Conference Summary

2023 Annual Meeting

June 25-28, 2023

Denver, Colorado, USA

For more details, join us at:

http://www.isapp-sfa.com

https://twitter.com/ISAPPSFA

@ISAPPSFA #ISAPP2023

About the SFA

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

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

Annual Meetings

The SFA began as an initiative by Gregor Reid and the students and fellows at the 2009 ISAPP meeting in Irvine, California. The SFA annual meetings are held in conjunction with the ISAPP annual meetings. Our first meeting began in 2010 in Barcelona, Spain, followed by: 2011 in Berkeley, California; 2012 in Cork, Ireland; 2013 in New York, New York; 2014 in Aberdeen, Scotland; 2015 in Washington D.C.; 2016 in Turku, Finland; 2017 in Chicago, Illinois, US; 2018 in Sinapore; 2019 in Antwerp, Belgium; 2022 in Sitges, Spain; and this year, 2023 in Denver, USA. The location of the 2024 meeting is to be determined.

Executive Summary

The SFA Conference Summary for the 2023 meeting is available here. On behalf of the SFA committee, we thank all SFA members who joined us in Denver, Colorado, USA for the 2023 annual meeting. Your involvement was indispensable for making this year a success and everyone benefited from the sharing of your research. We hope you had a great time!

2022-2023 SFA Executive Committee and Acknowledgements

SFA Executive Committee

2022-2023

President

Brendan Daisley, PhD.
University of Guelph, Canada

Vice-President

Daragh Hill, PhD.
APC Microbiome, Ireland

Secretary

Cathy Lordan, PhD.
Teagasc, Ireland

Communications

David Hourigan
APC Microbiome, Ireland

Treasurer

Dieter Vandenheuvel, PhD.
University of Antwerp, Belgium

Outreach

Sarah Ahannach, PhD.
University of Antwerp, Belgium

Local Organizer

Breanna Metras
University of Illinois Urbana Champaign, USA

Acknowledgements

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

Conference schedule

Overview:
This document represents the official **Students and Fellows (SFA)** schedule for the 2023 ISAPP Conference. It is designed specifically to help simplify and distinguish between the main ISAPP events and SFA-specific events (see Legend below). Details on SFA-relevant events are outlined in this document whereas ISAPP industry and/or board member events have been omitted. Maps of relevant locations are provided on last page of this document. Further details and general information about the conference are available at: http://www.isapp-sfa.com/2023-meeting

– Date of event –

Time SFA-only EVENTS
Location ISAPP EVENTS (SFA participating)
Event information
Chair/Presenter

LEGEND

Reminders:

- Locations:
  - Brown Palace is the main conference venue. All activities will be held in the Ballroom unless indicated otherwise.
  - Holiday Inn Express is the hotel with rooms reserved for SFA attendees. Basic expenses covered. Check-in is at 3pm on Sunday, June 25th. Check-out is at 11am on Wednesday, June 28th.
  - Wynkoop is where the SFA networking event will be held on June 25th.
  - University Club is where the Welcome Reception event will be held on June 26th.
  - History Colorado Center is where the Gala event will be held on June 27th.
- Meals:
  - Breakfast will be provided at the Holiday Inn Express between 7-8 AM daily.
  - Lunch/Dinner will be provided at the Brown Palace as indicated below
- Posters:
  - All SFA attendees will present a poster (including those giving oral presentations)
  - Posters are to be setup by noon on Monday June 26th and left up for rest of conference
  - Poster competition will include 3 prizes ranging from USD $50 - $150.
- **SFA =** Students and Fellows Association
- **IAC =** Industry Advisory Committee (all industry representatives)

Sunday, June 25

15:00 – 18:00 HOTEL CHECK-IN
Holiday Inn Express
Address: 1715 Tremont Pl, Denver, CO 80202, United States
Website: https://www.ihg.com/holidayinnexpress

17:00 – 18:00 REGISTRATION
Pick-up name badges and conference materials in hotel lobby.
Register in advance for discussion groups (see schedule for June 27th)

18:00 – 20:00 MEET AND GREET
Wynkoop
Social gathering to meet the other SFA members attending the conference. Informal dress attire. Meet in hotel lobby and travel to Wynkoop as a group.

Monday, June 26

8:00 – 8:45 **Brown Palace** *Georgetown*	**SFA INTRODUCTIONS** *This session is for SFA members only. The SFA Executive Committee will present background information on the conference and share guidelines for success.*
8:45 – 10:15	**SFA + IAC INNOVATION WORKSHOPS** **Chairs:** Mariya Petrova (Winclove Probiotics, Amsterdam, The Netherlands) and Brendan Daisley (University of Guelph, Canada).
10:00 – 12:00	**POSTER SETUP** *Posters are to be left up for the entire meeting.*
10:15 – 10:30	**BREAK**
10:30 – 11:00	**INNOVATION WORKSHOP REPORT BACK.** **Chairs:** Mariya Petrova and Brendan Daisley.
11:00 – 12:00	**INDUSTRY FORUM** *Microbiome endpoints for clinical trials***.** Jacques Ravel (University of Maryland School of Medicine, Institute for Genome Sciences, Baltimore, USA) and Sean Gibbons (Institute for Systems Biology, Seattle, WA, USA).
12:00 – 13:00	**LUNCH**
13:00	**WELCOME TO ISAPP 2022** **Chair:** Dan Merenstein, ISAPP President
13:00 – 14:30	**INTERACTIVE SESSION** *Game-changing insights from recent publications.* **Chair:** Anisha Wijeyesekera (University of Reading, UK)
14:30 – 15:00	*The vaginal microecology, immunity and the potential of probiotic interventions.* Jo-Ann Passmore (University of Cape Town, South Africa)
15:00 – 15:30	**BREAK AND POSTER VIEWING**
15:30 – 16:00	*Design of dietary and bacterial therapeutic interventions to enhance the resilience and health-promoting properties of the human gut microbiome.* Ophelia Venturelli (University of Wisconsin-Madison, USA)
16:00 – 16:30	*How the microbiome converses with the little brain and the big brain.* Premysl Bercik (McMaster University, Hamilton, Ontario, Canada)
16:30 – 16:45	**REFRESHMENTS**
16:45 – 17:45	**LATE BREAKING NEWS** **Chair:** Gregor Reid (Western University, Canada)
18:00 – 20:00 **University Club**	**WELCOME RECEPTION** Share a drink and gourmet appetizers with old and new friends. Semiformal dress attire.

Tuesday, June 27

7:45 – 8:30
Brown Palace

SFA KEYNOTE PRESENTATION
Learn to talk, walk, and hope: tips for a career in life.
Gregor Reid (Western University, Canada)

8:30 – 9:30

INTERACTIVE LIGHTNING TALK INTRODUCTIONS
This session is for SFA members only. The Executive Committee will present a 10 minute introduction. Subsequently, each SFA attendee will give a 90 second lightning presentation introducing themselves. Format will be 16:9 aspect ratio with 2 powerpoint slides – 1 on personal background and 1 on scientific research.
Chairs: Brendan Daisley (SFA President, University of Guelph, Canada) and Daragh Hill (SFA Vice-president, University College Cork, Ireland).

9:30 – 9:45

BREAK

9:45 – 10:45

ORAL PRESENTATIONS
Selected topic presentations from SFA attendees (15 min talk/5 min questions)

- *Orphan nisin immunity genes are widespread across the Bacillota.* Ivan Sugrue (University College Cork, Ireland)
- *Identifying novel probiotic candidates to counter kidney stone disease.* Gerrit Stuivenberg (Western University, Canada)
- *Lactiplantibacillus plantarum Plantaricin EF is a probiotic effector that protects barrier function in intestinal epithelial cells through an intracellular cation-linked mechanism.* Lei Wei (University of California, Davis, USA)

Chairs: Dieter Vandenheuvel (University of Antwerp, Belgium) and Breanna Metras (University of Illinois, USA)

10:45 – 11:00

BREAK

11:00 – 11:30

SFA SCIENTIFIC COMMUNICATION PRESENTATION
How to leverage social media and maximize the reach of your scientific findings. Kristina Campbell (Consulting Communications Director, ISAPP)

11:30 – 12:30

BREAKOUT GROUP SUMMARIES
One member of each breakout group will give a 3-5 minute presentation on their niche study area discussion to share with the rest of the group.

- Group 1: Probiotics and concept of using beneficial microbes for health
- Group 2: Prebiotics and concept of modulating fiber intake for health
- Group 3: Human microbiota and related bioinformatic analysis

Chairs: Cathy Lordan (Teagasc, Ireland) and Dave Hourigan (APC Microbiome, Ireland)

12:30 – 12:45

BREAKOUT GROUP SUMMARIES

12:45 – 13:00

NETWORKING ACTIVITIES
Reserved time for networking, idea generation, and collaborative planning.

13:00 – 14:00

LUNCH

14:00 – 14:15	**2023 GLENN GIBSON EARLY CAREER RESEARCHER PRIZE PRESENTATION** *Investigating the effects of short-chain fatty acids on the immune system and gut microbiota of healthy humans.* Paul Gill (Monash University, Melbourne, Australia)
14:15 – 14:30 *SFA highlight talk*	**PLENARY SESSION** *Immunometabolic effects of physicochemically-distinct dietary fibers in adults with excess body weight: towards precision nutrition strategies.* Anissa Armet (University of Alberta, Canada)
14:30 – 14:45 *SFA highlight talk*	*Identification of novel immunomodulatory components in Lacticaseibacillus rhamnosus GG.* Soyolmaa Jamiyanpurev (Shinshu University, Japan)
14:45 – 15:15	*Re-imagining the future of healthcare research registries: happening as we speak.* Khurram Nasir (The Methodist Hospital and Weill Cornell School of Medicine, Houston, TX, USA)
15:15 – 15:45	*Impact and personalized responses to prebiotics by human gut microbiota.* Lawrence David, Duke University, Durham, NC, USA
15:45 – 16:15	*Kill to prosper: intra-species competition of a probiotic.* Jan-Peter Van Pijkeren (University of Wisconsin-Madison, USA)
16:15 – 17:15	**POSTER VIEWING AND SFA COMPETITION** Authors are to be present at their posters while formal judging occurs.
17:30	**MEET IN BALLROOM**
18:00 – 21:00 **History Colorado Center**	**GALA SOCIAL EVENT** Assemble in Ballroom and then proceed to walk in groups to History Colorado Center (0.5 miles). Semiformal dress attire.

Wednesday, June 28

8:00 – 8:15 *IAC highlight talk*	**PLENARY SESSION** *Beneficial effects of multispecies probiotics on mood and cognition in clinical studies*. Annemarieke van Opstal (Winclove Probiotics, Amsterdam, the Netherlands)
8:15 – 8:30 *IAC highlight talk*	*Inulin-type fructans and 2'fucosyllactose alter microbial composition and alleviate stress-induced mood state in a working population: a randomized, controlled trial*. Jessica Van Harsselaar (BENEO-Institute, BENEO GmbH, Obrigheim, Germany)
8:30 – 9:00	*Microbiome maturation in premature infants*. Marie-Claire Arrieta (University of Calgary, Canada)
9:00 – 9:30	**STATUS REPORTS ON ISAPP PROJECT** • Association of live dietary microbes with health outcomes: a brief update. Bob Hutkins • The role of probiotics in restoring microbiota composition and function following antibiotic-induced perturbation. Hania Szajewska (Medical University of Warsaw, Poland)

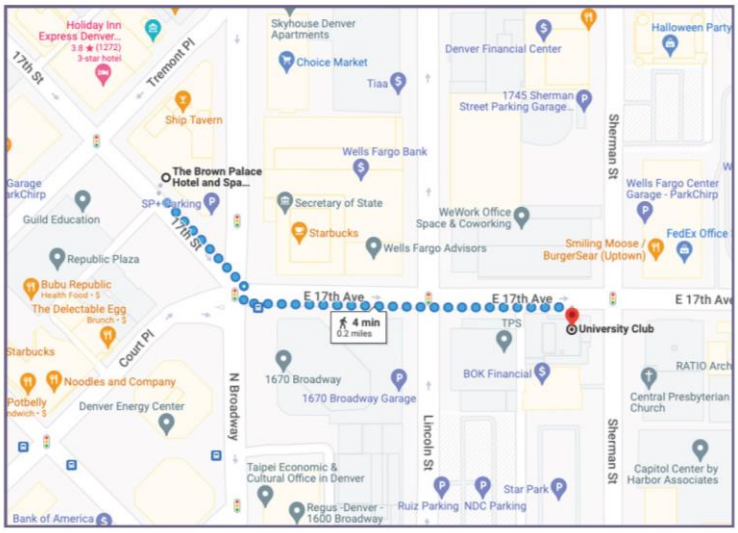
- Prebiotic criteria. Karen Scott, Rowett Institute (University of Aberdeen, UK)

9:30 – 10:00 Use of probiotics and prebiotics to restore microbiome homeostasis and treat GI diseases in companion animals. Karin Allenspach (Iowa State University, Ames, USA)

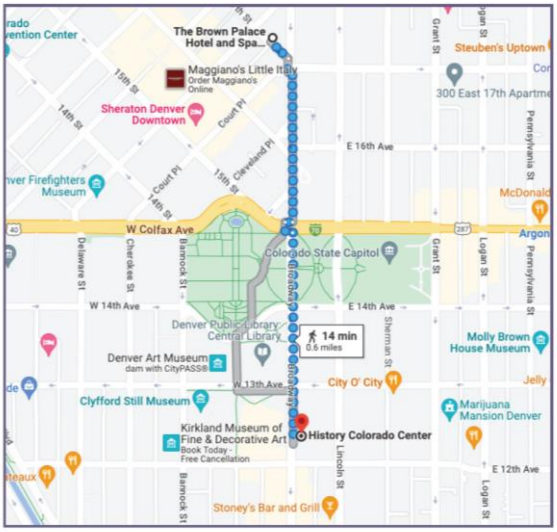
10:00 – 10:30 **BREAK AND POSTER VIEWING**

10:30 – 12:30 **SUMMARY REPORTS FROM DISCUSSION GROUPS**
Chair: Gabriel Vinderola (Instituto de Lactología Industrial, CONICET-UNL, Santa Fe, Argentina)

12:30 **MEETING ADJOURNED**



BROWN PALACE to UNIVERSITY CLUB

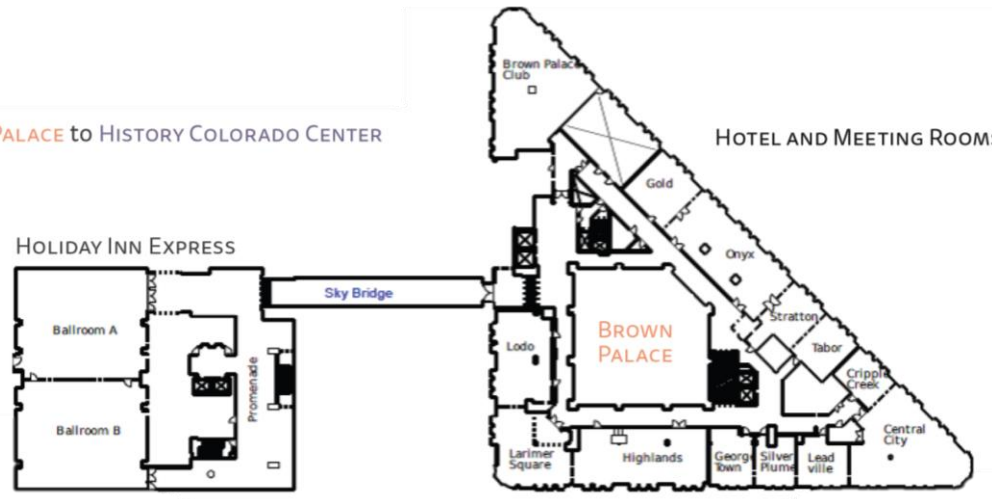


BROWN PALACE to HISTORY COLORADO CENTER

MAPS

BROWN PALACE to HISTORY COLORADO CENTER

HOTEL AND MEETING ROOMS MAP



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Participant abstracts

Gut microbiome composition and metabolic capacity differ by FUT2 secretor status

Alexander W. Thorman (University of Cincinnati, Cincinnati, USA), Grace Adkins(St. Jude's Graduate School of Biomedical Sciences, Memphis, USA), Shannon C. Conrey (University of Cincinnati, Cincinnati, USA)(Cincinnati Children's Hospital Medical Center, Cincinnati, USA), Allison R. Burrell (University of Cincinnati, Cincinnati, USA)(Cincinnati Children's Hospital Medical Center, Cincinnati, USA), Ying Yu (The University of Tennessee Health Science Center, Memphis, USA), Brendon White (Cincinnati Children's Hospital Medical Center, Cincinnati, USA), Rachel Burke (Centers for Disease Control and Prevention, Atlanta, USA), David Haslam (Cincinnati Children's Hospital Medical Center, Cincinnati, USA), Daniel C. Payne (Centers for Disease Control and Prevention, Atlanta, USA), Mary A. Staat (Cincinnati Children's Hospital Medical Center, Cincinnati, USA), David S. Newburg (University of Cincinnati, Cincinnati, USA) and Ardythe L Morrow (University of Cincinnati, Cincinnati, USA)(Cincinnati Children's Hospital Medical Center, Cincinnati, USA).

Risk of several gut diseases is influenced by a major polymorphism in the fucosyltransferase2 (FUT2) gene, but its impact on the microbiome of infants is understudied. In individuals with an active FUT2 enzyme (“secretors”), the intestinal mucosa is abundantly fucosylated, providing a rich endogenous source of fucose for mutualist bacteria. Similarly, maternal secretor status influences the abundance of fucosylated human milk oligosaccharides. Non-secretors lack the ability to create this enzyme and therefore have lower gut and milk fucosylation. We compared the impact of maternal secretor status, measured by FUT2 genotype, and infant secretor status, measured by FUT2 genotype and phenotype, on early infant fecal microbiome samples collected from 2-month-old breastfed and non-breastfed infants enrolled in the PREVAIL term birth cohort (n=211). Infant secretor status (22% non-secretor, 24% low-secretor, and 54% full-secretor) was more strongly associated with the infant microbiome than it was with the maternal FUT2 genotype. Alpha diversity was greater in full-secretors compared to the low- ($p=0.031$) or non-secretor infants ($p=0.045$). Three distinct microbial enterotypes corresponded to infant secretor phenotype ($p=0.022$) and to the dominance of *Bifidobacterium breve*, *Bifidobacterium longum*, or neither ($p<0.001$). Infant secretor status was also associated with microbial metabolic capacity, specifically, bioenergetics pathways. These patterns were modified by exclusive breastfeeding. We conclude that infant secretor status and breastfeeding status, but not maternal secretor status, is associated with infant microbial colonization and metabolic capacity. These findings indicate that the glycans help establish early colonizers of the gut.

Characterising *Lactobacillus* strains from African women with persistently optimal vaginal microbiota - framework for an African vaginal probiotic product development platform

Anika Chicken (Institute of Infectious Disease and Molecular Medicine (IDM), University of Cape Town, Cape Town, South Africa), Jo-Ann Passmore (IDM, University of Cape Town, Cape Town, South Africa; NRF-DST CAPRISA Centre of Excellence in HIV Prevention, Cape Town, South Africa; National Health Laboratory Service (NHLS), Cape Town, South Africa) Anna-Ursula Happel (IDM, University of Cape Town, Cape Town, South Africa), Christina Balle (IDM, University of Cape Town, Cape Town, South Africa), Linda-Gail Bekker (Desmond Tutu HIV Centre, University of Cape Town, Cape Town, South Africa), Katherine Gill (Desmond Tutu HIV Centre, University of Cape Town, Cape Town, South Africa), Heather B. Jaspan (IDM, University of Cape Town, Cape Town, South Africa; Seattle Children's Research Institute, Seattle, USA; University of Washington Department of Paediatrics and Global Health, Seattle, USA) and Brian Kullin (IDM, University of Cape Town, Cape Town, South Africa).

Bacterial vaginosis (BV) is associated with significant health risks in cisgender women (CW). The high rates of BV reoccurrence following antibiotic treatment necessitates the development of alternative treatments. While probiotic products are promising in this regard, there is an urgent need for the development of improved vaginal products containing strains with proven beneficial properties capable of persistently colonising the female genital tract. We collected vaginal specimens at 3 visits from a total of 86 CW (aged 15-19 years; uCHOOSE cohort). Strains were selectively isolated from participants with longitudinally stable *L. crispatus*-dominant communities (CST I) using VALENCIA and assayed for their ability to inhibit a BV-associated bacteria. Whole genome sequencing was performed on 3 isolates and their resistance to antibiotics used in BV treatment was determined. A total of 337 isolates were obtained from 3 participants with longitudinally stable CST I communities. Almost all isolates (n = 323 [95.8%]) showed some inhibition of *P. bivia* ATCC 29303 and local *P. biva* strains growth. Overall, 6 different bacteriocin related proteins (Class III: 3; Class II: 1; LAPs: 1) were identified. No resistance genes were identified (ResFinder & CARD) but all selected strains were phenotypically resistant to metronidazole. The proportion of CW with longitudinally stable, optimal vaginal communities in our setting is relatively low. However, targeting these women for the isolation of potential probiotic bacteria yielded a large number of isolates with inhibitory activity against a BV-associated pathogen, which can be characterised further. Phenotypic variation among isolates illustrates the importance of screening multiple strains of the same species per sample.

Immunometabolic effects of physicochemically-distinct dietary fibers in adults with excess body weight: towards precision nutrition strategies

Anissa M. Armet (University of Alberta, Canada), Fuyong Li (University of Alberta, Canada; City University of Hong Kong, China), Edward C. Deehan (University of Alberta, Canada), Daria Nikolaeva (Skolkovo Institute of Science and Technology, Russia; University College Cork, Ireland), Benjamin Seethaler (University of Hohenheim, Germany), Junhong Liu (University of Alberta, Canada), Janis L. Cole (University of Alberta, Canada), Yuan-Yuan Zhao (University of Alberta, Canada), Jonathan M. Curtis (University of Alberta, Canada), Spencer D. Proctor (University of Alberta, Canada), Stephan C. Bischoff (University of Hohenheim, Germany), Wendy V. Wismer (University of Alberta, Canada), Catherine J. Field (University of Alberta, Canada), Jeffrey A. Bakal (University of Alberta, Canada), Dan Knights (University of Minnesota, USA), Carla M. Prado (University of Alberta, Canada), and Jens Walter (APC Microbiome Ireland, University College Cork, Cork, Ireland).

Prebiotic dietary fibers (DF) elicit clinical benefits, but with significant inter-individual variation. It is unknown to what degree DF strategies can be improved through personalization based on the individual's gut microbiome. Our objective was to compare the immunometabolic effects of high doses of physicochemically-distinct DFs in adults with excess body weight and determine if individual microbiome signatures predict these effects using machine learning. In a six-week, parallel-arm, randomized controlled trial, adults with BMI 25-35 kg/m2 added 25g (females) or 35g (males) of one of three DFs to their usual diet daily: acacia gum (AG; soluble, fermentable; n=75), resistant starch type 4 (RS4; insoluble, fermentable; n=75), or microcrystalline cellulose (MCC; insoluble, non-fermentable control; n=45). A multi-omics approach was applied to assess clinical markers and microbiome composition, genes, and metabolism. AG and RS4 increased flatulence and overall gastrointestinal symptoms ($q<0.05$, linear mixed models), and AG increased fecal acetate ($q<0.01$), propionate ($q<0.05$), and total short-chain fatty acid levels ($q<0.01$). RS4 increased the anorexigenic hormone peptide YY ($q<0.001$), and AG decreased the orexigenic hormone ghrelin ($q<0.05$). Both AG ($q<0.001$) and MCC ($q<0.001$) reduced fecal calprotectin, a marker of gut inflammation. Coefficients of variation in immunometabolic markers ranged from 30-243%, signifying large inter-individual variation in responses to DF supplementation. We are currently exploring if baseline microbiome signatures and other metadata can predict clinical outcomes using machine learning. If successful, this study will help establish a framework to improve health through personalized DF use based on individual gut microbiome features.

Effects of commercial and traditional kefirs on apparent total tract macronutrient digestibility and fecal characteristics, metabolites, and microbiota of healthy adult dogs

Breanna N. Metras (University of Illinois at Urbana-Champaign), Patricia M. Oba (University of Illinois at Urbana-Champaign), Michael J. Miller (University of Illinois at Urbana-Champaign), and Kelly S. Swanson (University of Illinois at Urbana-Champaign).

Our objectives concerned the effects of commercial or traditional kefirs on apparent total tract macronutrient digestibility (ATTD) of a diet administered to healthy adult dogs and determine fecal characteristics, microbiota populations, metabolite, and immunoglobulin (Ig) A concentration. Dogs (n=12/group) were used in a replicated 3x3 Latin square design. A commercial diet was fed alongside allotments of 1 of 3 treatments: 2% reduced-fat milk treated with lactase (CNTL), commercial kefir (C-Kefir), or traditional kefir (T-Kefir) from 2% reduced-fat milk and kefir grains. Three 28-d periods were composed of a 22-d transition phase, a 5-d fecal collection phase, and 1 d for blood collection. Blood and fecal samples were collected for serum chemistry pH, dry matter, microbiota populations (16S rRNA gene amplicons), ATTD, metabolite, and IgA concentrations. Mixed Models procedure of SAS 9.4 was used, with main effects of treatment tested; significance set at $p<0.05$. T-Kefir had a higher ($p<0.0001$) CFU/mL count than CNTL and C-Kefir. Bacterial alpha diversity tended to be greater ($p=0.10$; Faith's PD) in dogs fed T-Kefir than CNTL. Beta diversity analysis (unweighted PCoA) identified a difference ($p<0.0004$) between dogs fed CNTL and C-Kefir. Dogs fed C-Kefir tended to have greater ($p=0.06$) relative abundance of Fusobacteriota; dogs fed T-Kefir had greater ($p<0.0001$) relative abundance of *Lactococcus*. Fresh fecal pH, DM, IgA and metabolite concentrations, and blood biomarkers were not affected by treatment. The supplementation of commercial or traditional kefir to healthy adult dogs had minor effects on fecal microbiota, fecal metabolite concentrations, stool quality, fecal IgA concentrations, and blood metabolites.

Emerging evidence for probiotic-based disease management in honey bees

Brendan Daisley (University of Guelph, Guelph, Canada), Andrew Pitek (Western University, London, Canada), Christina Torres (University of California, Davis, Davis, USA), Robin Lowery (University of California, Davis, Davis, USA), Bethany Adair (Western University, London, Canada), Kait F Al (Western University, London, Canada), Bernardo Niño (University of California, Davis, Davis, USA), Jeremy Burton (Western University, London, Canada), Emma Allen-Vercoe (University of Guelph, Guelph, Canada), Graham Thompson (Western University, London, Canada), Elina Niño (University of California, Davis, Davis, USA), Gregor Reid (Western University, London, Canada).

Managed honey bee (*Apis mellifera*) populations play a crucial role in supporting adequate pollination of food crops but are facing unsustainable colony loss as the result of rampant disease spread within agricultural environments. Antibiotics have failed to resolve the issue so far, whereas mounting evidence from in vitro experiments suggest that select lactobacilli strains (some of which are symbionts in honey bees) can inhibit a broad range of important pathogens via multifaceted mechanisms. Focusing on three select strains of lactobacilli (LX3), our group has performed several probiotic field studies in distinct regions across North America. Cumulative evidence indicates that the LX3 strains can significantly improve resistance to disease outbreaks, partially mitigate the negative effects of antibiotics in bees, and increase queen egg laying – the latter of which has the potential to increase the number of bees present in a hive and thus overall pollination capacity which is relevant to agricultural crop yields. Furthermore, our studies show that certain effects derived from LX3 treatment are directly dependent on the way in which the strains were administered to the hive. This highlights that consideration of delivery method is essential for deriving an expected probiotic benefit in honey bees. Overall, the collective scope of this work is expansive and broadly relevant to microbial disease management in terrestrial ecosystems.

Investigating select substrates on a gut microbial community using an ex vivo fermentation model

Cathy Lordan (Teagasc Food Research Centre), Geoffrey McCarthy (Teagasc Food Research Centre), Rita M. Hickey (Teagasc Food Research Centre), Mark Fenelon (Teagasc Food Research Centre), R. Paul Ross (APC Microbiome Ireland), Paul D. Cotter (APC Microbiome Ireland).

The contribution of the gut microbiota to health and disease is becoming increasingly apparent due to developments in both DNA sequencing and cultivation techniques. There has been a lot of focus on enhancing the growth of desirable microbes, especially bifidobacteria and lactobacilli, using prebiotics, i.e., non-digestible food substrates which are selectively utilised by beneficial bacteria. However, there remains much to be learned about the direct impact that different substrates have on the gut microbiota at the community level. In this study, we employed an ex vivo colonic model to test a range of substrates, including a formulated beverage, in various combinations on a gut microbial community. Ex vivo models provide a reproducible, rapid, and inexpensive means of assessing the colonic microbiota. Samples were obtained at 0h and 24h to establish the impact of these substrates before and after fermentation. Shotgun metagenomic sequencing was applied to unravel the composition and functional changes between time points. A combination of computational approaches were used, including species-level taxonomic classification, functional potential, and the generation of metagenome-assembled genomes. Substrates differed in their impacts on the microbiota, but some consistent patterns were revealed, such as oligosaccharides supporting the increased abundance of bifidobacteria and lactobacilli. Species-level alpha diversity was best maintained with lactose, a whey protein concentrate and xylo-oligosaccharide (XOS) combination, and XOS alone. This provides the basis for additional testing to determine the taxonomic and potential functional effects these substrates have on the gut microbial community.

A method for the enrichment of bacteriocin-associated genes across the bacterial pangenome.
Dave Hourigan (APC Microbiome Ireland, Ireland).

Bacteria rare live in isolation outside of the lab, usually inhabiting challenging ecosystems whilst having distinct ecological roles in their complex communities. Bacteriocins are ribosomally encoded antimicrobial peptides produced by bacteria with both broad and narrow spectrums of activity that can aid competition. Recent advancements in metagenomics have expanded our knowledge of the abundance and diversity of bacteriocin gene clusters whilst potentially giving us greater insight into their ecological role in these complex microbiomes. My research aims to investigate the proximity of genes or gene clusters to bacteriocin gene operons through Protein Family (PFAM) and Gene-Ontology functional enrichment to gain deeper insight into potential links between bacteriocin production and other bacterial properties. The non-redundant (nr) database was mined for lanthipeptide and circular bacteriocin (class IIc) gene clusters using RODEO2, a tool for evaluating the local genomic context of query proteins. PFAM co-localisation networks were constructed using “ggraph” and bacteriocin biosynthetic PFAMs were iteratively removed from the network to uncover PFAMs associated with bacteriocin biosynthetic gene clusters but not part of the core machinery. A negative control dataset was constructed using the refseq database without matches to known bacteriocin gene clusters as determined by blast hit vs BAGEL4 database, consisting of core and modification proteins (e-value; 1e-10). Protein domain and gene ontology enrichment analysis was performed using topGO.

Introducing a CRISPR-Cas9 based prime editing system for precision mutagenesis in lactobacilli

Dieter Vandenheuvel (University of Antwerp), Tom Eilers (University of Antwerp), Jelle Dillen (University of Antwerp), Eline Cauwenberghs (University of Antwerp), Peter Bron (University of Antwerp) & Sarah Lebeer (University of Antwerp).

Introduction: The Lactobacillaceae are key players in different ecological habitats, like food fermentations or important in the healthy state of diverse human, plant, and animal niches. Contrary to the industrial and medical importance of lactobacilli, there is surprisingly little known about the genetic factors behind these beneficial effects. One reason for this can be found in the limited amount of tools available for genetic modification, especially precision mutagenesis. Methods: Here, we employ a recent advancement on the classical CRISPR-Cas method, called 'prime editing'. Prime editing uses an nCas9-reverse transcriptase fusion protein, capable of selectively identifying a genetic target and replacing it with a desired mutation. The system will target two genes, the pili-related spaC gene and the nucleotide related thyA gene, resulting in easily screenable phenotypes. These genes will be knocked out with the introduction of in-frame stop codons. Results: First, the nCas9-reverse transcriptase fusion gene was codon optimized for lactobacilli, and synthetically produced. The fusion gene was cloned into an expression vector for lactobacilli. As a proof of concept, this system will now be introduced in *Lacticaseibacillus rhamnosus* GG, the type strain of the genus and one of the best studied probiotics. Discussion: After successful introduction in this type strain, the system will be introduced in other non-model strains isolated from food and the human vaginal niche in order to facilitate functional molecular studies.

Endolysins targeting the IBD-associated bacterium *Ruminococcus gnavus*

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Introduction: *Ruminococcus gnavus* is a bacterium that has a strong correlation with Inflammatory Bowel Disease (IBD). In IBD patients, an overabundance of *R. gnavus* is associated with increased inflammation. Endolysin (lysin) therapy is a method of targeted microbiome editing. These phage-derived proteins can be used to target specific bacteria in the gut, such as *R. gnavus*, without causing collateral damage to microbiome composition. Lysins are peptidoglycan hydrolases that attack the structure of their host cell wall and result in cell lysis. Methods: Lysin genes were predicted in the genomes of *R. gnavus* temperate phages. Lysins were cloned into *Escherichia coli* for recombinant expression. Lytic activity against *R. gnavus* was assessed by spot assays, turbidity reduction assays, and kill curves. Host range was established using a panel of commensal gut strains. Observation of apparent lysin-resistant mutants of *R. gnavus* led to the generation of true mutants. DNA extraction of these cultures, and Illumina sequencing was used to compare their genomes to that of the sensitive parent strain of *R. gnavus*. Results: All cloned lysins showed activity against *R. gnavus*. Host range analysis was performed on 20 relevant strains. The lysins were specific for *R. gnavus* with some variations. We observed that after 20h there appeared to be growth of lysin-resistant mutants. Whole genome sequencing revealed SNPs in the genomes of these mutants that are associated with the bacterial stringent stress response. Conclusions: This study resulted in the isolation of lysins targeting *R. gnavus*. Future work will assess the potential issues of lysin-resistant mutants, and the viability of these lysins as therapeutics to restore microbiome composition in IBD patients.

Development of a synergistic synbiotic containing arabinoxylan and *Bifidobacterium longum* using in vivo selection

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Colonization and metabolic activity of orally ingested bacteria in the colon rely on competitive ecological and niche-based factors that often limit functionality of commonly used probiotics. Synergistic synbiotics, which involve the parallel administration of a microorganism with its cognate substrate, have the potential to improve persistence and ecological performance of putative probiotic microbes. However, real synergism has not yet been established for synbiotics in human trials, and most synbiotic combinations have not been designed using an approach that accounts for the ecological constraints of the GI tract. Here we use in vivo selection (IVS) to identify strains of Bifidobacterium longum that are adapted toward the utilization of arabinoxylan (AX) in the human gut. To achieve this, bifidobacteria were quantitatively cultured from fecal samples collected during a human trial which showed that a high dose of corn bran AX leads to a significant but highly individualised increase of *B. longum.* Isolates were randomly picked and genotyped by a high throughput gyrB sequencing method. Bacterial counts and strain composition were compared between baseline and week 6, and representatives of B. longum strains enriched in vivo by AX were then tested through in vitro fermentations to investigate their growth on AX and its constituents. Our initial findings showed that strains grew well on the arabinose branches and xylose monomers of AX, but not on xylo-oligosaccharides (XOS). Future work will explore growth on native AX, and involve whole-genome sequencing and comparative genomic analysis of selected strains to characterize the genetic basis of AX degradation. This study demonstrates an ecologically relevant process for selecting improved synbiotic combinations.

Identifying novel probiotic candidates to counter kidney stone disease

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We have recently shown that the gut microbiota derived uremic toxins p-cresyl sulfate, indoxyl sulfate, and their precursors enhance calcium oxalate (CaOx) kidney stone production in vitro and in vivo. As these toxins build-up, they contribute to the production of reactive oxygen species which accelerate stone formation. Probiotic therapies can exploit the wealth of microbial diversity to reduce toxin accumulation and mitigate kidney stone incidence. Using in vitro culture techniques, we identified strains of lactobacilli and bifidobacteria that could resist or reduce uremic toxins in relevant culture media. Toxin clearance was measured using HPLC. To determine if uremic toxin resistance and clearance was associated with the ability to reduce CaOx kidney stone burden in vivo, the strains were assessed individually using an established *Drosophila melanogastor* model. We identified four bifidobacterial strains that could internalize uremic toxins and over ten strains of lactobacilli that could resist high concentrations of the toxins. Oral supplementation of toxin-clearing strains reduced stone burden in flies exposed to uremic toxins compared to controls. We also showed that all the uremic toxins tested increased reactive oxygen species in the Malpighian tubules (i.e., fly kidney) of exposed flies and some strains reduced this oxidative stress. This work highlights why dosing with certain probiotic strains may be clinically useful in kidney stone disease. The oral supplementation of toxin clearing probiotics may be useful in minimizing the incidence and recurrence of CaOx kidney stones and should be evaluated in humans. In addition to reducing stone burden, these strains have the potential to normalize the dysbiotic gut microbiota observed in stone formers.

Orphan nisin immunity genes are widespread across the Bacillota

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Nisin is the prototypical lantibiotic, widely utilized as a food preservative and often suggested as an alternative to antibiotics. Nisin resistance proteins can be found in some pathogens and are a potential limiting factor for the use of nisin and nisin producing probiotics as therapeutics. The gut derived *Streptococcus hyointestinalis* DPC6484 produces nisin H but was thought to lack a nisin immunity system. However, it is highly resistant to diverse nisin variants, warranting further investigation. *S. hyointestinalis* DPC6484 was fully sequenced and the resulting 2.32 Mb genome was subject to BLASTp with known nisin resistance and immunity protein sequences. This identified a 5.2kb region encoding a lantibiotic immunity protein (nshI), transporter (nshFP), and a two-component regulator (nshRK) located elsewhere on the genome. The predicted genes were expressed in *Lactococcus lactis* MG1614 where they were found to confer resistance to nisin A. Protein BLAST and gene neighbouring algorithms were used to determine the prevalence of this novel resistance cluster, identifying three instances across Streptococcus genomes. A further 70 clusters were detected encoding nisin immunity (lanI), transport (lanFEG) and regulation (lanRK) genes spread throughout the phylum Bacillota, including the gut phyla Lachnospiraceae, Clostridaceae, Lactobacillaceae, Oscillospiraceae, Eubacteraceae and Streptococcaceae. Structures of putative protein sequences were predicted using Alphafold2 and found to be similar to known nisin variant immunity proteins. The unexpected prevalence of nisin immunity and resistance genes across the phylum Bacillota suggests a central role for nisin in the gut microbiome and may impact the potential application of nisin and nisin producers as bio-therapeutics.

Protocol for the chemo-gut trial: a double-blind randomized controlled trial investigating the effects of a multi-strain probiotic on gut microbiota, gastrointestinal symptoms, and psychosocial health in cancer survivors

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Background: Survivors of cancer experience chronic gastrointestinal (GI) and psychosocial symptoms, and reduced gut microbial diversity. This may compromise therapy compliance and reduce wellbeing. No studies have investigated probiotics for managing GI and psychosocial symptoms and the gut microbiota in post-treatment cancer survivors. Aims: to investigate the effects of a probiotic vs. placebo on (1) abdominal pain and depressive symptoms (primary outcomes); (2a) GI (i.e. gas/bloating, diarrhea, constipation) and psychosocial symptoms (i.e. anxiety, cognitive function, fatigue, and general health; and (2b) gut microbiota composition; (3) relationships between microbiota, GI and psychosocial symptoms. Methods: This double-blinded, placebo-controlled, 2-arm, randomized trial will recruit N=66 participants for a 12-week trial. Adult survivors diagnosed with a solid tumour or blood cancer who completed chemotherapy, and show elevated GI or psychosocial symptoms will be included. The probiotic capsule contains *Lactobacillus helveticus* Rosell®-52, *Bifidobacterium longum* Rosell®-175 and *Lacticaseibacillus rhamnosus* Rosell®-11 strains ingested orally once daily. Stool samples are collected at baseline and week-12 and analyzed using GA-Map dysbiosis test and 16s rRNA gene sequencing. GI and psychosocial surveys are completed at baseline, weeks 6 and 12. Descriptive statistics, paired samples t-tests, linear mixed models, and Spearman's correlation analyses will be used. Implications: Our findings may improve symptom management and treatment adherence, while our commitment to patient-centered knowledge translation via creating patient materials (e.g. infographics, personal results summary) will enable patients to make informed decisions about their health.

Dietary inulin modulates host iron utilization and gut microbiota in high-iron milk formula fed neonatal piglets

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Iron nutrition imposes impacts on the mammalian physiology and gut microbiota, and host-microbe interactions are thought to be important in early-life development. Iron supplementation or fortification is a common method to resolve iron deficiency (ID) or iron deficiency anemia (IDA). However, iron-fortified infant formula provides excessive amount of iron compared to breastmilk and may have adverse effects on infant growth and development. The gut health is implicated in the adverse outcomes of neonatal iron supplementation, and dietary iron overexposure may exacerbate inflammation and dysbiosis because iron is an essential nutrient for bacterial growth and function and modulates gut microbiota. This study examined the efficacy of dietary inulin and inulin in combination with *Ligilactobacillus agilis* YZ050 (*L. agilis* YZ050) on the host iron utilization and gut microbiota in response to iron-fortified milk diet in a neonatal piglet model with the four dietary treatments: an iron-adequate formula (AI), a high-iron formula (HI), the HI formula supplemented with inulin (HIP), the HIP formula with *L. agilis* YZ050 (HIS). While HI formula resulted in hepatic iron overload and increased iron concentration in the colon digesta and feces, use of HIP and HIS formulas diminished iron deposition in the liver, colon digesta, and feces in piglets. We observed significantly different gut microbial clusters between non-inulin (AI and HI) and inulin (HIP and HIS) diet piglets and similar shifts in gut microbiota in HIP and HIS. Our findings shed light on appropriate use of dietary supplements and insights needed to purposely modulate gut microbiota via establishment of beneficial microbes linked to inulin and *L. agilis* YZ050 to mitigate risks of systemic iron overload at infancy.

An examination of the collateral damage caused to the gut microbiome by antimicrobials using an ex vivo distal colon model

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Introduction: Antimicrobials are commonly ingested by humans as food preservatives (sodium benzoate, potassium sorbate, sodium nitrite, sodium sulfite), antibiotics (fidaxomicin and vancomycin), bacteriocins (nisin and thuricin CD), and pesticides (glyphosate). Collateral damage to the gut can be caused when antimicrobials exhibit a broad range of activity. This study examines the antimicrobials listed above and the level of disruption they cause to the gut microbiome. Methods: MIC's were determined for the pharmaceuticals against a range of antibiotic resistant and gut bacteria, and for the food preservatives and pesticide against various Lactobacillus strains. These in vitro experiments were done to determine which concentration would be used to treat the micro-Matrix™ mini fermentation system (an ex vivo model of the distal colon). Each well of the micro-Matrix was treated with a different antimicrobial and incubated for 24 hrs. DNA was extracted from samples and sent for whole metagenomic sequencing from which absolute abundance was determined. Results: All food preservatives were minimally active against all Lactobacillus strains tested. Glyphosate was not active against any strain at the concentrations tested. Thuricin CD exhibited low MIC's (<3.1 µg/ mL) for C. difficile and B. firmus. Whereas fidaxomicin, vancomycin and nisin demonstrated lower MIC's for all other strains tested when compared to thuricin CD. These results were mirrored in the micro-Matrix™ system. Conclusion: All pharmaceuticals, except for thuricin CD, demonstrated a significant degree of collateral damage to the gut. In contrast, while all food preservatives exhibited activity against commensal gut bacteria, this activity was not observed at concentrations reflective of that found in food.

***Lactiplantibacillus plantarum* Plantaricin EF is a probiotic effector that protects barrier function in intestinal epithelial cells through an intracellular cation-linked mechanism**

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The two-peptide bacteriocin, plantaricin EF (PlnEF), made by *Lactiplantibacillus plantarum* is a potent antimicrobial with bactericidal activity due to its capacity to disrupt intracellular cation homeostasis. Recently, we showed that PlnEF elicits responses in intestinal epithelial cells that attenuate the effects of proinflammatory cytokines-mediated disruptions of intestinal epithelial cell function in a Caco-2 transwell model. To determine whether PlnEF alters intestinal cells in a manner similar to that observed for sensitive bacteria, we quantified the intracellular cation concentrations in Caco-2 cells using inductively coupled plasma mass spectrometry (ICP-MS). Exposure to 500 nM PlnEF for 32 h resulted in a significant 23.01% (2.11 x 10^10 to 1.62 x 10^10 atoms/cell) and 24.51% (2.48 x 10^11 to 1.87 x 10^11 atoms/cell) reduction in Mg2+ and K+, respectively. These changes were consistent with the 2- to 8-fold increase in expression of two genes encoding the divalent cation transport proteins, CNNM2 and CNNM4, which have an affinity for Mg2+ and homology to a putative binding site on the bacterial magnesium efflux protein CorC. Moreover, PlnEF treatment (500 nM) increased the expression of Caco-2 genes encoding tight junction proteins, CLDN1, CLDN3, CLDN4, occludin (OCLN), and ZO-1 and reduced the expression of pore-forming CLDN2 over time (reaching at least a 2-fold difference). These findings suggest that PlnEF is a probiotic effector that protects intestinal epithelial barrier integrity through a mechanism involving intracellular cation homeostasis.

The gut microbiome, mild cognitive impairment, and probiotics: a randomized clinical trial in middle-aged and older adults

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Advancing age coincides with changes in the gut microbiome and a decline in cognitive ability. Psychobiotics are microbiota-targeted interventions that can result in mental health benefits and protect the aging brain. This study investigated the gut microbiome composition and predicted microbial functional pathways of middle-aged and older adults that met criteria for mild cognitive impairment (MCI), compared to neurologically healthy individuals, and investigated the impact of probiotic *Lactobacillus rhamnosus GG* (LGG) in a double-blind, placebo-controlled, randomized clinical trial. A total of 169 community-dwelling middle-aged (52-59 years) and older adults (60-75 years) received a three-month intervention and were randomized to probiotic and placebo groups. Participants were further subdivided based on cognitive status into groups with intact or impaired cognition and samples were collected at baseline and post supplementation. Results: Microbiome analysis identified *Prevotella ruminicola*, *Bacteroides thetaiotaomicron*, and *Bacteroides xylanisolvens* as taxa correlated with MCI. Differential abundance analysis at baseline identified *Prevotella* as significantly more prevalent in MCI subjects compared to cognitively intact subjects (ALDEx2 P= 0.0017, ANCOM-BC P= 0.0004). A decrease in the relative abundance of the genus *Prevotella* and *Dehalobacterium* in response to LGG supplementation in the MCI group was correlated with an improved cognitive score. Our study points to specific members of the gut microbiota correlated with cognitive performance in middle-aged and older adults. Should findings be replicated, these taxa could be used as key early indicators of MCI and manipulated by probiotics, prebiotics, and symbiotics to promote successful cognitive aging.

Man's best friend: potentially novel antimicrobial compounds isolated from bacterial strains of canine source.

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Antimicrobial resistance (AMR) is a major risk to human and animal health requiring urgent attention. A contributing factor to AMR is the misuse/overuse of antibiotics adding to the development and spread of resistance mechanisms by pathogens. Greater efforts are needed not only to prevent additional AMR development but to reassess and identify alternative therapeutic options. One group of compounds that hold great promise are antimicrobial peptides (AMPs). AMPs produced by bacteria are termed bacteriocins, which can kill or inhibit bacterial strains closely-related or non-related to the producing strain. Notably, a diverse community of trillions of commensal bacteria inhabit the mucosal and epidermal surfaces of humans and animals and can be found in a multitude of sites; including the mouth, nose, skin, ears and intestines, many producing novel bacteriocins. Given their potent antimicrobial activity against pathogenic bacteria, immunomodulatory effects on their hosts, auto-regulation of their own production and self-immunity exhibited by the producing bacteria to their own peptides, this vast resource of bacteriocins and their beneficial characteristics show their potential as alternatives to traditional antibiotics in the fight against AMR. This study involves screening multiple sites from five canines in a bid to identify novel antimicrobial peptides which target a range of clinically relevant bacteria with a focus on drug-resistant pathogens particularly from veterinary settings.

Investigating the effects of the infant probiotic *Bifidobacterium infantis* and human milk oligosaccharides on the severity of anaphylaxis in a mouse model of peanut allergy

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Interactions between gut microbes and early-life immune programming may influence the development of food allergies, providing a potential explanation for the rapidly increasing prevalence of pediatric peanut allergy. Supplementation with Bifidobacterium has been shown to induce oral tolerance in conventional mouse models of food allergy; however, it has not been tested in mice harboring an early-life human microbiome. We hypothesized that treatment with *B. infantis* EVC001 + human milk oligosaccharides (HMO) would limit the severity of anaphylaxis in an infant microbiome-associated mouse model of peanut allergy and expand regulatory T cells (Treg). Germ-free mice received a human infant microbiome lacking Bifidobacterium and were then orally sensitized to peanut for five weeks. Beginning at colonization, mice were gavaged daily with either 1 x 10^8 CFU *B. infantis* or PBS and given drinking water with or without 5% w/v of the HMO Lacto-N-neotetraose (LNnT) for the duration of the study. Treatment with *B. infantis* + LNnT significantly decreased fecal pH and cecal acetic acid levels, increased abundances of *B. infantis* and lowered anaphylactic scores after challenge compared to sensitized mice treated only with *B. infantis*. No differences were observed in plasma levels of mast cell protease-1, total IgE, and peanut-specific IgE or in splenic Treg numbers. These results suggest that supplementation with LNnT enhances the ability of *B. infantis* to limit innate immune responses related to anaphylaxis but may not alter the peripheral adaptive immune response to peanut tested here. Moreover, our infant microbiome-associated mouse model of peanut allergy provides a novel framework for testing the efficacy of live therapeutic strategies in limiting allergic responses.

In vitro assessment of bacteriocins as microbiome modulators in a simplified human intestinal microbiota

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Bacteriocins are antimicrobial peptides produced by bacteria of many genera that have been studied for decades as food bio-preservatives or as alternatives to antibiotics. Bacteriocins are often narrow spectrum and can kill target organisms without causing collateral damage to other bacterial populations. That is why bacteriocins are gaining credibility as precise modulators of the human microbiome. However, rigorous evidence is required to determine if bacteriocins can act as microbiome-editing tools to shape communities in a desirable direction. The aim of this project is to assess the effect of different bacteriocins on a Simplified Human Intestinal Microbiota (SIHUMI), using a set of bacteriocin-producing strains (Bac+) and their corresponding isogenic non-producers (Bac-). Bacteriocins from different classes and spectrum of activity were selected, including lantibiotics and pediocin-like peptides. SIHUMI is a bacterial consortium of seven diverse human gut species that can be individually tracked in a complex media using qPCR. The Bac+ and Bac- strains were superimposed on the SIHUMI system, and samples were taken at intervals up to 48 h for genomic DNA extraction. The genome copy number of each SIHUMI member was evaluated using specific primers. We were able to determine the behavior of the consortium over time and evaluate how the system is impacted by different bacteriocin producers. Our results show that it is possible to shape the composition of the community in a predictable way by targeting multiple members or specific members with either broad or narrow spectrum bacteriocins. While we recognize that SIHUMI is a simplified model, it provides useful insights into the possible mechanisms by which the microbiome could be shaped by bacteriocins.

Healthy aging: Molecular bases for the development of a bioinnovative food prototype with psychobiotics

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One consequence of the increase in longevity is the appearance of diseases associated with aging such as dementia. Alzheimer's disease (AD) is the most common type of dementia. Currently, there is no definitive treatment for AD, cholinesterase inhibitors and memantine are the current mainstays of the treatment. Numerous nutritional interventions for AD are currently under study. To date, there is scientific evidence on the use of psychobiotics, those probiotics that provide a potential benefit to mental health. This work represent the first report on the daily oral administration (30 days) of *Lactobacillus delbrueckii* subsp. lactis CRL 581 (1x10E8), an in vitro AChE inhibitor and *Levilactobacillus brevis* CRL 2013 (1x 10E9), a GABA producer strain, on oxidative stress and cholinergic dysfunction in a scopolamine-mice model. Scopolamine, a cholinergic receptor blocker, produced memory loss, cognitive impairment and increased AChE activity, mimicking those alterations observed in AD. Administration of CRL581 showed a decrease in AChE activity in brain homogenates of scopolamine-treated mice; CRL2013 increased catalase activity and the amount of reduced glutathione. In addition, both strains were able to reduce malondialdehyde, an end-product of lipid peroxidation, considered to be one of the markers of reactive oxygen species generation. Shot-gun proteomic analysis of scopolamine- and psychobiotics-brain homogenates revealed unique differential expression patterns. Our results show that both strains evaluated here ameliorate oxidative stress markers in a scopolamine-mice model supporting the development of a functional supplement containing genetically and functionally characterized psychobiotics for non-pharmacological intervention of those affected with AD.

In vitro effects of human milk oligosaccharides (HMOs) on gut microbiota in irritable bowel syndrome (IBS)

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IBS is the most common gastrointestinal disorder in the Western world and a major public health concern. Despite extensive research, the psychological and physiological factors that contribute to the aetiology of IBS remain poorly understood. Recent evidence has presented HMOs as a potential treatment for IBS symptoms. We hypothesise that gut bacteria are integral contributors to cognitive as well as intestinal health, and that dietary interventions such as HMOs, which are known to positively alter the gut microbiota, have the potential to improve symptoms in IBS patients. This study will assess the impact of different HMOs on gut microbiota to streamline a novel IBS therapy. An in vitro HMO intervention, using a lab human gut model system is used to assess the potential therapeutic modulation of the microbiota in IBS. Molecular phenotyping using gas chromatography (GC) and fluorescent in situ hybridisation of fermentation samples provides assessment of impact of the intervention on gut microbial composition and activity. All 3 subtypes of IBS are under investigation, and one taken forward to a human study. Preliminary results for two healthy controls, two IBS-D, an IBS-C and an IBS-M donor show that the HMOs Lacto-N-tetraose, Lacto-N-neotetraose (LNnT) and 3'-sialyllactose have a bifidogenic effect. An HMO mix increases bifidobacteria in both healthy donors, IBS-D and IBS-C. Additionally, preliminary GC results show that in IBS-M, LNnT and 2'-fucosyllactose are the most successful in stimulating butyrate production. HMO supplementation shows promising results in altering the gut microbiota. Various HMOs produce different prebiotic effects on bacterial counts and short chain fatty acids and their potential in IBS needs to be confirmed in a prospective clinical trial.

Mining prebiotic active molecules using genetic analysis of plant foods with newly developed aims platform (automated in vitro microbiome screening)

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The human gut microbiome has the capacity for metabolizing fibers, lipids, proteins, polyphenols and other molecular components and plays critical roles in gut health and wellness. The abundance of these, frequently uncharacterized, microbiome-active components vary within individual plant foods. To identify and characterize potential prebiotic active molecules that alter the composition and function of the human gut microbiome, we developed a high throughput AiMS platform (Automated in vitro Microbiome Screening). AiMS platform has the capacity to screen hundreds of different grains via in vitro digestion and fermentation in a high throughput manner. The platform also leverages the vast diversity of prebiotic-active molecules that are naturally present in cereal grains. One such grain is sorghum, one of the most widely consumed grains in the developing world. Accordingly, we used the AiMS platform to conduct in vitro fermentations with 294 sorghum recombinant inbred lines as fermentation substrates. After sequencing and quantitative trait loci (QTL) mapping, we successfully identified 10 loci in the sorghum genome that were associated with variation in the abundance of microbial taxa and/or microbial metabolites. Two loci co-localized with sorghum genes involved in regulating the biosynthesis of condensed tannins. Many plant polyphenols, including condensed tannins have recently been recognized to have prebiotic potential. Genetic analysis in near isogenic lines and molecular complementation showed that condensed tannins stimulate the growth of Faecalibacterium. Our work illustrates the potential for genetic analysis to systematically discover and characterize novel molecular components with prebiotic potential in plant foods.

Metabolism of human milk oligosaccharides by infant gut microbiota

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The third largest solid component of human milk is a set of structurally diverse, complex carbohydrates known as human milk oligosaccharides (HMOs). Following consumption, HMOs enter the colon undigested, performing several functions critical to gut microbiota development. However, because of challenges in obtaining HMOs, research into the impact of HMO metabolism on microbial community structure and function is limited. Regardless, commercially available infant formula is already being supplemented with artificial 2-fucosyllactose (2-FL), a highly abundant HMO structure produced by most women. The aim of this study was to characterize the impact of pooled HMOs (pHMOs) and 2-FL on the composition and function of infant fecal-derived microbial communities. Seven communities were seeded from infant stool in bioreactors designed to mimic the conditions of the human distal colon. Steady-state communities were treatment with 4 g/L pHMOs, 0.5 g/L 2-FL, or a control under batch fermentation conditions. Degradation of 19 HMO structures was evaluated by HPLC-glycoprofiling while resultant changes in composition and metabolic output were assessed using metataxonomics (16S rRNA gene sequencing) and metabonomics (NMR) respectively. Despite the heterogeneity in composition, all communities maintained the capacity to degrade the majority of detectable HMO structures. Metataxonomic and metabonomic profiling demonstrated significant shifts in the composition and metabolic output of the communities as a result of pHMOs-treatment compared to 2-FL and the control, while 2-FL yielded results similar to the control. Overall, this study has considerably expanded our knowledge of HMO gut microbiota interactions and has provided deeper insights into the addition of HMOs to infant formula.

Identification of novel immunomodulatory components in *Lacticaseibacillus rhamnosus* GG

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Introduction: Recently, probiotic lactic acid bacteria have been reported to have numerous functional properties, but it has been pointed out that their effects vary greatly among individuals. We previously applied ribosome engineering to *Lacticaseibacillus rhamnosus* GG to enhance its probiotic functions; one of the obtained mutants, MTK56N, showed enhanced immunomodulatory activity. In the present study, we attempted to identify the immunomodulatory components of MTK56N, and to elucidate the mechanism of action. Methods: We examined the bacterial lavage fluid of MTK56N, and constructed a genetically modified lactic acid bacteria (gmLAB) that produced chaperone protein DnaK (DnaK) and 60 kDa chaperonin (GroEL), which were suspected to be the responsible proteins. The immunomodulatory effects of the purified proteins were investigated using RAW264.7. In addition, RAW264.7 was incubated with each protein, and the expression levels of cytokine-encoding mRNAs and cytokine secretion were analyzed by RT-qPCR and ELISA, respectively. Results: Western blotting analysis confirmed that the constructed gmLAB expressed recombinant proteins in a nisin-stimulation-dependent manner. The levels of the pro-inflammatory cytokines TNF-Î± and IL-6 were significantly increased when the purified proteins were added. Moreover, the ELISA results indicated that each purified protein enhanced TNF-Î± and IL-6 secretion into the supernatant. Discussion: Our results indicated that the purified recombinant DnaK and GroEL proteins promoted the expression of TNF-α and IL-6 and exerted immunomodulatory effects in RAW264.7. Thus, DnaK and GroEL appear to be immunomodulatory components of MTK56N. We plan to elucidate the mechanisms of action of these bacterial surface proteins in future studies.

Immunomodulatory effects of galacto-oligosaccharides

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Food allergies in infancy have become a growing public health concern. According to the CDC, food allergies are estimated to impact over 8% of children in the US. Galacto-oligosaccharides (GOS), structurally similar to human milk oligosaccharides, modulate the gut microbiota and have beneficial effects on host health. In an early pilot experiment, GOS feeding resulted in non-statistically significant reductions in peanut-specific IgE, anaphylaxis, intestinal permeability, and increased relative abundance of *Akkermansia* and *Bifidobacterium* in peanut-sensitized CC027/GeniUnc mice. Here, we showed in mice and primary human intestinal cells that GOS induced the expression of Lgals-1, which encodes Galectin-1 (Gal-1), a glycoprotein with immunomodulatory effects and an established role in suppressing allergic asthma. We first confirmed differential expression of Lgals-1 in mice fed GOS compared with control diets by reverse transcription-quantitative (q) PCR. GOS significantly increased Lgals-1 expression in the colon of young but not old mice. Computational molecular docking predictions confirmed the binding affinity between GOS and Gal-1, suggesting a direct interaction. We also showed that GOS interacted directly with human primary intestinal cells inducing Lgals-1 expression. Additionally, we showed that GOS induced Lgals-1 expression in the human intestinal cells by enhancing *Bifidobacterium*, providing an indirect induction pathway of Lgals-1 by GOS. Our study lays the groundwork for mechanistic research exploring a potential prebiotic role in modulating the immune system, specifically for preventing or treating food allergies.

