total flavonoid content, DPPH and ABTS radical scavenging antioxidant activities were analyzed. HPLC analysis was also conducted to determine the content of apigenin, myricetin, quercetin, kaempferol, catechin, and catechin derivatives.

Results: Summarize the results of the research

Total polyphenol content ranged from 5794 ± 349 to 1444.1 ± 29.8 mg GAE (gallic acid equivalent)/100g sample. In total flavonoid content, the highest value was 9590 ± 1035 mg CE (catechin equivalent)/100g and the lowest was 631.9 ± 57.0 mg CE/100g. DPPH radical scavenging antioxidant activities of samples ranged from 869 ± 414 to 10068.2 ± 77.6 mg AEAC (ascorbic acid equivalent antioxidant capacity)/100g. ABTS assay showed that antioxidative activities of samples ranged from 204 ± 27.6 to 689 ± 25.7 mM TEAC (trolox equivalent antioxidant activity)/g except only four samples which values were less than 50 mM TEAC/g.

Conclusions: State the main conclusions

Laphet contains high concentration of polyphenol and flavonoid conferring antioxidative activity. The wide spectrum of the value of total polyphenol, total flavonoid contents, and antioxidant activity of *Laphet* samples may be due to difference in age of tea leaves, microbial diversity, and fermentation time. This study demonstrates that *Laphet* is regarded as a health promoting fermented food product.

Keywords: *Laphet*; fermented tea leave; polyphenol; flavonoid; antioxidant activity

A mixture of Lactobacillus species isolated from traditional fermented foods promote recovery from antibiotic-induced intestinal disruption in mice

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Background and Rationale

Consumption of *Lactobacillus plantarum* was reported to alleviate the side effects of antibiotic therapy, including the risk of nausea and developing loose or watery stools.

Objectives: Indicates the purpose of the study

The objective of the present study was to evaluate the effectiveness of a mixture of four *Lactobacillus* species on restoration of gut microbiota and regulation of immunity in mice following treatment with antibiotic.

Methodology: Describe pertinent experimental procedures

Administration of the antibiotic ampicillin for 2 weeks. Then application of a probiotic cocktail of four *Lactobacillus* species (JUP-Y4; containing *L. plantarum*, *L. casei*, *L. rhamnosus* and *L. helveticus*) derived from fermented foods.

Results: Summarize the results of the research

Administration of the antibiotic ampicillin for 2 weeks decreased microbial community diversity, induced caecum tumefaction and increased gut permeability in mice. Application of a probiotic cocktail of four *Lactobacillus* species (JUP-Y4) modulated the microbiota community structure and promoted the abundance of potentially beneficial bacteria such as *Akkermansia*. Ampicillin administration led to a decline in *Bacteroidetes* from 46.6 ± 3.91% to 0.264 ± 0.0362%; the addition of JUP-Y4 restored this to 41.4 ± 2.87%. This probiotic supplementation was more effective than natural restoration, where the levels of *Bacteroidetes* were only restored to 29.3 ± 2.07%. Interestingly, JUP-Y4 treatment was more effective in the restoration of microbiota in faecal samples than in caecal samples. JUP-Y4 also significantly reduced the levels of D-lactate and endotoxin (lipopolysaccharide, LPS) in the serum of mice, and increased the expression of tight-junction proteins while reducing the production of inflammatory cytokines (TNF-α, IL-6, MCP-1, IFN-γ and IL-1β) in the ileum and the colon of antibiotic-treated mice.

Conclusions: State the main conclusions

Treatment with a cocktail of *Lactobacillus* species (JUP-Y4; containing *L. plantarum*, *L. casei*, *L. rhamnosus* and *L. helveticus*) derived from fermented foods restored the gut microbiota community structure, enhanced microbial diversity and reduced the intestinal inflammation caused by antibiotic administration.

Keywords: *antibiotics, diseases, immunology, intestinal microbiota, Lactobacillus.*

Milk Fat Globule Membrane Alone and in Combination with a Prebiotic Blend Moderates the Impact of Maternal Separation on Behavior and Gut Microbiota

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Background and Rationale

Nutritional interventions targeting the microbiota-gut-brain axis was proposed to modulate stress-induced dysfunction of physiological processes and brain development.

Objectives: Indicates the purpose of the study

To assess the effects of dietary supplementations through various nutritional interventions: milk fat globule membrane (MFGM) and a polydextrose/galactooligosaccharide prebiotic blend in maternally separated (MS) rats.

Methodology: Describe pertinent experimental procedures

The MS rats were separated from their mothers for 3 h /day from postnatal day (PND) 2 to 12. Starting at weaning, both non-separated (NS) and MS offspring were provided with or without supplementation of MFGM, prebiotic blend or a combination of both. Spatial memory, visceral sensitivity and stress reactivity were assessed in adulthood. Gene transcripts associated with cognition and stress were measured and the caecal microbiota composition was analysed.

Results: Summarize the results of the research

MS rats demonstrated visceral hypersensitivity which was ameliorated by MFGM and the combination of MFGM+prebiotic. MS rats received prebiotic, MFGM alone, as well as the combination showed improved cognitive performance in spatial and reference memory. Coinciding with these, plasma corticosterone was higher in MS rats following the acute stress and was reduced by prebiotic and the combination, suggesting moderation of stress reactivity. Moreover, MFGM and prebiotic supplementation alone, and in combination, induced changes in microbiome composition of MS rats. Peptostreptococcaceae was increased in the prebiotic+MFGM group compared to the control group. The relative abundance of *Barnesiella* was increased with MFGM supplementation in comparison with control and prebiotic groups. Clostridium cluster IV was increased in prebiotic+MFGM compared to MFGM and control. Finally, MFGM and prebiotic supplementation alone, and in combination, had a significant effect on beta diversity, with a more profound effect in MS animals compared to NS animals.

Conclusions: State the main conclusions

Given the impact of MS on the gut microbiota and behavior was modulated by MFGM and the probiotic blend, these dietary supplementations may offer a nutritional solution to early-life stress induced alterations in the microbiota-gut-brain axis.