

Annual Scientific Meeting Cork, Ireland • July 9-11, 2024

2024 MEETING GUIDE

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- Industry Advisory Committee Representatives
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On behalf of the ISAPP board, I'd like to welcome you to ISAPP's 2024 annual scientific meeting in Cork. This year's meeting is in an ideal location, given that Ireland – and especially Cork – has been a powerhouse in microbiome and biotics research over the past decade and continues to be on the cutting edge.

The field of biotic science has continued to grow in momentum this year, with ISAPP driving forward progress on important scientific questions and debates across many areas of biotic science. Projects over the past year have

included advancing a shared concept of postbiotics, developing criteria for prebiotic evaluation, evaluating the data on microbiome restoration with probiotics, as well as outputs addressing probiotic safety and the associations of live dietary microbes and fermented foods with health outcomes. We strive to advance the science in this field as well as provide timely and consistent messages about the science, and we thank all of those who have collaborated with us over the past year to share in the advancement of this mission.

Over the past year I was also happy to serve as ISAPP President during an important transition to our new Executive Director, Marla Cunningham. Transition is never easy, especially when an organization has had only one leader. Marla has stepped in and filled some big shoes and ISAPP has continued to move forward. I am confident with Marla's leadership and the Board's guidance that ISAPP will continue to fulfill our mission of advancing and translating the science of biotics.

Being part of the Board and being chosen as President is not an honor I took lightly. It was one of the privileges of my blessed career that I could play a small part in helping us achieve our mission. The learning environment of ISAPP is something I have seldom witnessed in my career. At the end of this meeting, I'll be welcoming Prof. Maria Marco into the role of President. Maria is a born leader, who has continued to push the board and me to think about what we want from ISAPP. I am excited to see where she helps take us in the next 3 years and am confident it will be to amazing places.

Daniel Merenstein, ISAPP President



It is my great pleasure to also extend a warm welcome to all of our new and returning members of the ISAPP community joining us here in Cork. The past year has personally been immensely rewarding for me in this new capacity working with the diverse and passion ate people who work and collaborate with the ISAPP organisation. Thank you to each and every one of you who has shared your time, support, collaboration and input over the past year.



This year for ISAPP's annual meeting we welcome close to 300 scientists from across the world working in the probiotic, prebiotic, postbiotic, synbiotic,

microbiome and fermented food fields. As a unique feature for this meeting, we have provided an open registration opportunity for scientists both new and familiar to ISAPP to participate in proceedings on days two and three of the meeting. The mixed meeting format this year provides delegates with enhanced opportunities to connect and learn from an expanded network of scientists and clinicians from academic, student, government and industry backgrounds, including many local attendees from the onsite University College Cork and APC Microbiome Ireland.

On day one of the meeting, we commence with a pre-meeting program organised by our industry member representatives. These pre-meeting activities provide an opportunity for members to engage with ISAPP board, bring together industry and student/fellow member scientists for interactive innovation-focused workshops, and address the issue of scientific requirements for new biotic substances with a regulatory-focused panel. The afternoon session will welcome invited academic and industry member experts to lead discussions and share perspectives at our concurrent discussion groups, advancing key questions in important and timely topics in the field. The evening welcome reception provides an opportunity for all delegates to gather and connect in the historic university Aula Maxima, with canapes, refreshments and optional campus tours available.

On day two, open meeting delegates will join for the meeting sessions, with a full day of stimulating and thought-provoking content. We will hear from scientists sharing their insights across a broad range of topics including mapping of microbiome transmission in early life, results from soil-based biotic interventions, sustainability implications of biotics in agriculture, as well as detailed insights into elusive areas of intestinal functionality such as the mucosal interface and small intestine. Through an expert panel of stakeholder perspectives, we will explore current challenges in the use of probiotics in preterm infants and priorities for scientific and clinical progress. Throughout the day, 2024 ISAPP award recipients will share their respective work on isolating bioactive fermented food components, exploring microbial mediators of gut-brain axis modulation, and implementing a large scale community probiotic program. Our poster session showcases 80+ contributions from across our assembled community of academic, student and industry scientists, providing delegates with a unique opportunity to gain insights into the work of leading labs across the globe. The day concludes with our popular and thought-provoking late breaking news session, showcasing new data and perspectives in an energy-filled session. Our evening social event at the local Cork City Gaol promises to be an evening of fun, sharing in local cuisine and musical talent, and perhaps even some Irish dancing.

On our concluding morning, featured talks from our industry and student members as well as highlights from top experts in gut-skin and gut-brain axis interactions start the morning. The meeting closes with the much anticipated sharing of perspectives and learnings from day one discussion groups.

ISAPP sincerely thanks each of you for joining our community in Cork this year to share, learn and collaborate in advancing biotic science with us, and wish you an inspiring and productive meeting ahead.



A warm welcome to all of you to Cork for the ISAPP 2024 meeting! We at APC Microbiome Ireland, based in University College Cork, are thrilled to host this event once again.

Last year, APC celebrated its 20th birthday, marking us as one of the oldest and largest microbiome research centres globally. To commemorate this milestone, we launched a 20-Year Impact booklet, highlighting our significant contributions across scientific, economic, and societal domains. We were honoured by the presence of An Tánaiste (Ireland's Deputy Prime Minister) Michael Martin TD at the celebration.

Our impact extends beyond academia. An econometric analysis revealed that APC returns €6.5 for every €1 invested by the State, thanks largely to our success in spinning out companies and our extensive research partnerships with industry.

APC has a long-standing and close relationship with ISAPP. In many ways, we have grown up together. Majority of our investigators, research staff, and postgraduate students are actively involved with the Association, contributing to its management, organization, and policy setting in the field. Furthermore, APC and ISAPP share numerous industry partners who value the knowledge base and opinion leadership that ISAPP provides.

I encourage you to make the most of your time in Cork and enjoy the conference. The program promises to be exciting, covering many of the hot topics and challenges in microbiome science. As a native of Cork, I can assure you there's plenty to see and do around the city. Take some time to explore, and please join us for a drink at the reception, compliments of the APC!

Paul Ross, Director, APC Microbiome





2024 ANNUAL MEETING PROGRAM

All program events will be held on campus at University College Cork in the Western Gateway Building (WGB) in the main auditorium (WGBG05) unless otherwise noted below. *Room numbers* are indicated in the left panel. *Abbreviations: IAC=Industry Advisory Committee (representatives of member companies); SFA=Students and Fellows Association*

TUESDAY JULY 9

Closed meeting for invited guests and industry members

07:30 - 13:00	Registration desk open
Pre-meeting pr 08:30 - 11:30:	ogram Open only to IAC, SFA and Board of Directors
08:30 - 09:15	IAC and Board of Directors meeting
09:15 - 10:45 WGBG02	IAC/SFA Innovation workshops (separate sign-up required). 1: Innovation in the gut-brain axis and potential role of biotics Mariya Petrova, Winclove Probiotics
WGBG15	2: Best practice in designing studies with biotics Shalome Bassett, Fonterra
WGBG04	3: Novel techniques in biotic research Cathy Lordan, Teagasc and APC Microbiome Ireland
WGBG18	4: Innovation outside the gut (and environmental applications) for biotics Brendan Daisley, University of Guelph
10:45 - 11:30 Atrium Cafe	Networking break Board & SFA IAC
11:30 - 12:30	Industry forum. From lab to market: Scientific requirements for new biotic substances in the changing regulatory landscape Bruno Pot, Yakult Europe BV, Vrije Universiteit Brussels, Belgium Diane Hoffmann, University of Maryland, USA Sarah Lebeer, University of Antwerp, Belgium Alison Winger, Novonesis, Ireland
12:30 - 13:15	Lunch break

2024 ANNUAL MEETING PROGRAM

13:15 - 17:30	Discussion groups (concurrent sessions) Open only to invited experts and IAC.
WGBG02	1: Characterization and quantification of postbiotics Gabriel Vinderola, National University of Litoral, Argentina and Seppo Salminen, University of Turku, Finland
WGBG04	2: How can we establish causal mediation in microbiome intervention studies? Daniel Tancredi, University of California, Davis, USA and Kristin Verbeke, Katholieke Universiteit Leuven, Belgium
WGBG16	3: The microbiome and neurodegenerative and neurodevelopmental disorders Eamonn Quigley, The Methodist Hospital and Weill Cornell School of Medicine, USA and Hania Szajewska, The Medical University of Warsaw, Poland
WGBG15	4: Evidence for candidate prebiotics, including polyphenols, resistant starch, and animal-derived substances Karen Scott, University of Aberdeen, UK and Kelly Swanson, University of Illinois at Urbana-Champaign, USA
WGBG18	5: How does digestion affect prebiotic and probiotic function? Anisha Wijeyesekera, University of Reading, UK and Maria Marco, University of California, Davis, USA
WGBG17	6: Next-generation probiotics by implementation of genetic engineering and other tools Sarah Lebeer, University of Antwerp, Belgium and Colin Hill, University College Cork, Ireland
17:30 - 20:00 Aula Maxima	Welcome reception. Aula Maxima, University College Cork In association with APC Microbiome Ireland. Includes Welcome to University College Cork from Paul Ross, APC Microbiome Ireland, 18:15 - 18:30.
	Social event with light refreshments. Open registration delegates are welcome to attend.

WEDNESDAY JULY 10

Open registration meeting

07:30 - 08:30	Registration desk open
08:30 - 08:35	Welcome
08:35 - 09:05	Health-associations in soil-based intervention trials – a probiotic and postbiotic perspective Aki Sinkkonen, University of Helsinki, Finland
09:05 - 09:35	Microbiota-human mucin interactions: Identification of key enzymes to prevent mucus barrier dysfunction. Ana Luis, University of Gothenburg, Sweden

2024 ANNUAL MEETING PROGRAM

09:35 - 09:55	The Sanders Award for Advancing Biotic Science 2024 Lecture: Yogurito: Challenges and achievements of a probiotic social assistance program Maria Pía Taranto, National Scientific and Technical Research Council (CERELA-CONICET), Argentina
09:55 - 10:25	Break
10:25 - 10:55	Mother-baby transmission of bifidobacterial strains: insights and prospects, Douwe van Sinderen, University College Cork, Ireland
10:55 - 12:25	Special Session: Probiotics and premature infants - Perspectives and paths forward Geoffrey Preidis, Baylor College of Medicine and Texas Children's Hospital, USA Mark Underwood, Providence Sacred Heart Medical Center and Children's Hospital, USA Hania Szajewska, The Medical University of Warsaw, Poland Janet Berrington, Newcastle Upon Tyne Hospitals NHS Foundation Trust, UK Marie Spruce, NEC UK Charity, UK Diane Hoffmann, University of Maryland, USA Greg Leyer, Biotic Solutions Consulting, USA
12:25 - 13:25	Lunch break
13:25 - 14:50 Atrium, WGBG14	Poster viewing and SFA poster judging. Authors will be present for all posters.
14:50 - 15:20	Developing models for the human small intestinal microbiota KC Huang, Stanford University, California, USA
15:20 - 15:50	The environmental implications of biotic use in agricultural animals Steven Ricke, University of Wisconsin-Madison, USA
15:50 - 16:10	The Glenn Gibson Early Career Researcher Award 2024 Lecture: Eat your (fiber-rich, fermentable) veg? A translational take on short-chain fatty acids as microbiota-gut-brain axis mediators in humans Boushra Dalile, Katholieke Universiteit Leuven, Belgium
16:10 - 16:30	The Gregor Reid Award for Outstanding Scholars in Developing Nations 2024 Lecture: Peptidome insights: Mining bioactive peptides in fermented dairy and non-dairy products in the Indian Himalayan Region Rounak Chourasia, National Agri-food Biotechnology Institute, India
16:30 - 17:30	Late Breaking News Gregor Reid, University of Western Ontario, Canada
17:30 - 18:30	Break
18:30 - 22:00 Cork City Gaol	Gala social event, Cork City Gaol, Convent Avenue, Sunday's Well, Cork City Tickets available for purchase for open registration delegates.

Program

2024 ANNUAL MEETING PROGRAM

THURSDAY JULY 11

Open registration meeting

09:00 - 09:15	IAC highlight: Human gut-associated Bifidobacterium species salvage exogenous indole, a uremic toxin precursor, to synthesize indole-3-lactic acid via tryptophan Toshitaka Odamaki, Morinaga Milk Industry Co Ltd, Japan
09:15 - 09:30	IAC highlight: An intestinal screening platform versus a clinical crossover intervention study: comparative evaluation with a dietary fiber mixture Frank Schuren, Netherlands Organization for Applied Scientific research (TNO), the Netherlands
09:30 - 09:45	SFA highlight: A multi-faceted exploration of lactobacillaceae-derived vitamin B2 in the vagina Caroline Dricot, University of Antwerp, Belgium
09:45 - 10:00	SFA highlight: Impact of probiotic yoghurt on gut microbiome dynamics: insights from in-vitro fermentation and metabolic profiling Choshani Dalukdeniya Arachchilage, Sabaragamuwa University of Sri Lanka
10:00 - 10:05	Announcement of poster award winners
10:05 - 10:35	Lactic acid bacteria and the gut-skin axis - a paradigm with therapeutic implications Catherine O'Neill, University of Manchester, UK
10:35 - 11:00	Break
11:00 - 11:30	Microbiome-gut-brain axis in health and disease - parsing causality John Cryan, University College Cork, Ireland
11:30 - 13:00	Summary reports from Discussion groups SFA: Students and Fellows Association report. Cathy Lordan, Teagasc, Ireland DG1: Characterization and quantification of postbiotics DG2: How can we establish causal mediation in microbiome intervention studies? DG3: The microbiome and neurodegenerative and neurodevelopmental disorders DG4: Evidence for candidate prebiotics, including polyphenols, resistant starch, and animal-derived substances DG5: How does digestion affect prebiotic and probiotic function? DG6: Next-generation probiotics by implementation of genetic engineering and other tools
13:00	Close

Program

2024 ANNUAL MEETING PROGRAM



Western Gateway Building to Aula Maxima - Reception venue



Western Gateway Building to Cork City Gaol - Gala venue

July 9 - 09:15-10:45 (concurrent workshops) Open only to SFA and IAC.

Innovation is critical to tackling the many scientific and clinical challenges facing the biotics field today. This session enables Industry Advisory Committee (IAC) and Students and Fellows Association (SFA) members to share their respective insights from their research and development work on the cutting edge of innovation across four highly topical areas.

Workshop 1: Innovation in the gut-brain axis and potential role of biotics

Chaired by Mariya Petrova, PhD, Winclove Probiotics, Netherlands

The last decades have marked biotic research expansion beyond traditional gut-focused context and gutrelated conditions. In fact, research focusing on the beneficial effects of biotics beyond the gut is growing exponentially. The gut-brain axis is one of the most prominent areas for biotic intervention and, in this way, benefits human health. This session will explore groundbreaking research in the area of the gut-brain axis, focus on novel aspects, and provide a future perspective regarding the potential role of biotics linked to the gut-brain axis.

Workshop 2: Best practice in designing studies with biotics

Chaired by Shalome Bassett, PhD, Fonterra, New Zealand

Significant time and resource go into clinical studies but sometimes the outcomes can't be commercialised, or IP can't be captured because of simple omissions, disclosures, or other factors. This session will focus on the key considerations that need to be made when designing clinical studies with biotics with a view to creating a valuable checklist for researchers. It will also explore the use of cutting-edge technologies such as wearables, and novel sampling devices and their potential to maximise trial outcomes.

Workshop 3: Novel techniques in biotic research

Chaired by Cathy Lordan, PhD, Teagasc and APC Microbiome Ireland

This session will involve discussing the latest advancements and methodologies related to biotics research. We will explore how different techniques and tools such as high-throughput bioreactor models, sequencing technology, computational analysis approaches, metabolic modelling and gut-on-a-chip applications are being employed to further investigate microbes and microbial communities.

Workshop 4: Innovation outside the gut (and environmental applications) for biotics

Chaired by Brendan Daisley, PhD, University of Guelph

This session will explore the expansive applications of biotics beyond traditional gut-focused contexts, with a spotlight on groundbreaking research and innovations relevant to respiratory and dermatological fields, as well as emerging uses of biotics in OneHealth approaches and environmental sustainability.



July 9 - 11:30-12:30 Open only to invited experts, SFA and IAC.

From lab to market: Scientific requirements for new biotic substances in the changing regulatory landscape

The regulatory landscape around novel biotic substances remains a hot topic with multiple unanswered questions. This session will address the impact of regulatory requirements on scientific development programs for new substance approvals, including novel probiotic strains and new prebiotic substances across food and medicinal frameworks. Although requirements differ among jurisdictions, some questions are common to many frameworks, including how to address safety, product identity, quality and efficacy. The session will focus on the scientific relevance behind various international requirements for new substance approvals and explore expert insights into incorporating regulatory requirements in study design.

Industry Forum Speakers:



Bruno Pot, Yakult Europe BV, Vrije Universiteit Brussels, Belgium

Bruno Pot completed his PhD in microbiology at the University of Gent, Belgium. In subsequent postdocs he performed research on lactic acid bacteria. In 1997 he joined the science department of the company Yakult as science manager Benelux. He worked as Research Director at the Institut Pasteur in Lille, France, from 2001 till 2016. During that time he was also Director of Business Development at the bioinformatics company Applied Maths NV and Food Microbiology Professor at the Vrije Universiteit Brussel. His major research topics have been bioinformatics, lactic acid bacteria, probiotics, bacteria-host interaction and health claim substantiation. Since November 2016 Bruno is back with Yakult as Science Director for Europe. Bruno Pot is member of the Taxonomic Subcommittee for *Lactobacillus, Bifidobacterium* and related taxa, former ISAPP Board member, former president of the Pharmabiotic Research Institute (PRI; currently VP), Narbonne, France, Board member of LABIP and Chair of the Board of ILSI-EU. He authored or co-authored >250 articles and >20 book chapters. More details on <u>https://www.researchgate.net/profile/Bruno-Pot</u>.



Diane Hoffmann, University of Maryland, USA

See <u>page 22</u> for bio.



Sarah Lebeer, University of Antwerp, Belgium

See <u>page 97</u> for bio.



Alison Winger, Novonesis, Ireland

Dr Alison Winger is the Head of Product Management in Human Health Biosolutions and Country Manager - UK/Ireland at Novonesis. Originally from New Zealand, Alison completed her PhD in Biochemistry from the University of Western Australia and went on to hold a number of academic research positions around the world before moving to Ireland in 2014. Since then she has been in senior leadership roles in various biotechnology and pharmaceutical companies before joining Novonesis (previously Novozymes) in 2021. Alison is author of a number of publications, including three enzyme patents and nine peer-reviewed papers in the fields of enzymes and probiotics. Alison is the 2024 Women in STEM diversity ambassador for Women Mean Business (WMB), a platform that supports and celebrates women in business in Ireland.



July 9 - 13:15-17:30 (concurrent sessions) Open only to invited experts and IAC.

Group 1: Characterization and quantification of postbiotics

Chaired by: Gabriel Vinderola PhD, National University of Litoral, Argentina and Seppo Salminen PhD, University of Turku, Finland

Postbiotics are defined in the ISAPP consensus definition as 'a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host'. Subsequent to the release of this definition, questions have been raised about how to quantify postbiotics according to this definition, and recommendations made that clarification of the approach to active components and their measurement is required for implementation in scientific and regulatory circles.

It is clear that characterization of postbiotic products may be challenging, especially with increased complexity that arises by use of multiple inanimate strains, inclusion of metabolic end-products, and the presence of whole and fragmented cells. In this sense, there is some uncertainty among stakeholders about postbiotics and how to implement the consensus definition. There is a need to clarify how to quantify postbiotics. The aim of this Discussion Group is to convene academic experts and industry scientists to discuss specific key issues about production, characterization, quantification and stability of postbiotics for practical and regulatory purposes, to produce a technical paper providing examples about how some postbiotic products are produced/characterized/quantified commercially and make some general recommendations about how to quantify postbiotics. We aim to discuss postbiotics ranging from "simple products" (just an inanimate single strain) to more complex formulations (a mixture of inanimate strains or cell fragments, plus metabolites); how processes are controlled to allow reproducibility; and to propose methodologic tools to characterize and quantify postbiotics, either at a single timepoint or over the duration of shelf life.



Group 2: How can we establish causal mediation in microbiome intervention studies?

Chaired by: Daniel Tancredi PhD, University of California, Davis, United States and Kristin Verbeke PhD, Katholieke Universiteit Leuven, Belgium

Many intervention studies targeting the microbiome have associated eventual changes in microbial composition or function to changes in a particular health outcome. Such associations do not necessarily establish that the microbiome change that was caused by the intervention was itself the cause of the eventual effect on the particular health outcome, because one has to be able to rule out confounding, reverse causality, and reporting bias as potential alternative explanations. Although randomization of a potential cause is a powerful technique for controlling for confounding of a simple cause/effect relationship, randomizing the upstream intervention does not by itself eliminate confounding of downstream cause-effect linkages.

Hence, demonstrating causal mediation is difficult and requires clever experimental design and analysis strategies to chain together two or more cause/effect relationships. In the context of microbiome intervention studies, the level of difficulty can be even more severe, due to the microbiome measurements potentially being high-dimensional and/or compositional. Additionally, absent the possibility of randomizing both the intervention and the microbiome manipulation, ruling out all potential confounders may require deeper systems knowledge of the microbiome than is currently available. Nevertheless, the establishment of causal mediation is important to advance the field and, potentially, to support health claims involving mediation, including those involving whether an intervention fulfills the definition of a prebiotic.

In this discussion group, we will discuss recent progress and remaining research gaps for causal mediation studies involving the microbiome. Our goal is to understand how to improve the quality of the evidence for a claimed mechanistic linkage involving the microbiome as a mediator of health effects caused by a biotic intervention. We will discuss experimental designs and analysis strategies aimed at causal mediation that can be adapted for use in microbiome intervention studies. We will consider how the overall evidence for a mediated causal linkage might be enhanced by experimental and observational studies involving animal models, genetic epidemiology, and systems biology.

Group 3: The microbiome and neurodegenerative and neurodevelopmental disorders

Chaired by: Eamonn Quigley MD, The Methodist Hospital and Weill Cornell School of Medicine, Texas, USA and Hania Szajewska MD, PhD, The Medical University of Warsaw, Poland

This workshop will address a very topical area – microbiome-CNS interactions – with the aim to explore how insights from microbiome research can be translated into clinical strategies for managing neuropsychiatric or neurodegenerative diseases. Topics for discussion will include advances in our understanding of the role of the microbiome in the development of the central nervous system and the intriguing issue of how the microbiome communicates with the CNS. We will explore how these microbiome-gut-brain connections/interactions impact on the human brain, as well as how interventions that modulate the microbiome can ameliorate neurodegenerative and neurodevelopmental disorders and related symptoms, such as altered mood.

The session will focus on bridging the gap between research and practice, investigating the application of microbiome study findings in real-world clinical settings to improve the management and treatment of these diseases. Relatively common disorders such as autism and Parkinson's disease will serve as examples of how microbiome-CNS interactions are being explored and insights gained.



Group 4: Evidence for candidate prebiotics, including polyphenols, resistant starch, and animalderived substances.

Chaired by: Karen Scott PhD, University of Aberdeen, Scotland and Kelly Swanson PhD, University of Illinois at Urbana-Champaign, Illinois, USA

The number of substances widely known in the prebiotic category has not changed greatly since the launch of prebiotics in 1995, namely FOS, inulin, and GOS. This is despite many publications describing the impact of a variety of different substances on the gut microbiota and subsequently on health, hence potentially acting as prebiotics. This discussion group will investigate the reasons behind this and explore what is required for this list to be expanded to include additional substances with described beneficial effects on health, mediated via the microbiota. We will discuss the existing evidence for specific 'candidate' prebiotics and establish how they fit the criteria and thus may move from candidate to accepted prebiotics, within the guidelines of the current definition. We will also try and outline a usable infographic outlining this descriptive process.

Group 5: How does digestion affect prebiotic and probiotic function?

Chaired by: Anisha Wijeyesekera PhD, University of Reading, United Kingdom and Maria Marco PhD, University of California, Davis, USA

Recent advances have revealed fascinating insights into digestive processes and the mechanisms through which 'biotics exert their effects. The further elucidation of some of these complex mechanisms (for example, physical and chemical (acid/bile) digestive processes) and the impact they have on diet and dietary substrates provide a step change in our understanding of the effects of digestion on 'biotics function. Other recent advances include the development of analytical approaches to uncover structural and functional characteristics of the intestinal environment and gut microbiota that may be exploited for personalised medicine. This includes technological innovation in the areas of ingestible in situ sampling and delivery devices for collection of novel spatial and temporal data from the gut environment. By better understanding gastrointestinal digestive physiology and inter-individual variations will pave the way to detect, monitor, treat and ultimately improve health outcomes.

These innovative analytical advances demonstrate huge potential for enhancing the viability and efficacy of 'biotics. This discussion group will review the latest science in this area, and discuss, debate and conclude on how digestion affects prebiotic and probiotic function, and recommendations of future research priorities.



Group 6: Next-generation probiotics by implementation of genetic engineering and other tools

Chaired by: Sarah Lebeer PhD, University of Antwerp, Belgium, Colin Hill PhD, DSc, University College Cork, Ireland

During the last two decades, the diversity of microbial strains and species that are being explored as probiotics is steadily increasing. At the same time, the genetic tools to precisely genome engineer and improve the functionality of specific microbial strains and whole communities are rapidly advancing. However, such next-generation probiotics and tools will not be rapidly followed by the necessary regulatory updates to allow market entry of these novel approaches.

In this discussion group, the following aspects will be discussed.

- What are potential benefits and pitfalls of such next-generation probiotics?
- Is the probiotic field waiting for such innovations? For which conditions and problems?
- Will genetically modified probiotics only have potential in the medical field but not in food and food supplements?
- What are the functional targets for GMO modifications how to determine particular microbial functions/traits to optimise for, what factors should be considered?
- Considering single microbe design vs ecosystem/consortia design
- Engineering safety beyond single microbe traits, considering safety in the ecosystem and foreseeing unintended effects



July 9 - 13:15-14:05 Oral Presentations (open only to SFA)

Chaired by: David Hourigan, APC Microbiome Ireland, Ireland and Breanna Metras, University of Minnesota Twin Cities, USA

Microbial metabolic responsiveness varies by individual and may be optimized by testing prebiotic combinations.

Alexander Thorman, PhD, University of Cincinnati, USA

Probiotic lactic acid bacteria associated with fermented millet based milk beverage (Brukina) and effects on the gut microbiome.

Bless Hodasi, University of Ghana, Ghana

Unlocking the Benefits of Probiotics for Bone Health - Evidence from Preclinical and Clinical Experiment.

Iskander Harahap, Poznan University of Life Sciences, Poland

isolateR: an R package for generating microbial libraries from Sanger sequencing data with potential for discovery of novel probiotic candidates.

Brendan Daisley, PhD, University of Guelph, Canada

July 9 - 14:05-15:00 Breakout Group Discussions (open only to SFA)

Chaired by: Sarah Ahannach, PhD, University of Antwerp, Belgium and Patricia Sanz Morales, University of Reading, UK

Group 1: Probiotics and the concept of using beneficial microbes for health

Group 2: Prebiotics and the concept of using beneficial substrates for health

Group 3: Microbiota and related bioinformatic analysis

July 9 - 15:15-15:55 Oral Presentation (open only to SFA)

Researching gut bugs for a living – a crap story. Glenn Gibson, PhD, University of Reading, UK

All Roads Lead to Rome: lessons learnt from a chequered career path.

Shalome Bassett, PhD, Fonterra, New Zealand





Aki Sinkkonen, University of Helsinki, Finland

Principal Scientist, Doc., Ph. D. Aki Sinkkonen has published over 90 international, peerreviewed papers in periodicals and book chapters in the fields of environmental ecology and medical sciences. He is a main and co-inventor in four patents. His group has been a global frontrunner in exposing urban dwellers to rich soil microbiota in intervention trials. The findings indicate that rich soil microbiota shifts commensal microbiota and improves immune regulation in intervention trials and in leukocyte studies. In this lecture, Dr. Sinkkonen presents the main findings and discusses the potential efficacy of certain environmental microbial taxa as pro- and postbiotics.

July 10 - 08:35-09:05

Health-associations in soil-based intervention trials – a probiotic and postbiotic perspective

Abstract: Frequent nature contacts have been associated with low-incidence of several noncommunicable diseases in comparative studies. Together with collaborators, my group has performed several intervention trials in which volunteers were exposed to microbially diverse nature elements. When we rewilded daycare yards, we found associations between skin Gammaproteobacteria and immune modulation in the intervention but not in the control group. We also did two placebo-controlled, randomized trials. In the first one, children were supervised to play for two weeks in microbially rich sand or microbially poor placebo sand. In the second one, urban volunteers cultivated vegetables indoors for four weeks in microbially rich soil or microbially poor placebo soil. In both trials, immune modulation was enhanced in the intervention but not in the placebo group. We have also explored the associations between green space and exposure to environmental microbiota. In our trials and exploratory studies, the key taxa have consisted of classes and even phyla. Only in rare cases, we found associations between certain strains and immune response, although the evidence was not particularly strong.

These findings support the role of environmental microbiota in immune modulation. Since soil microbiota consists mostly of slow-growing strains, our findings may also form a challenge to current practices to identify new probiotics. In the context of postbiotics, we did mouse trials in which we compared the effects of live and inactivated microbiota on mouse immune system. Despite minor differences, mice reacted to both live and inactivated soil similarly. This supports the use of rich soil microbiota as a postbiotic treatment in future studies. In parallel, we are currently running trials that target to find out whether rich soil microbiota reduces the incidence of certain non-communicable diseases.



Ana Luis, University of Gothenburg, Sweden

Dr. Ana Luis obtained her PhD at Newcastle University, UK. During this time, she studied the mechanisms of pectin degradation by the human gut microbiota. At the end of her PhD, she was awarded a Marie-Currie fellowship to develop her postdoctoral research at University of Michigan, USA, and University of Gothenburg, Sweden. During this time her research interests shift to mucin O-glycan active enzymes.

Since 2022, she has been an independent researcher at the Mucin Biology Groups at the University of Gothenburg, Sweden. Her research group is focused on the functional and structural characterization of enzymes and carbohydrate-binding modules in order to understand the mechanisms of mucin utilization by gut bacteria and the role of bacterial binding proteins in gut colonization. The goal of her research is to understand the basic mechanisms of mucin interactions.

July 10 - 09:05-09:35

Microbiota-human mucin interactions: Identification of key enzymes to prevent mucus barrier dysfunction.

Abstract: see next page



Abstract: The gastrointestinal mucus layer provides a critical barrier that separates gut microbes from the intestinal epithelium. Mucus is mainly composed of mucins glycoproteins containing ~10² different O-linked glycan structures. Some microbiota members are able to utilize colonic O-glycans. The combination of increased mucin degrading bacteria and the corresponding disruption of the mucus barrier have been proposed to promote inflammatory bowel disease (IBD). *Bacteroides thetaiotaomicron (B. theta*), a dominant member of human microbiota, has numerous Polysaccharide Utilization Loci (PULs) encoding dozens of predicted mucin-degradation enzymes. Significantly, the enzymatic mechanisms of mucin degradation by this and other gut bacteria remain unclear.

We hypothesized that "early" steps in depolymerization of O-glycans exist, which could block downstream metabolism of mucin glycans and may represent drug targets to block mucus degradation by the microbiota. Using biochemical and genetic approaches, we disclosed the first model of colonic O-glycan depolymerization by a single human gut bacterium. We established that utilization of O-glycans by *B. theta* can require the sequential action of at least 36 enzymes [glycoside hydrolases (GHs) and sulfatases]. Unexpectedly, *in vivo* studies of *B. theta* mutants revealed that multiple exo-active enzymes act as key enzymes in O-glycan utilization and have a major role in gut colonization. Overall, the characterization of the model of degradation O-glycans provides novel insights into the mechanism of mucin degradation by the microbiota allowing the identification of potential drug targets in the treatment of IBD.

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The Sanders Award for Advancing Biotic Science 2024 Lecture

María Pía Taranto, National Scientific and Technical Research Council (CERELA-CONICET), Argentina

Dr. Maria Pia Taranto PhD is a biochemist and Principal Investigator at the National Council for Scientific and Technical Research (CONICET) in Tucumán, Argentina. She is Co-Director of the PhD program in Biological Sciences at the National University of Tucumán (UNT), and Technical Director and Coordinator of the Yogurito Escolar Social Project and related projects. Dr. Taranto carries out her work at the Reference Center for Lactobacilli (CERELA). A specialist in probiotics, postbiotics, and synbiotics, she is experienced in basic and applied research on lactic acid bacteria as regulators of hyperlipidemia and producers of nutraceuticals. She has been involved in the design and development of probiotic products and has extensive expertise in technology transfer as well as biotechnological development between the public and private sectors.

July 10 - 09:35-09:55

Yogurito: Challenges and achievements of a probiotic social assistance program

Abstract: The YOGURITO program is an example of the application of functional nutrition using platforms of public policy. It was created in Tucumán-Argentina in 2008 when severe cases of child malnutrition motivated a group of scientists from CONICET-CERELA to give answer to society through Science and Technology.

This Program consists of the use of a probiotic strain belonging to the CONICET-CERELA Culture Collection, L. rhamnosus CRL 1505, for the development of functional foods (probiotic yogurt Yogurito, probiotic chocolate milk Chocolet, probiotic cheese QuesoBio) and a functional bioingredient (Biosec) for the improvement of the health and nutritional status of malnourished children. The CRL 1505 strain has numerous advanced scientific studies on immunostimulant capacity. The consumption of functional products stimulates the child's immune system through different mechanisms due to the action of the probiotic strain, generating an increase in natural defenses. In this way, the appearance (number and severity) of respiratory and intestinal infections that are more prevalent in childhood is reduced, a situation that is aggravated in vulnerable populations.





Since its implementation in 2010, the Program has remained active benefiting approximately 300,000 school children per year who attend 580 public schools and receive the benefits of the CRL 1505 strain during the school period (March-December). In almost 20 years of existence, this project has allowed millions of children and adolescents to access quality functional foods with strong scientific support. This has a high social impact since schoolchildren are better nourished and healthy and have greater intellectual development, allowing them equal opportunities in the future. Based on modern innovation systems, the YOGURITO program is a paradigm of interaction between the scientific, government, production and community sectors.



Douwe van Sinderen, University College Cork, Ireland

Prof. Douwe van Sinderen PhD is Full Professor of Molecular Microbiology in the School of Microbiology and a Principal Investigator and Founding member of APC Microbiome Ireland, both at University College Cork, Ireland.

Research interests include bifidobacterial functional and comparative genomics, in particular pertaining to microbe-host interactions and carbohydrate metabolism. He is the co-author of nearly 600 peer-reviewed publications, has contributed to 17 book chapters, has acted as co-editor for 2 books, and is listed as an inventor on 24 patents.

July 10 - 10:25-10:55

Mother-baby transmission of bifidobacterial strains: insights and prospects

Abstract: This presentation will provide an account on the phenomenon of microbial strain transfer between mother and baby, focussing specifically on bifidobacteria in a cohort of 135 mother-infant dyads¹. Extensive strain sharing is documented using a combination of metagenomic and cultivation methods and factors are identified that affect such apparent strain transfer. Furthermore, nearly 500 bifidobacterial strains were isolated from this cohort and their associated genome sequences allow insights into their genetic diversity and an exploration of their particular properties, some of which will be highlighted here^{2,3}.

¹Feehily et al., Nat Commun 14: 3015 (2023)
²Friess et al., Gut Microbes, in revision (2024)
³Sanchez Gallardo et al., submitted for publication



July 10 - 10:55-12:25

Special Session: Probiotics and premature infants- Perspectives and paths forward

A large body of literature exists on the use of probiotics in hospitalized preterm infants, with particular focus on the prevention of necrotising enterocolitis. At least 85 randomised clinical trials have evaluated the use of probiotics for this indication with systematic reviews and meta-analyses finding significant reductions in mortality and morbidity. However, probiotic administration is not without risk, esp. in vulnerable populations, and rare case reports of probiotic- and contaminant-induced sepsis have resulted in varying recommendations and responses from clinical organisations and regulatory bodies, and resulting disparity in neonatal clinical care practices internationally. This expert panel will explore the current state of practice and key barriers across the scientific, clinical, legal, regulatory and industry spheres, with a view to identifying key obstacles and priorities for advancement of science and coherent clinical application in this patient population.

This special session includes the following speakers:



Geoffrey Preidis, Baylor College of Medicine and Texas Children's Hospital, USA

See <u>page 98</u> for bio



Mark Underwood, Providence Sacred Heart Medical Center and Children's Hospital, USA

Dr. Mark Underwood, MD received his undergraduate degree in Italian from Brigham Young University and then attended the University of Texas Southwestern Medical School in Dallas followed by pediatric residency at UCLA. After 13 years as a general pediatrician in Montana and New Zealand, he completed a fellowship in neonatology at UC Davis and then joined the faculty there in 2006 and became the division chief in 2014. He retired from UC Davis in 2021 and now provides neonatal care in Spokane Washington. His research focuses predominantly on necrotizing enterocolitis, human milk oligosaccharides, the intestinal microbiome and probiotics. He also enjoys international collaborations and has taught and provided care across Africa, Central and Eastern Asia, Eastern Europe, South America and the Middle East.



Hania Szajewska, Medical University of Warsaw, Poland

See <u>page 99</u> for bio



Janet Berrington, Newcastle Upon Tyne Hospitals NHS Foundation Trust, UK

Dr. Janet Berrington MD is a neonatal consultant in Newcastle, UK. She has a background in neonatal immunology research in which she completed her MD. Her major research interests are necrotising enterocolitis and the role of the microbiome, human milk and constituents including IgA and human milk oligosaccharides. She is also the manager of the Great North Neonatal Biobank – a tissue and sample repository of more than 1000 infants less than 32 weeks gestation. This facilitates much of the translational research that she has undertaken alongside the Stewart Lab, and is open to external researchers to apply to use. She has also undertaken large randomised controlled trials in neonates and was a co-applicant on the SIFT, ELFIN, MAGPIE and AZTEC studies amongst others.





Marie Spruce, NEC UK Charity, UK

Biography coming soon.



Diane Hoffmann, University of Maryland, USA

Prof. Diane E. Hoffmann, JD MS, is the Jacob A. France Professor of Health Law, Distinguished University Professor, and Director of the Law & Health Care Program at the University of Maryland Carey School of Law. She received her law degree from Harvard Law School and her Master's degree from Harvard School of Public Health. She is currently Chair of the Maryland Stem Cell Commission, Co-Chair of the US Association for the Study of Pain (USASP) Advocacy Committee, a member of the Editorial Board of the Journal of Medical Regulation and a member of the Scientific Advisory Board for the Center for Gut Microbiome Research & Education at the American Gastroenterological Association. She is the recipient of three NIH grants, most recently an R01 from the National Human Genome Research Institute to evaluate the regulatory framework for direct-to-consumer microbiome-based tests. Hoffmann has published numerous articles on health law and policy issues in legal journals as well as scientific and medical journals including Science, JAMA, the NEJM, and the Annals of Internal Medicine.



Greg Leyer, Biotic Solutions Consulting, USA

Dr. Greg Leyer received his PhD from the University of Wisconsin – Madison from the Department of Food Microbiology and Toxicology in 1993 whereafter he began a 30-year career in the 'biotic' sciences. He spent five years at Abbott Nutrition developing innovative probiotic solutions for adults and children in the medical and pediatric nutrition space. Greg then held several probiotic-focused senior research and business development positions in a 15-year career at Danisco – DuPont. In 2013, Greg joined forces to acquire UAS Laboratories, a probiotic-focused dietary supplement company, where he held the role of Chief Scientific Officer with broad responsibilities while onboarding a state-of-the-art microbial fermentation and freeze-drying facility. In 2020, Chr. Hansen acquired UAS Labs and Greg fulfilled the role of Sr. Director –Scientific Affairs within the Human Health business unit until the end of 2023. Greg is now an independent consultant assisting a variety of companies in the 'biotic' space, and throughout his career has published multiple papers in the areas of probiotic clinical benefits, application know-how, and microbial safety parameters.





KC Huang, Stanford University, California, USA

Prof. KC Huang PhD was an undergraduate Physics and Mathematics major in Page House at Caltech, and spent a year as a Churchill Scholar at Cambridge University working with Dr. Guna Rajagopal on Quantum Monte Carlo simulations of water cluster formation. He received his PhD from MIT working with Prof. John Joannopoulos on electromagnetic flux localization in polaritonic photonic crystals and the control of melting at semiconductor surfaces using nanoscale coatings. During a short summer internship at NEC Research Labs, he became interested in self-organization in biological systems, and moved on to a postdoc with Prof. Ned Wingreen in the Department of Molecular Biology at Princeton working on the relationships among cell shape detection, determination, and maintenance in bacteria. His lab is currently situated in the departments of Bioengineering and Microbiology & Immunology at Stanford, and his current interests include cell division, membrane organization, cell wall biogenesis, and the organizational principles of bacterial communities. He has been director of the Biophysics Graduate Program since 2015, and the chair of the DEI committee for the Aspen Center for Physics board.

July 10 - 14:50-15:20

Developing models for the human small intestinal microbiota

Abstract: Our understanding of region-specific microbial function within the gut is limited due to reliance on stool. Using our recently developed capsule device, we exploit regional sampling from the human intestines to develop models for interrogating small intestine (SI) microbiota composition and function. In vitro culturing of human intestinal contents produced stable, representative communities that robustly colonize the SI of germ-free mice. During mouse colonization, the combination of SI and stool microbes altered gut microbiota composition, functional capacity, and response to diet, resulting in increased diversity and reproducibility of SI colonization relative to stool microbes alone. Using a diverse strain library representative of the human SI microbiota, we constructed defined communities with taxa that largely exhibited the expected regional preferences. Response to a fiber-deficient diet was region-specific and reflected strain-specific fiber-processing and host mucus-degrading capabilities, suggesting that dietary fiber is critical for maintaining SI microbiota and its role in disease and dietary responses.



Steven Ricke, University of Wisconsin-Madison, Wisconsin, USA

Dr. Steven C. Ricke PhD received his B.S. and M.S. from the Univ. of Illinois, Champaign-Urbana, IL. and Ph.D. from the Univ. of Wisconsin, Madison, WI. Dr. Ricke was a USDA-ARS postdoctorate in the Microbiology Department at North Carolina State Univ. then joined Texas A&M Univ. as a professor in the Poultry Science Dept. In 2005, he became the first holder of the new Donald "Buddy" Wray Endowed Chair in Food Safety and Director of the Center for Food Safety at the University of Arkansas (UA) and was a faculty member of the Dept. of Food Science and Cellular/ Molecular Graduate program. In 2020 he became the Director of the Meat Science and Animal Biologics Discovery Program in the Animal and Dairy Sciences Dept. at the University of Wisconsin-Madison. Dr. Ricke conducts studies on the growth, survival, and pathogenesis of pathogens in the poultry gut and their interactions with gut microbiota.

July 10 - 15:20-15:50

The environmental implications of biotic use in agricultural animals

Abstract: There is an increasing need to manage the environmental impact of agricultural livestock more effectively. The impact on land and water ecosystems by animal agriculture can occur in a multitude of direct and indirect routes and pathways. Nitrogen and phosphorous emissions from animals can directly contaminate waterways and ground water as well as indirectly when animal manure and poultry litter are applied as fertilizers to the soil.



Animals can also be a source of gaseous emissions such as ruminants producing methane that enters the atmosphere as a greenhouse gas. Food animals harbor a wide range of pathogens in their gastrointestinal tracts including most of the foodborne pathogens of public concern such as pathogenic *Escherichia coli, Salmonella* spp., and *Campylobacter* spp. that can be introduced to soils and water reservoirs. The introduction of biotic-based feed additives represents a potentially complex series of environmental outcomes depending on the animal and the type of biotic. Some outcomes are relatively straightforward. For example, an obvious benefit of biotic compounds is their respective ability to reduce pathogen colonization and in turn, reduce their entry into the environment. Others are less obvious. While administration of certain biotic compounds to improve feed efficiency can lead to decreases in waste emissions from the animal, they may have unintended negative consequences if nutritional formulation is not re-calibrated to account for the improved digestive efficiency. In summary, several factors must be taken into account when including biotic additives into animal diets to determine the environmental consequences.



The Glenn Gibson Early Career Researcher Award 2024 Lecture Boushra Dalile, KU Leuven, Belgium

Dr. Boushra Dalile PhD is a Postdoctoral Researcher at the Laboratory of Biological Psychology at KU Leuven, Belgium. She was trained in psychology (Swinburne University of Technology, Australia) and cognitive neuroscience (University of Skövde, Sweden; The Max Planck Institute for Human Cognitive and Brain Sciences, Germany), before being awarded a PhD in Biomedical Sciences in 2021 at the Translational Research Center for Gastrointestinal Disorders at KU Leuven under supervision of Prof. Kristin Verbeke. Since her PhD, she investigates the effects of dietary fiber and the role of short-chain fatty acids (SCFAs) on stress and anxiety, and is currently mapping out their putative mechanisms of action in humans. Her latest research seeks to harness butyrate's neuro-psychopharmacological potential in modulating learning and memory to advance translational research on anxiety and help shape treatment options and dietary recommendations. Her work was published in Nature Reviews Gastroenterology & Hepatology, The Lancet Planetary Health, Neuropsychopharmacology, and Psychoneuroendocrinology.

July 10 - 15:50-16:10

Eat Your (Fiber-rich, Fermentable) Veg? A Translational Take on Short-Chain Fatty Acids as Microbiota-Gut-Brain Axis Mediators in Humans

Abstract: The gut microbiota can produce and modify neuroactive metabolites and thereby impact brain function via the bidirectional microbiota-gut-brain axis. Short-chain fatty acids (SCFAs) are the major products of dietary fiber fermentation by the gut bacteria in the colon and are one of the most studied neuroactive bacterial metabolites in rodents. However, their role in modulating psychobiological processes that underlie the development of stress- and anxiety-related disorders is scarcely studied in humans. Over the past years, we have harnessed the use of capsules with a pH-dependent coating to deliver SCFAs via the oral route directly to the colon, in a precise, chronic, and non-invasive manner, which allows mimicking the fermentation of dietary fiber by gut bacteria into SCFAs. This talk will highlight the results of three RCTs that address the effects of colonic SCFA administration and the modulation of their circulating concentrations in healthy participants on stress responses, fear conditioning and extinction, and subjective mood-related outcomes. Furthermore, the exploration of unstimulated interindividual differences in circulating SCFAs and their associations with the above-mentioned outcomes from the pooled sample of healthy participants will be presented.





The Gregor Reid Award for Outstanding Scholars in Developing Nations 2024 Lecture Rounak Chourasia, National Agri-food Biotechnology Institute, Mohali, Punjab, India

Dr. Rounak Chourasia PhD currently serves as a Research Associate at the National Agri-food Biotechnology Institute (NABI) in Mohali, India. He earned his PhD in Biotechnology in 2023 under the mentorship of Dr. Amit Kumar Rai and Prof. Dinabandhu Sahoo and achieved first rank in both his BSc and MSc (Microbiology) from the University of North Bengal. Rounak dedicated his Ph.D. work towards functional enhancement of traditional chhurpi cheese of the Sikkim Himalayas. He devised a method to produce milk chhurpi using specific lactic acid bacterial strains, unveiling novel bioactive peptides with potential nutraceutical benefits. Additionally, he developed a bioactive peptides-enriched novel soybean cheese tailored for individuals with lactose intolerance and provided a foundation towards entrepreneurial opportunities for local farmers. Presently, his research focuses on evaluating the metagenomics and metabolomics of traditional fermented millet foods in India, with the goal of creating functional fermented millet products. With 18 published papers, seven book chapters, an average impact factor of 5.6, and over 600 citations, his contributions have been widely recognized. Rounak received the Anna University - Centre for Biotechnology (AU-CBT) Excellence Award in 2021 and The Association of Microbiologists of India (AMI) Young Scientist Award in 2023, highlighting his commitment to advancing fermented foods research.

<u>July 10 - 16:10-16:30</u>

Peptidome insights: Mining bioactive peptides in fermented dairy and non-dairy products in the Indian Himalayan Region

Abstract: Traditional fermented foods, renowned for their multifaceted health benefits derived from bioactive compounds, face challenges related to the presence of antibioticresistant and pathogenic microbes, and the inability to economically produce chhurpi throughout the year. To address this, we utilized native lactic acid bacteria (LAB) isolated from various stages of traditional Sikkim Himalayan chhurpi production as defined starters for controlled milk fermentation. This process resulted in chhurpi with notable antioxidant and ACE inhibitory activity. Expanding the spectrum, we developed a novel soy chhurpi cheese using selected LAB strains, catering to lactose-intolerant individuals. The controlled fermentation using Lactobacillus delbrueckii WS4 as a starter yielded outstanding results in terms of chhurpi and soy chhurpi production, showcasing high peptide content, antioxidant activity, and ACE inhibition. Simulated in vitro gastrointestinal digestion augmented peptide content, enhancing the functional attributes of both dairy and non-dairy cheeses. Whey and soy whey demonstrated comparable activities, indicating the potential utility of byproducts in the nutraceutical and functional food industry. Peptidome analysis unveiled several bioactive peptides in the fermented foods. Noteworthy multifunctional peptides, such as YQEPVLGPVR and PVVVPPFLQPE, were identified in undigested chhurpi, GI-digested chhurpi, and whey. In soy chhurpi, nine antioxidant and ACE inhibitory peptides were identified, with high potential for novel bioactive peptides. In silico bioactivity prognosis further supported the potential of chhurpi, soy chhurpi, and their GI digests in the functional food industry. Subsequent synthesis and in vitro analysis validated the antioxidant and ACE inhibitory activity of specific peptides from *chhurpi* (HPHPHLSFM, LKPTPEGDL) and sov chhurpi (SVIKPPTDE, SFLVPPQESQ). Chhurpi peptides demonstrated a non-competitive type mixed inhibition, while soy *chhurpi* peptides acted as competitive inhibitors of ACE. Notably, HPHPHLSFM and SVIKPPTDE were identified as true inhibitors, escaping GI digestion and displaying high therapeutic potential. While promising, these findings warrant further validation through animal studies and clinical trials. Successful validation could establish L. delbrueckii WS4 chhurpi and soy chhurpi as robust functional food options, offering North-East India affordable, nutritious sustenance while also fostering entrepreneurial opportunities and socioeconomic advancement within the local population. Additionally, the identification of bioactive peptides presents an opportunity to develop specialized nutraceutical formulations.





IAC Highlight

Toshitaka Odamaki, Morinaga Milk Industry Co Ltd, Japan

Toshitaka Odamaki is a professional affiliated with Morinaga Milk Industry Co., Ltd., specializing in the research of gut microbiota and probiotics. He holds a PhD from the University of Tokyo. His career at Morinaga Milk Industry has seen him progress from a factory role finally to a senior research scientist. He currently serves as a Section Head. His academic contributions extend to Juntendo University, University of Tokyo, Kansai University, and Kyoto University. He has also spent time as a company-sponsored research trainee at RIKEN, Japan, and a researcher at APC Microbiome Institute, Ireland. He is currently a Visiting Professor at Kyoto University.

July 11 - 09:00-09:15

Human gut-associated Bifidobacterium species salvage exogenous indole, a uremic toxin precursor, to synthesize indole-3-lactic acid via tryptophan

Abstract: Indole, produced in the gut from dietary tryptophan via bacterial tryptophan-indole lyase, not only instigates biofilm formation and antibiotic resistance in gut microbes, but also contributes to kidney dysfunction progression post-intestinal absorption and liver sulfation. Given that tryptophan is a vital amino acid for humans, these occurrences appear unavoidable. However, our proof-of-concept study demonstrates that exogenous indole can be transformed into an immunomodulatory tryptophan metabolite, indole-3-lactic acid (ILA), through an unexplored microbial metabolic pathway involving tryptophan synthase β subunit and aromatic lactate dehydrogenase. Selected bifidobacterial strains were shown to convert exogenous indole to ILA via tryptophan (Trp), as evidenced by incubating bacterial cells with (2-13C)-labelled indole and L-serine. Disrupting the genes responsible led to varying effects on the efficiency of indole bioconversion to Trp and ILA, contingent on the strains. Database searches across 11,943 bacterial genomes representing 960 human-associated species revealed that the co-occurrence of tryptophan synthase β subunit and aromatic lactate dehydrogenase is unique to human gut-associated Bifidobacterium species, thus exposing a new aspect of bifidobacteria as probiotics. Consequently, indole, previously considered an end-product of tryptophan metabolism, may serve as a precursor for the synthesis of a host-interacting metabolite with potential beneficial activities within the intricate gut microbial ecosystem.

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IAC Highlight

Frank Schuren, Netherlands Organization for Applied Scientific research (TNO), the Netherlands

Frank Schuren received his PhD at Groningen University in 1987 on the molecular biological analysis of fungal development. After a postdoc in Groningen and at the ETH Zurich in Switzerland he joined TNO in 1996. At TNO he started the implementation of microarray technology in applied research which led to successful implementation in applied microbial research. These include novel diagnostic tools such as the Legionella chip and the analysis of complex microbial populations (such as the intestinal and vaginal microbiota). Furthermore Frank has extensive experience with different approaches for novel antimicrobial strategies. Currently Frank is senior scientist in the Microbiology team within TNO and actively involved in activities towards better understanding the role of the microbiome in human health and especially in innovative ways to modulate gut microbiome in order to improve human health, with a specific focus on dietary fibers and innovative approaches for dealing with individual variation.

<u>July 11 - 09:15-09:30</u>

An intestinal screening platform versus a clinical crossover intervention study: comparative evaluation with a dietary fiber mixture

Abstract: Large individual variation in gut microbiome composition and in response to interventions limits the development of novel microbiome targeted supplements. *In vitro* models reflecting this individual variation and predicting individual clinical intervention responses would allow for pre-study assessment of potential efficacy. Here we investigated if *in vitro* microbiome modulation by a dietary fiber mixture is translational to clinical study outcome.

A 12-week double blind, randomized, placebo-controlled, crossover study with a dietary fiber mixture of acacia gum (AG) and carrot powder was performed in healthy volunteers (N=54, 45-70 years, BMI 27.3 ±1.4) to modulate their microbiome. Analysis of fecal samples collected at multiple timepoints during the intervention period showed a significant effect on microbiota composition albeit with strong individual variation. The fiber intervention was mimicked in vitro by exposing individual participants' anaerobically cultured microbiota baseline samples to the same dietary fiber ratio as used for 12 weeks in vivo and culture these microbiome samples for 24h. The *in vitro* results showed no correlation with baseline human microbiome data, a trend became visible with human microbiome data after 4 weeks intervention and statistically significant correlations could be shown with human microbiome data after 8 and 12 weeks intervention (p= 0.003 and 0.0107). Microbial taxa responding to the intervention in vitro and in vivo also showed clear overlap. Translatability was not limited to microbiome data but surprisingly also extended to systemic biomarker response for which in vitro microbiome results predicted (biomarker) subgrouping with 91% accuracy. These results clearly show the potential for pre-study selection of donors whose microbiomes respond to specific intervention products and may thereby support more efficient fiber intervention study designs.

Authors and affiliations (presenter underlined): Femke P.M. Hoevenaars¹, Tim J van den Broek¹, Boukje C. Eveleens Maarse², Matthijs Moerland², Ines Warnke³, Hannah M. Eggink¹, Frank H.J. Schuren¹ 1 Unit Healthy Living and Work, Netherlands Organization for Applied Scientific research (TNO), Leiden, the Netherlands 2 Centre for Human Drug Research, Leiden, The Netherlands 3 DSM-Firmenich, Kaiseraugst, Switserland





SFA Highlight

Caroline Dricot, University of Antwerp, Belgium

Caroline Dricot is a third year PhD candidate at the Lab of Applied Microbiology and Biotechnology, University of Antwerp (Belgium). Soon after graduating with a Master's degree in Bioscience engineering, she obtained a FWO-SB fellowship to study the role of B-vitamin production by Lactobacillaceae sourced from other environments than the gut, with the guidance of Prof. Sarah Lebeer and Prof. Irina Spacova. She currently focuses on the role of riboflavin (vitamin B2) producing lactobacilli, isolated from the Isala project, in vaginal health. More specifically, within her PhD, she investigates whether Limosilactobacillus-derived riboflavin supports the dominance of health-associated microbiota members, including L. crispatus, and whether it can reinforce the vaginal epithelial barrier function. In this regard, Caroline has learned many different techniques from collaborating research groups in and outside of University settings, including a research stay at the Zimmermann group (Metabolic microbiome-host interactions), European Molecular Biology Laboratory (Heidelberg).

<u>July 11 - 09:30-09:45</u>

A multi-faceted exploration of lactobacillaceae-derived vitamin B2 in the vagina

Abstract: It is well-established that Lactic Acid Bacteria (LAB) play a pivotal role in healthy vaginal ecosystems by preventing pathogen colonization and engaging in symbiotic interactions with commensals and the host. However, the exact mechanisms behind their metabolic activities and functionalities are not yet fully understood. Here we focused on the role of B-vitamin production by LAB in the vagina due to their known role in gut microbiome regulation, anti-oxidation, and anti-inflammation. Using targeted metabolomics (HILIC), the prevalence of B-vitamins in the vagina was monitored in 257 healthy women. Interestingly, this could not be correlated with dietary habits or lifestyle factors, such as age and contraceptive method. However, riboflavin (B2) and its intermediate RL-6,7-diMe were elevated in health associated L. crispatus dominated profiles compared to non-optimal vaginal microbiome profiles (Gardnerella or Prevotella). In addition, we observed that these flavins were involved in crossfeeding between beneficial LAB in synthetic vaginal bacterial communities. To validate interactions of B2-producing LAB with the host, the surfaceupregulation of the B2 host-immune receptor, Major histocompatibility complex class Irelated protein (MRI), was evaluated for vaginal epithelial cells (VK2) and overexpressing mutants (VK2.hMR1). Interleukin 7 and 12, associated with protective MAIT-cell activation by flavins, were increased upon co-incubation with B2-producers. We also observed that the intracellular concentration of B2 was dose-dependently increased in VK2 cells exposed to B2producing LAB, indicating the capacity of the vagina to absorb luminal B2 similar to the gut. Altogether, this indicates that B2 production by LAB has an important role in vaginal health.



SFA Highlight Choshani Dalukdeniya Arachchilage, Sabaragamuwa University of Sri Lanka

Choshani Dalukdeniya Arachchilage is a lecturer in the Department of Food Science and Technology at Sabaragamuwa University of Sri Lanka. She has submitted her PhD thesis and is awaiting her viva at the Department of Food and Nutritional Sciences, University of Reading, under the supervision of Prof. Anisha Wijeyesekara and Prof. Glenn Gibson. Her research focuses on unravelling the functional capabilities of the human gut microbiota using a combination of microbiological and analytical chemistry approaches. In her PhD research, Choshani investigated the effects of probiotic yogurt on gut microbiota, examining both pure cultures and mixed consortia to design a synthetic gut microbial consortium. Additionally, she analysed a human intervention trial to assess the impact of probiotic yoghurt on school children, aiming to gain metabolic insights through in vitro experiments and metabolic profiling. Her research interests include understanding functions of gut microbiota and developing functional foods that positively influence gut microbiota, with the goal of enhancing the quality of life in developing countries.



<u>July 11 - 09:45-10:00</u>

Impact of probiotic yoghurt on gut microbiome dynamics: insights from in-vitro fermentation and metabolic profiling

Abstract: This study explored the effect of probiotic yoghurt consumption on gut microbiome modulation and metabolite production. Here, yoghurt was selected for use with the probiotic due to its accessibility and longevity. pH controlled *in-vitro* anaerobic batch culture experiments with nutrient rich medium were used to mimic the colonic environment. Five conditions—control, starch, inulin, probiotic, and probiotic yoghurt with *Lacticaseibacillus rhamnosus* GG were studied. Sampling at T0, T12, and T48 occurred for 16S DNA sequencing, fluoresecent in situ hydridisation and ¹H-NMR spectroscopy for microbiome and metabolite analyses.

Findings revealed elevated *Lacticaseibacilli* in all vessels and suppressed pathogenic *Clostridium* growth in both probiotic and probiotic yoghurt treatments compared to starch and inulin. *Bacteroides* increased at Tl2 but decreased again at T48. Inulin showed the highest response towards bifidobacteria. Levels of other bacteria tested were approximately equal at the end of fermentation in all vessels. Metabolite analyses indicated increased acetate, propionate and butyrate production.

This research demonstrates the potential of yoghurt to impact on the gut microbiota compared to starch and inulin. Understanding the change of bacterial diversity and metabolite production helps to elucidate microbiota-host dynamics which can inform targeted interventions for gut microbiota modulations.



Catherine O'Neill, University of Manchester, UK

Prof. Cath O'Neill PhD is Professor of Translational Dermatology and Associate Dean for Business Engagement in the Faculty of Biology, Medicine & Health at the University of Manchester. She received both her B.Sc (Hons) and PhD from the University of Wales (Bangor) where she studied Biochemistry. Cath's research work focuses on the human microbiome and whether it can be harnessed as a therapy for skin in health and disease. Her work is very translational and several basic laboratory findings have been developed and tested in human clinical studies. This translational approach has led to the creation of two spin out companies: Curapel in 2011 and SkinBioTherapeutics plc in 2017 which she led as CEO and subsequently as CSO until 2024. Caths' current interests are centred on the impact of sunlight on skin and its microbiome and how the microbiome modulates skin response to ultraviolet radiation.

July 11 - 10:05-10:35

Lactic acid bacteria and the gut- axis - a paradigm with therapeutic implications

Abstract: It has long been known that the gut and the skin participate in a bi-directional relationship (the gut-skin axis) that is at least in part, modulated by the gut microbiome. Hence, orally consumed lactic acid bacteria are emerging as potential new therapies to improve the skin in health and alleviate the symptoms of disease. Similarly, topically applied lactic acid bacteria (sometimes as lysates - postbiotics) have also been demonstrated to improve the barrier function of skin, diminish the signs of photoageing and improve the appearance of skin in conditions such as acne vulgaris. At present, the true potential of either topical or enteral lactic acid bacteria for skin is limited mostly due to the choice of organism which often appears to lack a systematic basis. In this presentation, I will describe the available data from human and animal studies and discuss whether better screening of organisms for functionality that matches skin requirements, may be the next step in utilising lactic acid bacteria for skin health.





John Cryan, University College Cork, Ireland

Prof. John F. Cryan PhD is Professor & Chair, Dept. of Anatomy & Neuroscience, University College Cork and was appointed Vice President for Research & Innovation in 2021. He is also a principal investigator in APC Microbiome Ireland. Prof. Cryan has published over 650 peer-reviewed articles and has a H-index of >162 (Google Scholar). He is a Senior Editor of Neuropharmacology and of Neurobiology of Stress and is on the editorial board of a further 10 journals. He has co-edited four books and is co-author of the bestselling "The Psychobiotic Revolution: Mood, Food, and the New Science of the Gut-Brain Connection". He has been on the Highly Cited Researcher list in 2014 and from 2017 to the present. He was elected a Member of the Royal Irish Academy in 2017; has been a TEDMED & TEDx Speaker.

<u>July 11 - 11:00-11:30</u>

Microbiome-gut-brain axis in health and disease- parsing causality

Abstract: The microbiota-gut-brain axis is emerging as a research area of increasing interest for those investigating the biological and physiological basis of neurodevelopmental, agerelated and neuropsychiatric disorders. The routes of communication between the gut and brain include the vagus nerve, the immune system, tryptophan metabolism, via the enteric nervous system or via microbial metabolites such as short chain fatty acids. Studies in animal models have been key in delineating that neurodevelopment and the programming of an appropriate stress response is dependent on the microbiota. Developmentally, a variety of factors can impact the microbiota in early life including mode of birth delivery, antibiotic exposure, mode of nutritional provision, infection, stress as well as host genetics. Stress can significantly impact the microbiota-gut-brain axis at all stages across the lifespan. Moreover, animal models have been key in linking the regulation of fundamental brain processes ranging from adult hippocampal neurogenesis to myelination to microglia activation by the microbiome. Finally, studies examining the translation of these effects from animals to humans are currently ongoing. Further studies will focus on understanding the mechanisms underlying such brain effects and developing nutritional and microbial-based psychobiotic intervention strategies and how these interact with various systems in the body across the lifespan.



July 10 - 16:30- 17:30

The Late Breaking News session provides an opportunity for ISAPP meeting delegates to share interesting data, perspectives, or developments in 5-minute talks. **Start times** are approximate for this fast-moving session.

Chaired by Gregor Reid, Lawson Health Research Institute, London, Canada.

16:35: Yogurt and reduction of type 2 diabetes: New FDA authorized qualified health claim Miguel Freitas, Danone North America, USA

16:40: Unlocking the gut-brain connection: Exploring the potential of probiotics in autism management Maria Stolaki, Winclove, Netherlands

16:45: From predictive modelling to functional analysis focusing on appetite regulation Cristina Cuesti Marti, APC Microbiome, Ireland

16:50: Spatially and temporally precise capsule-based sampling of the small intestine Joseph Wang, Nimble Science Ltd., Canada

16:55: Which, if any, probiotic or prebiotic can impact jejunal Crohn's? Gregor Reid, University of Western Ontario, Canada

17:00: Transcriptional recording captures bacterial nutrient sensing Katie Guzzetta, ETH Zürich, Switzerland

17:05: Importance of integrated human datasets: Perspectives from the GutBrainGABA study Bhismadev Chakrabarti, University of Reading, UK

17:10: Comparison of acute impacts of fermented foods, prebiotics and probiotics on the human gut microbiome and metabolome Liam Walsh, Teagasc, Ireland

17:15: Strain level quantitation of a B. infantis probiotic using metagenomics Michael Shaffer, Bill & Melinda Gates Medical Research Institute, USA

17:20: Assessment of the safety of "probiotics" in food supplements Mary O'Connell Motherway, University College Cork, Ireland

17:25: Live microbe intakes and health benefits: An emerging theme with industry significance? Maria Marco, University of California, Davis, USA



Please note that the abstracts are grouped topically into four different sections which correspond with how the posters are located for viewing.

SECTION 1: Fermented Foods and Synbiotics

Poster #1 SFA

Multi-omics approaches in unmasking the seasonal microbial variation and health-promoting properties in the soft chhurpi cheese of Sikkim Himalayan region

Authors and affiliations (<u>presenter underlined</u>): <u>Md Minhajul Abedin</u> (National Agri-Food Biotechnology Institute (NABI), Sector-81 (Knowledge City), S.A.S. Nagar, Mohali, Punjab-140306); Rounak Chourasia (National Agri-Food Biotechnology Institute (NABI), Sector-81 (Knowledge City), S.A.S. Nagar, Mohali, Punjab-140306); Loreni Chiring Phukon (National Agri-Food Biotechnology Institute (NABI), Sector-81 (Knowledge City), S.A.S. Nagar, Mohali, Punjab-140306); Sudhir P. Singh (Center of Innovative and Applied Bioprocessing (CIAB), Sector-81 (Knowledge City), S.A.S. Nagar, Mohali, Punjab-140306); Amit K. Rai (National Agri-Food Biotechnology Institute (NABI), Sector-81 (Knowledge City), S.A.S. Nagar, Mohali, Punjab-140306)

Abstract: Soft chhurpi cheese, a traditional fermented milk product consumed in the Sikkim Himalayan region of India, includes lactic acid bacteria (LAB) and yeasts associated with various health benefits. In this study, microbial populations were identified through metagenome analysis, revealing prevalent yeast species in seasonal chhurpi samples. The dominant yeast species include Kazachstania unispora, Yarrowialipolytica, Wickerhamomycesanomalus, and Kluyveromyces marxianus. Lentilactobacillus parabuchneri and Lactococcus lactis emerged as the predominant LAB species in the samples. LC-MS/MS based peptidome analysis identified 1803 peptides in seasonal soft chhurpi, with 62 peptides exhibiting diversefunctionalities. Notably, 126 peptides were predicted as antihypertensive, and 66 peptidesas antioxidant. Additionally, 287 fragments from different bacteriocins were identified, with 128 peptides predicted as antimicrobial. Molecular docking predicted 20 peptides each with antihypertensive, antioxidant, and antimicrobial activities, demonstrating binding with target catalytic residues. Noteworthy peptides, such as VYPFPGPIH and PVLGPVRGPFPI, exhibited strong binding affinity towards ACE and MPO, respectively. Several antimicrobial peptides displayed strong binding with transcriptional regulator and surface proteins of food-borne pathogens (Escherichia coli, Listeria monocytogenes, and Staphylococcus aureus). These findings provide valuable insights into the microbial composition of chhurpi across different seasons, highlighting the presence of generally recognized as safe (GRAS) microbial species, along with the presence of bioactive peptides and add value to soft chhurpi cheese as a functional food.

Poster #2 SFA

Impact of Probiotic Yoghurt on Gut Microbiome Dynamics: Insights from *In-Vitro* Fermentation and Metabolite Profiling

Authors and affiliations (<u>presenter underlined</u>): <u>Choshani Dalukdeniya</u> (University of Reading, UK), Anisha Wijeyesekera (University of Reading, UK), Glenn Gibson (University of Reading, UK)

Abstract: This study explored the effect of probiotic yoghurt consumption on gut microbiome modulation and metabolite production. Here, yoghurt was selected for use with the probiotic due to its accessibility and longevity. pH controlled *in-vitro* anaerobic batch culture experiments with nutrient rich medium were used to mimic the colonic environment. Five conditions—control, starch, inulin, probiotic, and probiotic yoghurt with *Lacticaseibacillus rhamnosus* GG were studied. Sampling at T0, T12, and T48 occurred for 16S DNA sequencing, fluoresecent in situ hydridisation and ¹H-NMR spectroscopy for microbiome and metabolite analyses.



Poster #2 cont.

Findings revealed elevated *Lacticaseibacilli* in all vessels and suppressed pathogenic *Clostridium* growth in both probiotic and probiotic yoghurt treatments compared to starch and inulin. *Bacteroides* increased at Tl2 but decreased again at T48. Inulin showed the highest response towards bifidobacteria. Levels of other bacteria tested were approximately equal at the end of fermentation in all vessels. Metabolite analyses indicated increased acetate, propionate and butyrate production.

This research demonstrates the potential of yoghurt to impact on the gut microbiota compared to starch and inulin. Understanding the change of bacterial diversity and metabolite production helps to elucidate microbiota-host dynamics which can inform targeted interventions for gut microbiota modulations.

Poster #3 SFA

Development of Ogi-Pro: A probiotic Nigerian fermented food with diarrhoea-mitigation potentials

Authors and affiliations (<u>presenter underlined</u>): <u>Rachael T. Duche</u> (Joseph Sarwuan Tarka University Makurdi-Nigeria), Anamika Singh (Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India), Vikas Sangwan (Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India) Manvesh Kumar Sihag (Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India), Tochukwu N. T, Nwagu (University of Nigeria Nsukka, Nigeria), Harsh Panwar (Guru Angad Dev Veterinary and Animal Sciences University of Nigeria Nsukka, Nigeria), Lewis. I. Ezeogu (University of Nigeria Nsukka, Nigeria.)

Abstract: The Nigerian indigenous fermented foods constitute the major staple in most homes which appear as excellent means for probiotics delivery. This study developed a probiotic product by incorporating the fermented indigenous food and probiotic lactobacilli. Two indigenous Lactobacilli strains; Lb. pentosus 15ST2 and Lb. casei KN3 exhibited potential probiotic characteristics, which included acid resistance in simulated gastrointestinal juice (pH 2), bile tolerance in 2% bile salt concentration and in vitro adherence to HT-29 epithelial cell lines. The strains also demonstrated antimicrobial activity against some food-borne pathogens (E. coli 10536TM, S. Typhimurium 51812, L. monocytogenes 13932 and S. flexneri 9199), with no β -hemolytic activity on sheep blood agar and transferrable antibiotic genes. These were used in the formulation of probiotic-Ogi using underutilized cereals. The base materials were assessed for 6 weeks for their effects on growth, survival, acidification rates and, the probiotic lactobacilli strains tolerance to simulated gastrointestinal conditions, and finally, sensory properties. Maize 'Ogi' in combination with the mixed strains was selected for the final product formulation. The product demonstrated good probiotic growth recovery after 42 d of storage at 4°C with viability above the minimum recommended for probiotic foods (1x106CFU/mL). The highest probiotic counts (2.14x107) were recorded on day 28, with acid stability at pH-2 after 3 h, and 2% bile solutions after 4 h of exposures. The product had a pH of 3.00, moisture content 9.94%, firmness 9.031, consistency 210.979 and overall acceptability from sensory evaluations. Probiotic-Ogi made from maize, may serve as an inexpensive suitable vehicle for the delivery of probiotics.



Poster #4 SFA

Synbiotic effect of probiotic Lactobacillus with Allium sativum L. Extract against Salmonella: A Metabolomic and Electron Microscopy Insights

Authors and affiliations (<u>presenter underlined</u>): <u>Olalekan S. Fadare</u> (Elizade University, Ilara Mokin, Ondo State, Nigeria), Onaiwu I. Enabulele (Uniersity of Benin, Benin City, Edo State, Nigeria), DIwas Pradhan (National Dairy Research Institute, Deemed University, Karnal, Haryana, India)

Abstract: Initially, lactobacilli were screened from the faeces of Indian and Nigerian human subjects for probiotic properties and anti-Salmonella effects. The selective effect of garlic extract (GE) against probiotic lactobacilli and Salmonella species was then tested in broth supplemented with different GE doses, and their viability was assessed using plating methods. The metabolomic profile of each probiotic strain cocultured with Salmonella in GE-supplemented broth was collected using GC-MS, and the killing effect of the combination against Salmonella strains was studied using a scanning electron microscope (SEM). Further, haematological and blood biochemistry tests were also checked to determine the safety of high doses of GE in Wistar rats. Aqueous garlic extract had strong, concentration-dependent inhibitory action against both S. Typhi and S. Typhimurium. The bactericidal dosage of 125 mg/ml GE against Salmonella had no impact on the Lactobacillus strains; therefore, it was used for further study. The combination of garlic and lactobacilli also showed a synbiotic effect against Salmonella. SEM analysis revealed visible cellular damages as well as a significant density reduction of S. Typhi and S. Typhimurium when treated with the combination as compared to single treatments of garlic-lactobacilli only. GC-MS analysis also showed significant upregulation of certain antimicrobial metabolites, such as pentanoic acid, pyrrolo[1,2-a]pyrazine-1,4-dione, thymol-beta-D-glucopyranoside, etc., in the garlic-lactobacilli combination. The in vivo safety parameters also demonstrated the safety of high garlic doses. Overall, the study demonstrates the synbiotic action of probiotic Lactobacillus strains and G in tackling Salmonella infections with no harmful effects.

Poster #5 SFA

In vitro fermentation characteristics of dietary fibers using starter bacterial culture, grain kefir culture, or canine feces as inoculum

Authors and affiliations (<u>presenter underlined</u>): <u>Breanna N. Metras</u> (Division of Nutritional Sciences, University of Illinois Urbana-Champaign), P. M. Oba (Department of Animal Sciences, University of Illinois Urbana-Champaign), L. L. Bauer (Department of Animal Sciences, University of Illinois Urbana-Champaign), M. J. Miller (Division of Nutritional Sciences, University of Illinois Urbana-Champaign), M. J. Miller (Division of Nutritional Sciences, University of Illinois Urbana-Champaign), M. J. Miller (Division of Nutrition, University of Illinois Urbana-Champaign), R. N. Dilger (Division of Nutritional Sciences, University of Illinois Urbana-Champaign), R. N. Dilger (Division of Nutritional Sciences, University of Illinois Urbana-Champaign), K. S. Swanson (Division of Nutritional Sciences, University of Illinois Urbana-Champaign), K. Animal Sciences, University of Illinois Urbana-Champaign), K.

Abstract: Traditional grain kefir is produced from the fermentation of milk with yeast- and bacteriacontaining cultures. To maintain consistency and adhere to food safety guidelines, industrial producers sell kefir products based on starter bacterial cultures (typically no yeast). Bacterial profiles of starter vs. grain kefirs differ, but their influence on health effects are unknown. The objectives of this experiment were to determine the in vitro fermentation characteristics of common dietary fibers using 1) a starter bacterial culture or a grain kefir culture as inoculum and 2) fecal inoculum from dogs supplemented with starter- or grain-based kefirs.



Poster #5 cont.

Twelve adult beagle dogs were given one of 3 treatments (n=4/group; 60 mL/d): 2% reduced-fat milk treated with lactase (control); starter kefir (S-Kefir; Champions Choice); or grain kefir (G-Kefir; Kefir Garden Grains). After 14 d, fresh fecal samples were collected and immediately frozen in a 20% glycerol solution. Fecal samples were thawed for the in vitro experiment, heated to 39oC, pooled by treatment, and diluted 1:4 (wt/vol) in anaerobic diluting solution under CO2. Blended, diluted feces then inoculated tubes containing semi-defined medium and one fiber source: cellulose (CEL, negative control), pectin (PC, positive control), beet pulp (BP), or chicory pulp (CP). Fibrous substrate triplicates were incubated at 39oC for 0, 6, 12, or 18 h. Incubation was stopped at each interval and processed for measurement of pH, shortchain fatty acid (SCFA) concentrations, and microbiota populations using 16S rRNA gene amplicon sequencing. A second in vitro experiment was conducted using similar methods and measurements, but by using S-Kefir and G-Kefir as inoculum sources. Effects of treatment, time, and treatment*time interactions within fiber source were tested using SAS, with significance considered P<0.05. Using fecal inoculum, BP and PC were rapidly fermented, leading to large pH reductions, SCFA increases, and microbiota shifts. pH change was of greater (P<0.05) magnitude (PC) and higher (P<0.05) kinetic rate (CP) when using feces from dogs fed S-Kefir or G-Kefir than controls. Butyrate increases were greater (P<0.05) in tubes inoculated with G-Kefir feces than S-Kefir or control feces. When PC and BP were fermented, tubes with S-Kefir feces had greater (P<0.05) acetate, propionate, and total SCFA increases than G-Kefir or control feces. Fermentations were slower using starter and kefir cultures, but some differences were noted. Butyrate was higher (P<0.05) in CEL and CP tubes fermented by S-Kefir, but higher (P<0.05) in PC and BP tubes fermented by G-Kefir. Bacterial beta diversity and relative abundances shifted over time within each substrate and were unique to inoculum source. These data suggest that the activity of kefir bacterial populations differ and that kefir consumption changes the abundance and activity of the fecal microbiota of dogs, justifying in vivo investigation.

Poster #6

Effect of lactose in RGP and EPS receptors in streptococcal phages interactions

Authors and affiliations (<u>presenter underlined</u>): <u>Natalia Diaz-Garrido</u>¹, Dowve van Sinderen¹, Jennifer Mahony¹

1 School of Microbiology & APC Microbiome Ireland, University College Cork, Cork, TI2YT20, Ireland.

Abstract: Streptococcus thermophilus is the most extensively applied thermophilic lactic acid bacterial species in industrial dairy fermentations. The consistent and intensive use of strains of this species in these fermentations is associated with a persistent threat of bacteriophage infection. Phage infection of starter cultures may cause inconsistent, slow or even failed fermentations with consequent diminished product quality and/or output. The phage life cycle commences with the recognition of, and binding to, a specific host-encoded and surface-exposed receptor, which in the case of S. thermophilus can be the rhamnoseglucose polysaccharide (RGP; specified by the rgp gene cluster) or exopolysaccharide (EPS; specified by the eps gene cluster). This study aimed to evaluate if EPS influences the streptococcal phage-host interactions in comparison to RGP receptors. To this end, two EPS-producing strains of S. thermophilus (Nect13 and St50) were analysed for the concentration of produced EPS in the presence of 1%, 5% and 10% lactose in the growth medium using the Dubois colorimetric method. Moreover, gene expression was also assessed by RT-qPCR and phage sensitivity was evaluate by the double agar plaque assay method. Comparative analysis of the eps gene clusters of S. thermophilus Nect13 and St50 was also undertaken to explore their genetic relatedness. Our results showed that higher concentrations of soluble EPS were extracted from cell free supernatants of cultures grown in 10% lactose in comparison to the control for both strains. Moreover, gene expression of epsA and epsB genes was higher in presence of 10% lactose. Plaque assays revealed a mild increase in phage sensitivity in lactose rich media (5% and 10%) compared to the control. In conclusion, our findings expand our understanding of the diversity of the gene clusters that encode EPS biosynthesis and the impact of EPS on interactions with phages of this species.



Poster #7

Factors governing the dominance of lactic acid bacteria in vegetable fermentations

Authors and affiliations (<u>presenter underlined</u>): <u>Tom Eilers</u>, Wannes Van Beeck, Tim Van Rillaer, Katrien Michiels, Ines Tuyaerts, Thies Gehrmann, Stijn Wittouck, Sarah Lebeer Laboratory of Applied Microbiology and Biotechnology (LAMB), University of Antwerp, Antwerp, Belgium

Abstract: Different types of vegetables can be fermented, resulting in products with different organoleptic and microbiological properties. Bacterial community dynamics play an important role in the flavor and safety of these microbially-rich foods, but the various factors influencing community dynamics in fermented foods are not yet well understood. Vegetable substrate, addition of salt, and the carbon dioxide could influence these dynamics. 16S rRNA amplicon sequencing was used to explore the impact of these factors on the dynamics of the active community during vegetable fermentations.

Eleven different vegetables and fruits, with different locations on the plant (e.g. rhizosphere, carposphere, and phyllosphere), were fermented and were characterized by a dominance of lactic acid bacteria (LAB), with Leuconostoc being the most abundant genus. Substrate influenced the microbial dynamics the most, with difference between rhizosphere (sunroot, carrot, parsnip, and beetroot) and the phyllosphere and carposphere. Root vegetables had a more robust and LAB dominated microbial community dominated by *Leuconostoc, Lactiplantibacillus,* and *Lactococcus,* similar to previous studies. Additionally, an uncharacterized *Enterobacteriaceae* genus BIGb0383 was detected in tomato and fennel fermentations, a genus worth researching for safety purposes.

Furthermore, the impact of lower salt concentrations was explored, demonstrating that reduced salt levels resulted in a slower dominance of LAB and a slower microbial succession of *Leuconostoc* and *Lactiplantibacillus*. Lower salt concentration also led to a higher abundance of *Weissella* and various *Enterobacterales* taxa. CO2 was explored as an alternative to lower salt concentration. CO2 injection reduced *Enterobacterales*' relative abundances and increased the overall abundance of *Lactobacillales*. This study revealed the importance of vegetable substrates and proposed a novel strategy to improve the safety of fermented vegetables, which should be further investigated for scalability.

Poster #8

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

Authors and affiliations (<u>presenter underlined</u>): <u>Lisa Friess</u>^{1,2}, Sandra M Kelly^{1,2}, Jose Munoz³, Francesca Bottacini⁴, Douwe van Sinderen^{1,2}

- 1 APC Microbiome Ireland, University Collage Cork, Ireland
- 2 School of Microbiology, University Collage Cork, Ireland
- 3 Department of Applied Sciences, Northumbria University, England
- 4 Biological Sciences, Munster Technological University, Ireland

Abstract: Members of the genus *Bifidobacterium* can be found in the human gut and are purported to elicit health benefits to their host. Strains belonging to *B. longum* subsp. *longum* (*B. longum*) can utilise plant-derived glycans, common to the adult diet, that would otherwise not be digested by the human host. In order for *B. longum* to metabolize such complex dietary glycans, it will need to encode for specific carbohydrate-active enzymes.


Poster #8 cont.

Due to their narrow substrate specificity, multiple enzymes are required to utilise and fully break down large carbohydrates, particularly if they contain various substitutions, such as is the case for arabinoxylan. In the current study, we describe seven enzymes, encoded by in two genetically distinct clusters, and represented by AbfII_1, AbfII_2, AbfII_3, AbfII_4, AbnA2 as well as AbfIII_1 and AbfIII_2. Synthetic and natural substrate assays were performed, and the obtained enzymatic data together with an *in-silico* analysis suggest that: (i) AbfII_1 represents an extracellular exo-arabinofuranosidase; (ii) AbfII_2 is an intracellular exo-arabinofuranosidase; (iii) AbfII_4 represents an intracellular β -L-arabinofuranosidase; (v) AbnA2 is an intracellular exo-arabinosidase; (vi) AbfII_1 operates as an extracellular α -L-arabinofuranosidase, and (vii) AbfII_2 acts as an extracellular α -L-arabinofuranosidase. This study provides a further understanding of the metabolism of a *B. longum* subsp. *longum* strain on dietary glycans containing arabinose.

Poster #9

Adaptations and community changes in milk and water kefir microbiomes in response to environmental parameters (Kefir4All-Citizen Science Project).

Authors and affiliations (presenter underlined): Liam H. Walsh^{1,2}, Samuel Breselgem^{1,3}, Guilherme L.P.

Martins⁴, Mairéad Coakley¹, Eimear Ferguson^{1,5}, Aimee Stapleton³, Fiona Crispie¹, Kieran N. Kilcawley¹, Paul W. O'Toole^{2,3} and Paul D. Cotter^{1,3,5}*

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Abstract: Milk kefir and water kefir are fermented beverages traditionally produced in a household setting through the inoculation of milk or a sugar rich solution, respectively, with a symbiotic microbial ecosystem contained within a milk or water kefir grain. Although traditionally consumed in some regions, the global popularity of, and demand for, both milk and water kefir has increased in recent years. While this has prompted greater research of these beverages and their associated microbiomes, researchers have also studied milk and water kefirs by virtue of their relevance as interesting model microbial communities. Here we apply genome-resolved metagenomic analysis to milk and water kefir metagenomes derived from the same initial microbial population of bacteria, yeast and bacteriophage to study compositional change and microbial evolution at the species and strain level. This was facilitated through the contributions of citizen scientists who used the common source of milk or water kefir grain to carry out fermentations using different substrates and growth conditions for up to 21 weeks. We observed an initial period of rapid compositional change over weeks one (wk01) to nine (wk09) in both the milk and water kefir liquid and grain microbiomes, including in some instances changes in community subtype as represented by the dominant species present, followed by a period of greater stability for many, though not all, metagenomes for the remainder of the study. We document evolutionary change in a number of prevalent species within the milk and water kefir microbiome, which revealed higher mutation rates on average in the initial periods (wk01-wk09), compared to the latter timeframes of the project (wk13-wk21). Both milk and water kefir metagenomes frequently contained bacteria not typically regarded as being part of milk or water kefir communities and presumably acquired from the environment. Although no potentially pathogenic species persisted in the kefir grains throughout the 21-week project, a number did persist over an extended period. Ultimately, our combined findings provide a new insight into how microbial ecosystems change and evolve over successive fermentations.



Protective effect of synbiotic combination of *Lactobacillus* sp. and polyphenols against Benzo[a]pyrene-induced intestinal toxicity

Authors and affiliations (<u>presenter underlined</u>): <u>Shivani Popli</u>¹, Kanthi Kiran Kondepudi²* & Chakkaravarthi Saravanan¹*

1 Department of Basic and Applied Sciences, National Institute of Food Technology Entrepreneurship and Management, Sonipat, Haryana 131028, India

2 Healthy Gut Research Group, Food and Nutrition Biotechnology Division, National Agri-Food Biotechnology Institute, S.A.S Nagar, Punjab, 140306, India

Abstract: Benzo[a]pyrene (B[a]P) is an emerging global food contaminant whose exposure is often linked with microbial dysbiosis as well as the increasing occurrence of colorectal cancers. The present study is focused on utilizing a novel approach of synbiotic combination of Lactobacillus sp. and polyphenols to counteract B[a]P-induced intestinal toxicity. Initially, a highly efficient B[a]P-adsorbing Lactiplantibacillus plantarum MTCC 25433, capable of removing 86% of B[a]P within 2 hours from aqueous solutions, was identified using HPLC. FTIR spectra and chemical pre-treatments unveiled the crucial role of polysaccharides, proteins and lipids in B[a]P adsorption, causing 9%, 16% and 20% reduction in adsorption upon pre-treatment with SDS, sodium m-periodate and mutanolysin, respectively, compared to untreated viable cells. Moreover, >50% of B[a]P was adsorbed by the extracted peptidoglycan of MTCC 25433 alone, indicating a primary role of peptidoglycan in B[a]P adsorption. In-vitro assays showed varying potencies of polyphenols (resveratrol, curcumin and quercetin) in mitigating B[a]P-induced colonic damage. Furthermore, the synbiotic combination of MTCC 25433 and quercetin demonstrated superior efficacy in mitigating B[a]P-mediated toxicity through modulation of redox markers: GSH (17-30 %), SOD (50-88 %) and catalase (19-45 %), suppression of IL-8 secretion (19-28 %) and maintenance of barrier function. In conclusion, this study emphasizes a novel and promising approach of using a synbiotic combination of Lactobacillus sp. and polyphenols to mitigate the intestinal toxicity triggered by B[a]P exposure.



SECTION 2: Microbiota, Metabolome and Gut Health Insights

Poster #11 SFA

Lactobacilli on intimate skin microbiome in Isala citizen science project

Authors and affiliations (presenter underlined): Sarah Ahannach (Laboratory for Applied microbiology and Biotechnology, University of Antwerp, Belgium), Leonore Vander Donck (Laboratory for Applied microbiology and Biotechnology, University of Antwerp, Belgium), Thies Gehrmann (Laboratory for Applied microbiology and Biotechnology, University of Antwerp, Belgium), Lize Delanghe (Laboratory for Applied microbiology and Biotechnology, University of Antwerp, Belgium), Camille Nina Allonsius (Laboratory for Applied microbiology and Biotechnology, University of Antwerp, Belgium), Eline Cauwenberghs (Laboratory for Applied microbiology and Biotechnology, University of Antwerp, Belgium), Eline Cauwenberghs (Laboratory for Applied microbiology and Biotechnology, University of Antwerp, Belgium), Joke Van Malderen (Laboratory for Applied microbiology and Biotechnology, University of Antwerp, Belgium), Joke Van Malderen (Laboratory for Applied microbiology and Biotechnology, University of Antwerp, Belgium), Irina Spacova (Laboratory for Applied microbiology and Biotechnology, University of Antwerp, Belgium), Gilbert Donders (University Hospital of Antwerp, department of Gynaecology and Obstetrics, Antwerp, Belgium), Veronique Verhoeven (Department of Primary and Interdisciplinary Care, University of Antwerp, Belgium), Sarah Lebeer (Laboratory for Applied microbiology and Biotechnology, University of Antwerp, Belgium)

Abstract: Lactobacilli are highly abundant in the vaginal microbiome but are also found on the skin, although in much lower abundances. The high-oxygen levels on the skin result in a suboptimal environment for the growth of lactobacilli, which raises questions whether they really have a habitat on the skin. Yet, a reduction in the total number of abundances of different taxa of lactic acid bacteria appears has been associated with skin problems, such as acne vulgaris and atopic dermatitis. These findings indicate a potential functional role that remains to be explored.

In our large-scale citizen science project, Isala (<u>https://isala.be/en/</u>), we investigated the occurrence of lactobacilli on intimate skin areas. 275 women provided detailed surveys and swabs from various intimate skin sites during the luteal phase of the menstrual cycle. Here, we found that the skin microbiome around the mouth and the groin skin constitute of a mixture of typical skin bacteria as well as bacteria present in the saliva and the vagina respectively. Surprisingly, we also observed a higher relative abundance of typical vaginal lactobacilli, i.e. Lactobacillus crispatus and Lactobacillus iners, on the skin around the breasts compared to publicly available data of other skin sites that are also not in direct contact with vaginal fluid. These results suggest a stochastic dispersal of vaginal lactobacilli onto intimate skin sites, resulting from intimate hygiene and sexual activity, contributes to their elevated abundance.

To substantiate this hypothesis, ongoing analysis involves leveraging extensive survey data from Isala participants regarding their general and sexual lifestyles, as well as intimate hygiene practices such as vaginal douching and pubic shaving. However, it is also feasible that lactobacilli are promoted on particular skin sites due to other factors, such as increases in estrogen secretion around the breast, or that during vaginal birth maternal vaginal lactobacilli establish a niche on the skin. Ongoing research aims to further elucidate the underlying factors influencing lactobacilli dispersal and their implications for skin health. Our study is not only highly relevant for skin health, intimate hygiene practices, but also on offering a novel perspective on the dynamics between vaginal and skin microbiomes.



Poster #12 SFA

isolateR: an R package for generating microbial libraries from Sanger sequencing data with potential for discovery of novel probiotic candidates

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Abstract: Here we present isolateR, an R package designed to streamline microbial isolation workflows and uncover novel probiotic taxa. Sanger sequencing of taxonomic marker genes (e.g., 16S/18S/ITS/rpoB/cpn60) represents the leading method for identifying a wide range of microorganisms including bacteria, archaea, and fungi. However, the manual processing of sequence data and limitations associated with conventional BLAST searches impede the efficient generation of strain libraries essential for cataloging microbial diversity and discovering novel probiotic species. isolateR addresses these challenges by implementing a standardized and scalable three-step pipeline that includes: 1) automated batch processing of Sanger sequence files, 2) taxonomic classification via global alignment to type strain databases in accordance with the latest international nomenclature standards, and 3) straightforward creation of strain libraries and handling of clonal isolates, with the ability to set customizable sequence dereplication thresholds and combine data from multiple sequencing runs into a single library. The tool's user-friendly design also features interactive HTML outputs that simplify data exploration and analysis. In silico benchmarking done on two comprehensive human gut genome catalogues (IMGG and Hadza hunter-gather populations) showcase the proficiency of isolateR in uncovering and cataloging the nuanced spectrum of microbial diversity, advocating for a more targeted and granular exploration within individual hosts to achieve the highest strain-level resolution possible when generating candidate probiotic culture collections. isolateR is available at: https://github.com/bdaisley/isolateR.



Poster #13 SFA

A Multi-faceted Exploration of Lactobacillaceae-derived Vitamin B2 in the Vagina

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Abstract: It is well-established that Lactic Acid Bacteria (LAB) play a pivotal role in healthy vaginal ecosystems by preventing pathogen colonization and engaging in symbiotic interactions with commensals and the host. However, the exact mechanisms behind their metabolic activities and functionalities are not yet fully understood. Here we focused on the role of B-vitamin production by LAB in the vagina due to their known role in gut microbiome regulation, anti-oxidation, and anti-inflammation. Using targeted metabolomics (HILIC), the prevalence of B-vitamins in the vagina was monitored in 257 healthy women. Interestingly, this could not be correlated with dietary habits or lifestyle factors, such as age and contraceptive method. However, riboflavin (B2) and its intermediate RL-6,7-diMe were elevated in health associated L. crispatus dominated profiles compared to non-optimal vaginal microbiome profiles (Gardnerella or Prevotella). In addition, we observed that these flavins were involved in crossfeeding between beneficial LAB in synthetic vaginal bacterial communities. To validate interactions of B2producing LAB with the host, the surface-upregulation of the B2 host-immune receptor, Major histocompatibility complex class I-related protein (MRI), was evaluated for vaginal epithelial cells (VK2) and overexpressing mutants (VK2.hMR1). Interleukin 7 and 12, associated with protective MAIT-cell activation by flavins, were increased upon co-incubation with B2-producers. We also observed that the intracellular concentration of B2 was dose-dependently increased in VK2 cells exposed to B2-producing LAB, indicating the capacity of the vagina to absorb luminal B2 similar to the gut. Altogether, this indicates that B2 production by LAB has an important role in vaginal health.

Poster #14 SFA

The role of the gut microbiota in neuroblastoma tumour-bearing animals with varying immunotherapy response activities

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Poster #14 cont.

Abstract:

Introduction: Evidence suggests a role for the gut microbiota as a modulator in anti-tumour immunity and enhancing the efficacy of immune checkpoint inhibitors (ICIs). However, the specific impact of the microbiota is contingent upon its composition, cancer type, and individual variables. This study focuses on elucidating the interplay between the gut microbiota and the immune phenotype of induced immunologically hot neuroblastoma tumours, after induced mismatch repair deficiency (idMMR).

Methods: Immunocompetent 6-8-week-old female A/J mice were either administered phosphate-buffered saline (PBS) or an antibiotic cocktail (ABX). Subsequently, mice were subcutaneously inoculated with N2a cells. Tumour volumes were monitored at 2–3 day intervals. Flow cytometry was used to assess the immunophenotypes of tumour-infiltrating lymphocytes (TILs), while 16S rRNA gene sequencing was used to analyze gut microbial composition.

Results: ABX treatment led to a less diverse gut microbiota composition, correlating with higher tumour volumes at 15-, 18-, and 20-days post-tumour cell inoculation compared to PBS-treated mice (p<0.05). A depleted gut microbiota promoted idMMR N2a tumour growth, evidenced by larger tumour volumes. Additionally, ABX-treated mice exhibited reduced levels of CD3+ -TILs and these cells were more exhausted and dysfunctional.

Conclusion: This study demonstrates that in idMMR N2a tumours, a depleted gut microbiota promotes tumour growth and compromises overall survival. Gut microbiota depletion diminishes functional tumour-specific T-cell populations, rendering them more exhausted and dysfunctional. These findings advance our comprehension of the gut microbiota's role in shaping the tumour immune microenvironment, potentially impacting ICI sensitivity.

Poster #15 SFA

Nisin-like biosynthetic gene clusters are widespread, abundant and diverse in nature

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Abstract: Bacteriocins are gathering traction as potential alternatives to traditional antibiotics due to their narrow and broad spectrums of inhibition and high degrees of potency against clinically relevant pathogens. One broad spectrum bacteriocin with recognised potential for biotherapeutic application is nisin, a commercially available food preservative.



Poster #15 cont.

Although evidence is amounting of the biotherapeutic application of bacteriocins against relevant pathogens – limited knowledge is known of the widespread nature of their production among competing species, the breadth of variants that exist in nature and their spectrums of inhibition. These may be deciding factors in determining the suitability of bacteriocins as prospective therapeutics. Here we take nisin, the most heavily studied bacteriocin to date, show how widespread nisin variants are found in Genbank and MGNIFY databases. Our research discusses evidence showing nisin biosynthetic gene clusters are more widespread than previously known. We also characterise nisin-VP, a novel natural nisin variant from the strict gut anaerobe Velocimicrobium porci.

Poster #16 SFA

Prenatal exposure to omega-3 fatty acids shapes neonatal gut microbiome and improves long-term metabolic and neurocognitive development

Authors and affiliations (<u>presenter underlined</u>): <u>Saurabh Kadyan</u> (Florida State University, Florida, USA), Gwoncheol Park (Florida State University, Florida, USA), Nathanial Hochuli (Florida State University, Florida, USA), and Ravinder Nagpal (Florida State University, Florida, USA)

Abstract: Maternal dietary habits, particularly in westernized lifestyles, can lead to gut dysbiosis in infants, contributing to adverse cardiometabolic outcomes. We herein investigated whether incorporating polyunsaturated fatty acids (PUFA) in maternal diet protects the newborn from the negative long-term effects of saturated fatty acids (SFA). C57BL/6 mice breeders were fed with a westernized diet (WD) enriched with either: 20% of anhydrous milk fat (SFA control), 20% corn oil (ω 6), or 19% olive oil plus 1% fish oil (ω 3). Offspring exposed to these diets during gestation and nursing were fed with WD for 10-weeks. The offspring of PUFA-fed groups displayed a lower body weight per diet intake compared to the SFA control group. Both PUFA-fed groups showed significant microbiome differences compared to SFA control, both at weaning and 8-weeks. The abundance of Bacteroidota was lower, while that of Firmicutes was higher in PUFA-fed groups. ALDEx2 analyses revealed predominance of genera Erysipelotrichaceae in ω3 group, and Blautia and Eubacterium_nodatum in $\omega 6$ group, both at weaning and 8-weeks. $\omega 6$ group exhibited elevated serum cholesterol and LDL levels. Gut permeability was lowest in $\omega 3$ group, accompanied by lower ileal expression of pore-forming claudins-2 and -15. Improved spatial learning and memory were observed in the ω3 group. Gene expression related to tight-junctions (ZO, OCLN), neuronal signaling (BDNF, TrKB, CREB) and plasticity (PSD95, DCX, Egrl) were enhanced in PUFA-fed groups either in hypothalamus or hippocampus. Notably, neuroinflammatory genes were downregulated in $\omega 3$ (GFAP, Casp1) and upregulated in $\omega 6$ (MCP1, CD11b, CD16) groups. The study underpins the benefits of $\omega 3$ fatty acids in maternal diets for healthier neonatal gut-brain development.



Beneficial effects of multi-mineral supplements on the intestinal barrier

Authors and affiliations (<u>presenter underlined</u>): <u>Garance Coquant</u>^{1,2}, Sinéad Corr^{1,2} 1 Trinity College Dublin, Ireland 2 APC Microbiome Ireland, University College Cork, Ireland

Abstract: A dietary calcium- and magnesium-rich multimineral food supplement obtained from mineralised red marine algae, has previously been shown to exert beneficial effects on the gut, including observations of improved digestive health with an enhanced gut microbial diversity and reduced susceptibility to the development of colitis in a human cohort(1). Food supplements have an increased popularity but further research is needed to back up health benefits.

We are assessing the effects of the multimineral supplements on gut intestinal mucin production and barrier function. To do so, we used an *in vitro* model of cocultured Caco- 2/HT29-MTX, respectively enterocyte and goblet cell lines, grown on transwell plates. We showed that the magnesium-rich supplement could boost trans-epithelial electric resistance (TEER) and reduce FITC-dextran-4kDa translocation, illustrating a lower permeability to ion and macromolecules respectively. The supplements were also able to rescue LPS-induced barrier dysfunction by counter-acting the LPS-induced TEER drop. Secreted-protein MUC2 mRNA was increased when exposed to either of the mineral blends. Transmembrane mucins mRNA such as MUC12 and MUC13 were also increased with the calcium- or magnesium-rich supplements. Our next step is to measure the effects at the protein level and a targeted strategy to identify cellular receptors that might be involved.

Overall, the multimineral supplement showed a beneficial effect on intestinal barrier function, by boosting tight junctions and mucin production, which could explain the improvement of digestive health observed previously. Boosting mucus production would ultimately have an impact on the gut microbiome by promoting beneficial mucus-feeding bacteria. Given that patients affected by pathologies such as inflammatory bowel disease obesity, colon cancer are affected by micronutrient deficiencies, supplementing patients with multimineral blends that have proven beneficial impact could be a co-therapeutic strategy.

1 Aslam MN, Bassis CM, Bergin IL, Knuver K, Zick SM, Sen A, Turgeon DK, Varani J. A Calcium-Rich Multimineral Intervention to Modulate Colonic Microbial Communities and Metabolomic Profiles in Humans: Results from a 90-Day Trial. Cancer Prev Res (Phila). 2020 Jan;13(1):101-116. doi: 10.1158/1940-6207.CAPR-19-0325. Epub 2019 Nov 26. PMID: 31771942; PMCID: PMC7528938.

Profiling the bacterial microbiota of a diet fed in meal or pelleted form, delivered as dry, wet/dry or liquid feed and its impact on the faecal and intestinal microbiome of grow-finisher pigs

Authors and affiliations (<u>presenter underlined</u>): <u>James T. Cullen</u>^{1,2}*, Peadar G. Lawlor², Paul Cormican³, Gillian E. Gardiner¹

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Abstract: The aim of this study was to profile the intestinal and faecal microbiome of grow-finisher pigs provided with the same diet in meal or pelleted form when delivered as dry, wet/dry or liquid feed and to investigate whether the differentially abundant bacterial taxa found are correlated with the growth, feed efficiency and/or carcass quality of these pigs. The study involved 216 Danavil Duroc x (Large White x Landrace) pigs penned in same sex (entire males or females) pen groups of 6 pigs of similar weight (average starting weight of ~33.3 kg). Pen groups were blocked by sex and weight before being randomly assigned to 1 of 6 dietary treatments in a completely randomised block design: (1) Dry meal diet; (2) Dry pelleted diet; (3) Liquid meal diet; (4) Liquid pelleted diet; (5) Wet/dry meal diet; and (6) Wet/dry pelleted diet. The diets were fed on an ad-libitum basis for 64 days. The experiment was a 2 x 3 factorial arrangement, with two factors for feed form (meal and pellets) and three factors for feed delivery (dry, liquid and wet/dry feeding). Bacterial richness was lower in the pelleted diet, compared to meal ($P \le 0.05$). Several lactic acid bacteria (LAB), including Weissella, Leuconostoc and Lactococcus were more abundant in the residual-trough sampled feed compared to the mixing tank and fresh trough-sampled feed. The ileal microbiome of meal-fed pigs was more diverse ($P \le 0.01$) than pellet-fed pigs, with increased relative abundances of Megasphaera, Mitsuokella and Prevotella, while Streptococcus and Escherichia-Shigella were more abundant in pellet-fed pigs (P \leq 0.01). Lactobacillus was enriched in the caecal digesta and faeces of pigs fed liquid meal ($P \le 0.05$), corresponding with its predominance in the liquid meal diet consumed. Liquid meal, liquid pellet and wet/dry pellet-fed pigs had the highest average daily gain (P < 0.001), while average daily feed intake (ADFI) was highest in liquid meal and liquid pellet-fed pigs (P < 0.001). Feed conversion efficiency (FCE) was improved in the dry pellet-fed pigs compared to liquid-fed pigs (P <0.001). Leuconostoc was most abundant in the ileal digesta and faeces of liquid-fed pigs and correlated with increased ADFI and poorer FCE across treatment groups (P \leq 0.05), which may, in part, explain the poorer FCE of liquid-fed pigs. This study associated the poorer FCE of liquid-fed pigs with increased ileal and faecal abundance of Leuconostoc, a LAB associated with spontaneous fermentation in liquid feed.



Identification of a gut microbiota signature in patients with drug-resistant epilepsy upon ketogenic diet treatment

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Abstract: Epilepsy is a neurological disease characterized by seizures that affects 50 million people worldwide. Around 30% of the patients do not respond to the current drugs. Ketogenic diet (KD), highly enriched in fats, is an established therapy for drug-resistant epilepsy (DRE) that shows a reduction of >50% of seizures frequency in ~50% of DRE patients. Interestingly, microbiota from DRE patients responsive to KD is different from KD-non- responder DRE patients and it is still unclear why some patients do not respond to KD. Our research project will address microbiota differences in DRE patients, responders and non-responders to KD, and identify strains of bacteria with potential anti-seizure effect. For this purpose, fecal samples will be collected from patients with DRE before and during treatment with KD up to 6 months together with clinical and dietary information. Stool microorganism composition will be analysed through shotgun metagenomic sequencing, stool and blood metabolites (including ketone bodies) will be measured through targeted and untargeted metabolomics, and biochemical and inflammatory parameters (e.g., Creactive protein, procalcitonin, cytokines) from serum samples will be analyzed. We expect that >30-50% of the patients under KD will have >50% reduction of seizures, ~30% will experience <50% reduction of seizure frequency and ~5-10% will not show any improvement or eventually will get worse upon KD introduction. Healthy relatives and patients with DRE without KD will be analyzed as controls. The ultimate goal of this project is to describe which microbes are associated with KD-dependent epilepsy amelioration and to test their effect in vivo in preclinical animal models of epilepsy. Identification of those microbes could be used as probiotics for a potential treatment for KD-non-responder DRE patients, as well as alternative to KD, which cannot be kept long-term. We believe that these results will shed light for diet-microbiota based treatments for other neurological diseases and metabolic disorders.

Poster #20

The impact of iron and lactoferrin on the pre-weaning infant and adult gut microbiota

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Abstract: Anaemia affects 42% of children aged 6 to 59 months globally, according to the World Health Organisation. Despite known adverse effects of high-dose iron supplementation on the gut microbiota, iron fortification in staple foods is a common practice to meet nutritional needs. In the UK, infant formula must contain 0.5 mg of iron per 100 ml of milk to compensate for the low iron content of cow's milk (formula's primary ingredient). Mother's milk, though lower in iron, has higher bioavailability, partly due to lactoferrin, an iron-binding protein with 'prebiotic' and antibacterial properties. Understanding the impact of iron and lactoferrin on the infant gut microbiota is crucial as the first thousand days of life are key in establishing the gut microbiota which has an important influence on long-term health. Our study investigated how different iron and lactoferrin regimens, alone or in combination, affect the infant and adult gut microbiota using in vitro models.



Poster #20 cont.

Preliminary 24-hour fermentation experiments (n=4) testing the amount of iron and lactoferrin equivalent to the contents of formula and breast milk (at distinct stages) revealed significant (p<0.001) lactoferrininduced increases in Lactobacillaceae abundance in both infants and adults by 670- and 500-fold, respectively, whereas iron alone resulted in more modest increases of 47- and 57-fold in infants and adults, respectively. Additionally, Enterobacteriaceae abundance was significantly (p<0.001) increased in adults by iron and lactoferrin (7.5- and 32-fold, respectively). The combined use of iron and lactoferrin had no notable synergistic effect on the microbiota. Subsequent experiments with continuous infant models (n=3; 6-day, two-vessel fermentation), assessing the same treatments as above, highlighted a significant increase (39-fold increase; p=0.007) in the Prevotellaceae family (in the proximal gut vessel) which are known for their production of beneficial short-chain fatty acids. In adult continuous models (n=3, 16-day, three-vessel fermentation), where the impact of daily doses of iron and lactoferrin as health supplements were explored, the role of iron in increasing Lactobacillaceae abundance was confirmed within the proximal, transverse and distal colon vessels (by 240, 51 and 4.1-fold, respectively; p<0.05). Lactoferrin consistently maintained alpha diversity more efficiently compared to both the control and iron groups.

The above findings thus reinforce previous studies suggesting that lactoferrin supplements improve gut health. This study underscores the complex interactions between dietary supplements and gut microbiota, emphasizing the need for further research to optimize lactoferrin and iron supplement/fortification dosages for maximum benefits across age groups.

Poster #21

Early-life resistome and mobilome exploration in infant gut microbiome datasets from different geographical locations

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Abstract: Early life stages are pivotal for the development of the human body, where the mode and timing of microbiota colonization play a crucial role. Besides aiding the host through bioactive metabolites production and competition towards pathogens, this complex ecosystem also acts as a reservoir for antibiotic resistance genes (ARGs), namely the resistome, which can also be detected without antibiotic treatment. This poses a serious concern as ARGs can be transferred from commensals to pathogens, increasing the chances of diseases, such as multi-drug resistant infections, whose severity can also result in mortality.

Several studies reported a significantly different geographic variation in the prevalence and type of ARGs in infant microbiota, mainly due to environmental and lifestyle factors, making the resistome exploration a challenge.



Poster #21 cont.

Due to the increasing spread and abundance of antibiotic treatments, the present study aims to investigate ARGs through an in silico screening on eight selected infant gut microbiome datasets with a special focus on their potential genetic mobility.

The whole dataset contains 1,358 samples coming from different geographical locations especially North-Eastern European countries and the USA, with different feeding regimens (formula or breast) born by Csection or vaginal delivery. The shotgun raw reads were downloaded from public repositories, then assembled using MEGAHIT v1.2.9 and annotated with Prokka v1.14.6. For the exploration of ARGs, the proteins obtained by the annotation were aligned using DIAMOND against ResFinder 4.1 and with the RGI (Resistance Gene Identifier) tool, based on CARD (Comprehensive Antibiotic Resistance Database) v3.2.5. Efflux pumps and housekeeping genes with chromosomal point mutations were removed to exclude intrinsic traits. The results were then merged selecting the best percentage of identity and query coverage. Finally, the duplicates were removed, and a gene catalog containing around 20,000 proteins was obtained.

Data obtained so far will be employed to conduct a correlation analysis between antibiotic resistance determinants and bacterial genera/families to explore the distribution of ARGs across the countries, the feeding regimen, and the sampling day. Finally, a more accurate metagenome re-assembly of samples with the highest number of antibiotic resistances will be carried out to estimate the probability of horizontal gene transfer.

Data obtained set the stage to unravel ARG's persistence in early life; this plays a crucial role in developing targeted strategies to limit their spread.

Poster #22

Monitoring changes in exhaled volatile organic compounds following iron supplementation for anemia treatment

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Abstract:

Background: There are hundreds of volatile metabolites exhaled in breath that originate from both microbial and human metabolism. Supplements, including pre- and probiotics, can induce shifts in these metabolites by modulating the composition and activity of the gut microbiome. Volatile metabolites are transported from the gut to the lungs, via the circulatory system, where they are volatilized and exhaled. Short- term intervention can transiently shift microbiome and metabolite profiles, while long-term interventions can result in longer-lasting impacts on the diversity and function of the microbiome and, by extension, associated metabolites. Due to the non-invasive nature of breath collection, high-frequency serial sampling at short intervals can be easily implemented into research to gain an improved understanding of microbiome dynamics associated with short- and long-term supplementation.



Poster Abstracts

Poster #22 cont.

Method: Forty-eight nominally healthy participants provided breath samples at four time points before and after 28 days of iron supplementation using a ReCIVA® mask and Breath Biopsy® system. Each participant provided breath samples on day 1 before and after ingesting lactulose to assess baseline levels of intestinal carbohydrate fermentation. Then, volunteers took iron supplements daily for 28 ± 2 days and again provided breath samples before and after ingesting lactulose. Changes to volatile metabolites due to 28 days of iron supplementation were assessed using Wilcoxon signed-rank test for paired samples and linear regression models. Acceptance criteria were set as 2 standard deviations above blank.

Results: Levels of acetic acid on breath significantly differed between day 1 and day 28 of iron supplementation, including the short-chain fatty acid, acetate. Noting when these metabolites changed after lactulose ingestion provided insights into geography-specific fermentation dynamics, further enhancing understanding of the impacts of supplementation on gut microbiome metabolism.

Conclusion: The ability to detect changes in microbiome-associated metabolites in breath due to supplementation represents a pivotal advancement in microbiome modulation research. This non-invasive method offers accessible means for examining both short- and long-term effects of pre- and probiotic interventions. Moreover, when integrated with complementary tools like 16S sequencing and metagenomics, it can provide a more comprehensive understanding of the intricate interactions between pre- and probiotics and the microbiome.

Poster #23

Acute stress, sodium butyrate and the microbiota-gut-brain axis: focus on microbial regulation of barrier function and hippocampal plasticity

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Abstract: Acute stress exposures are the building blocks of chronic stress. Unravelling the mechanisms underpinning the whole-body response to acute stress is critical to understand this neglected aspect of health and disease. A single acute stress exposure rapidly modulates gut-brain axis signalling including intestinal permeability and hippocampal plasticity. The gut microbiota is increasingly recognized as an important modulator of mood and cognition, and microbially-produced metabolites such as butyrate can be a key regulator of brain and gut function via the gut-brain axis. The aim of this work is to determine if acute stress regulates the microbial production of butyrate in the gut to modulate stress-induced alterations in barrier function and hippocampal plasticity. Mice underwent acute restraint stress, and butyrate production was analysed in caecal content at contemporaneous timepoints to LTP measured by electrophysiology. Colonic T84 epithelial and mouse brain endothelial (bEnd3) cells were used to evaluate barrier function by measuring transendothelial electrical resistance (TEER) and fluorescein isothiocyanatedextran (FITC) flux with or without lipopolysaccharide (LPS) as a disrupting agent. Butyrate and acetate production were reduced in the caecal content at 45 min after the acute stress. Butyrate prevented LPSinduced disruption of gut and brain barrier function in a dose-dependent manner. We also expanded our evaluation of key electrophysiological indices of hippocampal plasticity, showing that LTP was modified following acute stress. In conclusion, acute restraint stress reduced the production of butyrate and altered hippocampal plasticity with implications for gut and brain barrier disruption. Future work will evaluate the role of butyrate in acute stress-induced alterations in hippocampal plasticity.



Conditioning of Lactococcus Lactis with nisin and sucrose

Authors and affiliations (<u>presenter underlined</u>): <u>Killian Scanlon</u>, Paul Ross and Colin Hill, APC Microbione Ireland and School of Microbiology, University College Cork.

Abstract: Classical conditioning is a learning process where an unconditioned stimulus is paired with a neutral stimulus, leading to an association that results in a conditioned response. A famous example is Pavlov's experiments with dogs, where the ringing of a bell was paired with the presentation of food. Over time, the dogs associated the bell with food, salivating at the sound of the bell even in the absence of food. This phenomenon is commonly observed in animals and to our knowledge, has yet to be detected in bacteria. To test this hypothesis, we applied classical conditioning concepts to bacteria by pairing a neutral stimulus with an unconditioned stimulus such as a carbon source, heat, or other forms of cellular stress. By administering these stimuli at timed intervals as training cycles, and subsequently only adding the neutral stimulus while measuring for a physiological response to the unconditioned stimuli, we aim to confirm a conditioned response. Our initial work is focused on Lactococcus lactis using Nisin (as the neutral stimulus) and Sucrose (as the unconditioned stimulus). These two inducible systems are co-located on a ~70kb integrative conjugative element known as the Nisin-Sucrose transposon. Initial work has been carried out to ensure the systems are independently expressed, determining the optimal stimuli concentrations and have conducted a pilot experiment to observe a potential conditioned response. The primary aim of this work is to demonstrate memory and learning in a single bacterial organism. Additional project goals include investigating learning and memory in co-culture and in complex microbial communities. If bacterial memory can be harnessed, it could enable the training of bacteria and bacterial communities to deliver functional benefits in the gut or other specific environments. Potential applications include conditioning therapeutically important bacterial strains to anticipate their environments and training the microbiome to interact favourably with drugs.

Poster #25

Simba capsule captures small intestinal luminal content for metagenomics analysis and maps spatial differences along the GI tract as validated by saliva, duodenal endoscopy and feces

Authors and affiliations (<u>presenter underlined</u>): <u>Gang Wang</u>¹, Cedoljub Bundalovic-Torma¹, Sabina Bruehlmann¹, Oksana Lukjancenko², Heidi H. Hau³, Yasmin Nasser⁴, Matthew Woo⁴,

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Abstract:

Introduction: The small intestine (SI) microbiome plays a crucial role in nutrient absorption and emerging evidence indicates that fecal microbiome is insufficient to represent the SI microbiome. Ingestible sampling capsule technologies are emerging. However, potential contamination becomes are major limitations of these devices [].



Poster #25 cont.

Aims and Methods: We previously reported the Small Intestinal MicroBiome Aspiration (SIMBA) capsules as effective means for sampling, sealing and preserving SI luminal contents for 16s rRNA gene sequencing analysis [1-3]. A subset of the DNA samples, including SI samples collected by SIMBA capsules (CAP) and the matched saliva (SAL), fecal (FEC) and duodenal endoscopic aspirate (ASP) and brush (BRU) samples, from 16 participants recruited for an observational clinical validation study [3] were sent for shotgun metagenomic sequencing. The aims are 1) to compare the sampling performance of the capsule (CAP) compared to endoscopic aspirates (ASP) and 850 small intestine, large intestine and fecal samples from the Clinical Microbiomics data warehouse (PRJEB28097), and 2) to characterize samples from the 4 different sampling sites in terms of species composition and functional potential.

Results: 4/80 samples (1/16 SAL, 2/16 ASP, 0/16 BRU, 1/16 CAP and 0/16 FEC) failed library preparation and 76 samples were shotgun sequenced (average of 38.5 M read pairs per sample). Quality assessment demonstrated that despite the low raw DNA yield of CAP samples, they retained a minimal level of host contamination, in comparison to ASP and BRU (mean 5.27 % vs. 93.09 - 96.44% of average total reads per sample). CAP samples shared majority of the species with ASP samples as well as a big fraction of species detected in the terminal ileum samples. ASP and CAP sample composition was more similar to duodenum, jejunum and saliva and very different from large intestine and stool samples.

Functional genomics further revealed GI regional-specific differences: In both ASP and CAP samples we detect a number of Gut Metabolic Modules (GMMs) for carbohydrate digestion and short-chain fatty acids. However, probiotic species, and species and genes involved in the bile acid metabolism were mainly prevalent in CAP and FEC samples and could not be detected in ASP samples.

Conclusion:

CAP and ASP microbiome are compositionally similar despite of the high level of host contamination of ASP samples. CAP appears to be of better quality to reveal GI regional-specific functional potentials than ASP. This analysis demonstrates the great potential for the SIMBA capsule for unveiling the SI microbiome.

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Gut Microbiome Differences in Individuals with PTSD: A Systematic Review

Authors and affiliations (<u>presenter underlined</u>): <u>Chantelle Winder</u>, University of Surrey & University of Sheffield, Kathrin Cohen Kadosh, Social Brain and Development Lab, University of Surrey, Melissa Basso, University of Surrey

Abstract: Post-traumatic stress disorder (PTSD) is a common mental health disorder that can occur following exposure to a traumatic event, which is characterized by symptoms including intrusive memories, dissociation, nightmares and sleep disturbances. PTSD poses significant suffering on the individual and can reduce quality of life substantially, however, its mechanisms are not fully understood. It has also been associated with gut abnormalities, such as with irritable bowel syndrome, indicating possible involvement of the gut microbiome and gut-brain axis. Whereas previous research has implicated the gut microbiome and gut-brain axis in various mental health disorders, the relationship between gut microbiome function and PTSD is unclear. Specifically, little is known about whether specific gut microbiome compositions can increase the risk of developing PTSD, or, vice versa, present as a protective factor for the individual. Here, I will present the results of a systematic review of the literature on gut microbiome differences in individuals with PTSD, including differences in overall diversity, phylum, genus and species. The systematic review will be reported following the PRISMA guidelines. Implications for potential mechanisms behind PTSD (e.g. susceptibility and resilience) and how this could be used to inform potential novel treatments, such as psychobiotic and dietary interventions will be discussed.



SECTION 3: Prebiotic and Fibre-related Interventions

Poster #27 SFA

The efficacy of prebiotic HMOs in improving renal transplant outcomes

Authors and affiliations (<u>presenter underlined</u>): <u>Kait F. Al</u> (Department of Microbiology and Immunology, Western University; Lawson Health Research Institute), Mikhaela Moore (Lawson Health Research Institute), Cadence Baker (Lawson Health Research Institute), Jeremy P. Burton (Department of Microbiology and Immunology, Western University; Lawson Health Research Institute; Department of Surgery, Division of Urology, Western University), Alp Sener (Department of Microbiology and Immunology, Western University; Lawson Health Research Institute; Department of Surgery, Division of Urology, Western University)

Abstract: Nearly 3 million people worldwide suffer from end-stage renal disease. There are more patients on the transplant wait list than available organs, and approximately 20% of transplanted patients subsequently return to dialysis due to poor graft function. Therefore, one of the major goals of the transplant community has shifted to ensuring the longevity of transplanted organs. This study investigated the potential benefits of a human milk oligosaccharide (HMO) on the graft function and gut and urinary microbiomes in renal transplant patients, aiming to improve overall outcomes and reduce complications commonly associated with immunosuppressive therapy. Renal transplant recipients were enrolled in a randomized, double-blind, placebo-controlled trial, where participants received either 4g twice daily of the HMO 2'-fucosyllactose or a placebo. Beginning the day after transplant, participants consumed the product for 90 days, followed by 90 days of observation. Fecal and urine samples were collected, and quality of life, patient satisfaction, and graft function were assessed during 8 study visits spanning the 6-month study period. There have been no serious adverse events as a result of the study product. Preliminary analysis of a subset of 10 patients indicates that the HMO group trended towards decreased gastrointestinal symptom severity compared to placebo controls. 16S rRNA gene sequencing analysis is currently ongoing, but based on the preliminary clinical trends it is likely that the HMO group will demonstrate alterations in the gut and urinary microbiota. These findings will have important implications for optimizing post-transplant outcomes and overall wellbeing in this vulnerable patient population.

Poster #28 SFA

Daily consumption of galactooligosaccharide gummies ameliorates constipation symptoms, gut dysbiosis, degree of depression and quality of life among sedentary university teaching staff: A double-blind randomized placebo control clinical trial

Authors and affiliations (<u>presenter underlined</u>): <u>Kankona Dey</u> (The Maharaja Sayajirao University of Baroda, Vadodara, India), Mini Sheth (The Maharaja Sayajirao University of Baroda, Vadodara, India)

Abstract: Functional constipation affects approximately 10% of the Indian population and may reduce the quality of life (QOL) and increase gut dysbiosis. The study aimed at assessing the impact of galactooligosaccharide (GOS) gummy supplementation on gut health, depression status and QOL of constipated subjects. A double-blind placebo control clinical trial (CTRI/2021/10/037474) was conducted on sedentary constipated adults (n=35), who were split into an experimental group (n=17) and a control group (n=18), supplemented with 10 g GOS and sugar gummies, respectively, for 30 days. Relative abundance of fecal gut microbes, including Bifidobacterium, Lactobacillus, Clostridium and Bacteroides and phyla Bacteroidetes and Firmicutes using real-time polymerase chain reaction and short-chain fatty acids, was analyzed pre and post supplementation. Constipation profile was studied using Rome IV criteria and the Bristol stool chart.



Poster #28 cont.

Depression status was studied using the Becks Depression Inventory. The QOL was assessed using patient assessment of constipation. GOS gummy supplementation increased Bifidobacterium and Lactobacillus by 1230% and 322%, respectively, with reduced Clostridium by 63%, phylum Firmicutes by 73% and Bacteroidetes by 85%. The GOS-supplemented group demonstrated a higher F/B ratio (4.2) indicating improved gut health with reduced gut dysbiosis and constipation severity. GOS gummies enhanced acetic acid and butyric acid levels compared to the control group (p<0.01; p<0.001). Post supplementation, there was 40% reduction in depression (p<0.01) and 22% improvement in QOL(p<0.05). This research validates the predicted beneficial benefits of short-term GOS consumption on constipation profile, gut microflora, depression status and quality of life of constipated subjects.

Poster #29 SFA

Establishing 2'-Fucosyllactose as a Prebiotic Candidate in Ulcerative Colitis using an in vitro Fermentation Model

Authors and affiliations (<u>presenter underlined</u>): <u>James M Kennedy</u> (University of Reading, Royal Berkshire NHS Foundation Trust), Aminda De Silva (Royal Berkshire NHS Foundation Trust), Gemma E Walton (University of Reading), Carlos Poveda (University of Reading), Glenn R Gibson (University of Reading)

Abstract: Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) characterised by chronic inflammation of the colon. Patients with UC have an altered gut microbiota compared to healthy controls. Key differences include a reduction in Lactobacillus spp. and Bifidobacterium spp., and increased sulphate-reducing bacteria, e.g. Desulfovibrio spp. Using prebiotics in patients with UC may help reduce inflammation and improve symptoms. Limited in vitro studies and small clinical trials of prebiotics in UC have been carried out with variable outcomes.

This study explored the effect of three prebiotic candidates, the human milk oligosaccharide (HMO) 2'-Fucosyllactose (2'-FL), a fructo-oligosaccharide (FOS), and a galacto-oligosaccaride (GOS) mixture, on the faecal microbiota from patients with UC using in vitro batch culture fermentation models. pH controlled fermentation was carried out over 48 hours on samples from three healthy controls and three patients with active UC with each of the three substrates. Changes in bacterial groups were measured using fluorescence in situ hybridisation with flow cytometry. Short chain fatty acid (SCFA) quantification was performed using gas chromatography mass spectrometry.

All substrates had a positive effect on the gut microbiota and significantly increased total SCFA and propionate concentrations at 48 hours. 2'-FL was the only substrate to significantly increase acetate and led to the greatest increase in total SCFA concentration at 48 hours. 2'-FL best suppressed Desulfovibrio spp. While 2'-FL, FOS and GOS all showed promise as prebiotic candidates in UC in this in vitro study, 2'-FL demonstrated the most relevant improvements in microbiology and metabolism and would be a suitable choice to take forward to a human intervention trial.



Poster #30 SFA

Assessment of the impact of oat and fruit-based foods and ingredients and on a human gut microbial community using an ex vivo distal colon model

Authors and affiliations (<u>presenter underlined</u>): <u>Cathy Lordan</u> (Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland), Aoife McHugh (Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland), Thomas Boileau (PepsiCo Inc.), Yvonne Collins (PepsiCo Inc.), Paul D. Cotter (Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland; APC Microbiome Ireland, Cork, Ireland)

Abstract: The human gut microbiome plays a key role in health and there is an ever-increasing interest in identifying modulators, including foods and ingredients that have a beneficial impact on this community of microbes. This study focused on assessing the impact of ingredients and foods primarily derived from oats and fruits on the human gut microbiome, assessed using an exvivo gut model.

Oat bran, which consists of high levels of water soluble β -glucan, is associated with numerous health benefits such as lowering cholesterol and improving blood-glucose levels in diabetic patients. Fruit pomace, a side product of fruit juice production composed of fruit pulp and membranes, is often discarded, or used as animal feed. Typically, this material is rich in fibre, pectin, minerals, and polyphenols. These food components, individually and in combination, have value as gut microbiome modulators.

In this study, a high-throughput fermentation model, the microMatrix, was employed to evaluate the impact a variety of oat- and fruit-based ingredients and foods on a gut microbial community. Through shotgun metagenomic sequencing these microbial shifts were determined, both at the microbial compositional and potential functional levels. The relative abundance of *Bifidobacterium adolescentis* increased between 0 hours and 24 hours following supplementation with Oat Flour (Universal), Fermented Apple Juice, and Soluble Oat Bran Cereal. Similarly, *Bifidobacterium longum* relative abundance was higher following supplementation with Grapefruit and Fermented Apple Juice at 24 hours compared to 0 hours. When categorised into ingredient-based groups, the overall species alpha diversity was highest in the fruit ingredients group at 24 hours in comparison to the oat and food groups. This study provides an insight into how different microbes in a human gut microbial community respond to supplementation with different oat and fruit-based ingredients and foods.

This study was funded by PepsiCo, Inc. The views expressed in this abstract are those of the authors and do not necessarily reflect the position or policy of PepsiCo, Inc.

Poster #31 SFA

Creation of a knock-in mouse model that synthesizes 2'-fucosyllactose in milk

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Abstract: The trisaccharide 2'-fucosyllactose (2'FL) is the most abundant human milk oligosaccharide produced by humans with a functional FUT2 gene, commonly known as secretors. Because of the prebiotic, antibacterial, and immunomodulatory effects demonstrated by 2'FL in vitro, there is much commercial interest in its application in early life nutrition. However, animal studies have been constrained by the absence of 2'FL in the milk of most mammals, including mice, as well as challenges to orally gavaging mouse pups. Consequently, our understanding of the immediate and long-term health effects of 2'FL is limited.



Poster #31 cont.

To assess the impact of 2'FL on infant development, we used CRISPR/Cas 9 genome editing to insert the human FUT2 gene into the C57BL/6 mouse genome under the mammary gland- and lactation-specific whey acid protein promotor. Both FUT2+/+ and FUT2+/- mice reached adulthood and reproduced normally with no apparent pathologies. HPLC-characterization of knock-in mouse milk revealed a 2'FL concentration of approximately 8 g/L that remained consistent throughout the course of lactation. Concentrations of 3'SL and 6'SL produced by knock-in mice were lower than wild type mice. We currently investigate the impact of FUT2 expression on mammary gland biology and milk synthesis and composition. Furthermore, we use multiple cross-foster approaches with the newly generated line and the already available FUT2 systemic knockout (KO) line to study the effects of dietary 2'FL and endogenous al,2-linked glycans in the offspring intestine, which are present in wild type (secretor) offspring but absent in the FUT2 KO line. Our newly established mouse model will revolutionize research on the effects of early life exposure to prebiotic 2'FL on short- and long-term health outcomes.

Poster #32 SFA

A focus group study to assess perspectives of patients with Irritable Bowel Syndrome on human milk oligosaccharides and lifestyle insights

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Abstract:

Background: Irritable Bowel Syndrome (IBS) is a highly prevalent disorder of gut-brain interaction with significant morbidity and a major public health/economic concern. Our in vitro (gut models) evidence has suggested human milk oligosaccharides (HMOs) as a potential treatment for IBS symptoms. However, little is known about potential barriers to the use of prebiotic HMOs as therapy for IBS.

Objective: To explore the impact of IBS on quality of life (QoL) measures, with specific focus on symptoms experienced on their impact on diet, lifestyle, and mood. Insights into triggers of IBS flare-ups and opinions on the use of prebiotics, in particular HMOs, will be used to inform the design of a prospective intervention trial.

Design: Five virtual focus groups were held between March 2022 and January 2023. Thirteen females and eleven males were recruited around Berkshire, UK and through social media to attend a single, same-sex focus group. Thematic analysis of transcripts was undertaken. Themes were organised using a semantic coding tree.



Poster #32 cont.

Results: Low QoL in IBS was apparent. Triggers which resulted in worsening symptoms or flares discussed by the groups were all consistent with well-recognised triggers for IBS in the literature and clinical practice. Few participants (6 out of 24) had tried biotic therapies and knew little to nothing about HMOs. Most participants showed interest in HMOs as a new therapy option, with a preferred duration of weeks/months instead of years.

Conclusion: This study suggests IBS sufferers are interested in using HMOs to improve their gut health and IBS symptoms, although they have unanswered questions about efficacy. These results will be taken into consideration in the design of a prospective clinical trial investigating potential new IBS dietary therapies with HMOs.

Poster #33 SFA

Microbial metabolic responsiveness varies by individual and may be optimized by testing prebiotic combinations

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Abstract: Exclusive breastfeeding promotes infant health but is not always possible. Human milk oligosaccharides (HMOS) influence the infant microbiota. 2'-fucosyllactose (2'FL) is the most abundant HMOS and is being tested in infant formula trials alone and in combination with other oligosaccharides. Using in vitro fermentation, we studied shifts in metabolites produced by infant microbial communities given 2'FL alone, galacto-oligosaccharide (GOS) alone, or 2'FL and GOS together. We hypothesized that the combination would increase microbial metabolic response. To test this hypothesis, we analyzed 28 fecal samples collected from 2-4-week-old infants who were either fed formula (FF, n=19) or exclusively breastfed (EBF, n=9). Fecal samples were cultured anaerobically in the presence of 5g/L 2'FL, 5g/L GOS, or 2.5g/L 2'FL + 2.5g/L GOS. Metabolites at 16 hr were analyzed using NMR metabolomics. Shifts were observed in the overall metabolome when an oligosaccharide was given, but not dependent on which oligosaccharide was tested. Differences in oligosaccharide utilization were reflected by microbial butyrate production, defined as [Butyrate]prebiotic-[Butyrate]No sugar>0.1mM. When given 2'FL or GOS individually, the same proportion of microbial communities were responders: 16/28 [57%]. When 2'FL and GOS were given together, responsiveness increased (19/28 [68%]); 22/28 [79%] infants responded to one or more of the oligosaccharide conditions. Microbial metabolic response to a prebiotic was not associated with infant secretor or feeding type. These data indicate that the individual response to prebiotics is varied and can be assessed by in vitro fermentation analysis, which can be used to find oligosaccharide combinations to optimize microbial prebiotic response.



Poster #34 SFA

Solutions to Enhance Health with Alternative Treatments (SEHAT): a double-blinded randomized controlled trial for gut microbiota-targeted treatment of severe acute malnutrition using rice bran in ready-to-use therapeutic foods in Indonesia

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Abstract: Formulations of ready-to-use therapeutic foods (RUTFs) to treat severe acute malnutrition (SAM) in children have focused on weight gain. Due attention, however, has not been given to the gut microbiome dysbiosis experienced by children even after SAM recovery. Recent emphasis has been placed on prebiotic foods in treating gut dysfunction. Rice bran is rich in prebiotics that positively influence the gut microbiome and support healthy growth. This study investigates the effects of rice bran in a novel RUTF for the gut-microbiota-targeted treatment of SAM in Jember, Indonesia. Children aged 6–59 months (n=200) with uncomplicated SAM or approaching SAM (weight-for-height z-scores <-2.5, or mid-upper arm circumference <115mm) were enrolled in a double-blinded, randomised controlled trial. Children received either a locally produced RUTF or the same RUTF with 5% rice bran, and were provided with 8 weeks of treatment, and monitored for 8 weeks. Anthropometric data, stool samples, and dried blood spots (DBS) were collected. Primary outcomes include the effectiveness of RUTF as measured by changes in growth. Secondary outcomes include modulation of the gut microbiome and blood metabolome, and recovery and relapse rates. In an intention-to-treat analysis, improved growth outcomes were found in both treatment arms, indicating responsiveness to the RUTFs. Significant differences in weight and weight gain velocity were observed between RUTFs. Analysis will be performed to elucidate associations between growth outcomes and changes in gut microbiota and metabolites over time and between treatment arms. This investigation will provide new insights into metabolic changes related to rice bran as a prebiotic in the gutdirected treatment of malnutrition.

ClinicalTrials.gov:NCT05319717

Poster #35 IAC

The Effect of Arabinoxylan Polyphenol Content on Short Chain Fatty Acid Production, Gas Production & Microbial Composition: Ex-vivo SIFR® technology

Authors and affiliations (presenter underlined): Hannah Ackermann, COMET

Abstract:

Objective: Arabinoxylan (AX) can exhibit significant structural heterogeneity with respect to the ratio of A:X, substitution pattern of arabinoses, content of feruloyl groups and molecular size.(1) The research objective of the study was to characterize the impact of the polyphenol content of different grades of arabinoxylans extracted from wheat straw on short-chain fatty acid (SCFA), gas production and microbial composition.

Method: Using Cryptobiotix's ex vivo SIFR® technology(2), the impact on metabolite production (key fermentation parameters (pH, gas, SCFA, bCFA)) and microbial composition (shallow-shotgun sequencing) of the gut microbiota of 6 different donors was assessed after 24 hours. Two grades of commercial AX (COMET) with low polyphenol content (AX V, 75% total dietary fiber, 65% AX, 2% polyphenols + lignin) at 2/3/5 g dose and AX with high polyphenol content (AX P, 80% total dietary fiber, 60% AX, 16% polyphenols + lignin) at 3/4/5/8 g dose, were tested against a no-substrate control and the reference prebiotic inulin (IN) at 5 g dose.

Results: AX V and AX P significantly decreased pH, bCFA and stimulated the production of gas, acetate, propionate, butyrate (and thus, total SCFA) in a dose-dependent manner. Comparing AX V/AX P to IN at the same dose (5 g) showed that AX P and especially AX V exhibited significantly higher total SCFA production. Further, while AX V/IN more strongly stimulated butyrate, AX P specifically boosted propionate. AX V/AX P resulted in significantly less gas production compared to IN.

All test subjects exerted bifidogenic effects, mostly boosting B. adolescentis. All test products, but especially AX P stimulated acetate/propionate producers Bacteroides spp, Phocaeicola spp., Parabacteroides and Phascolarctobacterium spp. AX P also promoted Prevotella spp which could explain the stronger acetate/propionate/valerate stimulation by AX P.

All test products also stimulated butyrate-producing gut microbes including species with recognized health benefits such as Anaerobutyricum hallii, Faecalibacterium prausnitzii. AX P and AX V specifically promoted Roseburia intestinalis and Blautia obeum. Roseburia inulinivorans was more consistently promoted by IN and AX P. Anaerobutyricum hallii was particularly linked to the most strongly enhanced butyrate production for AX V and IN.

Conclusion: Results indicate that the polyphenol content of wheat straw arabinoxylan impacts SCFA production and microbial composition. Furthermore, results indicate that arabinoxylan overall stimulates higher SCFA production and greater microbial diversity with less gas production compared to inulin at equivalent dose.

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Poster #36 IAC

The efficacy of prebiotic inulin-type fructans at improving mood in the context of gut-brain axis: results from two randomized human trials

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Abstract: Depression and anxiety are major mental health problems affecting people worldwide, causing a significant loss of productivity in the global economy. There has been a growing interest in the scientific community to understand the interconnection between gut and brain health. Prebiotics are defined as substrate[s] that [are] selectively utilized by host microorganisms conferring a health benefit. Preclinical evidence have indicated that inulin-type fructans might have the capability to modulate stress responsiveness via the so-called gut-brain axis mechanism(s). With the purpose of translating the mechanistic evidence from preclinical studies, we hypothesized that inulin-type fructans might have a potential benefit to modulate stress responsiveness in humans. We aimed to substantiate their efficacy by conducting two randomized human trials with distinct study designs. In the first study (the SYN-ReduSTRESS Study), healthy men (n=204) were randomly assigned to receive 10 q/d of either oligofructoseenriched inulin (Orafti®Synergy], BENEO) or control (maltodextrin) for 4 weeks. At the end of the intervention period, participants were subjected to the Trier Social Stress Test (TSST) considered as golden standard protocol for measuring stress responsiveness. The SYN-ReduSTRESS Study found that oligofructose-enriched inulin supplementation for 4 weeks significantly reduced anxiety (P = 0.04) and cortisol levels (P = 0.002) during the social stress paradigm (TSST). The second study (the EFFICAD Study) involved 96 healthy participants with mild-to-moderate anxiety who were randomly allocated to receive either oligofructose/OF (Orafti®P95, BENEO; 8 g/d), 2'FL (2 g/d), mixed oligofructose (8 g/d) and 2'FL (2 g/d) or maltodextrin as control (10 g/d). The results showed that both OF alone and mixed of OF and 2'FL outperformed 2'FL alone in several mood aspects (BDI-II, PANAS-SF, and STAI YI and Y2), compared to the control group. Following 4 weeks intervention, cortisol awakening response was also significantly reduced in OF alone (P < 0.001) and mixed OF and 2'FL (P = 0.03) groups, compared to the control group. Both studies support the efficacy of prebiotic inulin-type fructans in improving mood (depression and anxiety) and reducing stress-related hormone cortisol in the context of the gut-brain axis.

Poster #37 IAC

Shaping the infant gut: The influence of HMO-enriched infant formulae

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Abstract: In cases where breast-feeding is not possible, or when the nutrient demands of the infant surpass what is provided by human milk, fortified infant formulas which aim to mimic the functions of breast milk have been developed. Incorporation of human milk oligosaccharides (HMO) represents an important strategy aimed at replicating the beneficial effects of breast-feeding. HMOs serve as prebiotic components supplying metabolic substrates necessary for beneficial bacteria to thrive, and thereby support the development of a health-promoting microbial community. It has been anticipated that incorporation of more and more distinct HMOs, as they become more readily available for infant milk formulation, will revolutionize the landscape of paediatric nutrition. The objective of this study was to investigate whether combinations of commercially available HMO impart superior microbiome modulating effects when compared to individual HMO structures.



Poster #37 cont.

Initially, infant milk formula (IMF) containing 2'-fucosyllactse (IMF-2'FL) or a blend of HMO (IMF-6HMO) was developed. These IMF formulations were then passed through infant gastrointestinal digestion (INFOGEST) and dialysis before assessing the influence of these digested formulae on the gut microbiota of breast-fed infants using SIFR® fecal fermentation technology. Shifts in the composition of the infant microbiome were assessed using 16S rRNA gene profiling. Key fermentative parameters (pH, gas production, short-chain fatty acid production) were monitored throughout. Subsequently, the influence of metabolites produced during fermentation of IMF-2'FL and IMF-6HMO on gastrointestinal health (gut permeability, tight junction health, and intestinal inflammation) was assessed using advanced *in vitro* cell models.

The microbiome and metabolite profiling performed here allowed for examination of the structure-function relationships between HMOs consumed in early life and infant intestinal development. Findings from this study confirm that the prebiotic effects of HMO are strongly influenced by the type and number of HMO structure applied. As the use of HMO in nutritional settings continues to expand, we confirm that combinations of novel methodologies (INFOGEST digestion, SIFR® fecal fermentation, and advanced in vitro functional testing) are essential in unravelling the multifaceted interactions between HMOs and the infant gut microbiota and their collective impact on immune function and prevention of multiple disease states.

Poster #38 IAC

A randomized controlled intervention trial in healthy women demonstrates the beneficial impact of low dosages of prebiotic galacto-oligosaccharides on gut microbiota composition.

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Abstract:

Background: Multiple dietary intervention studies have demonstrated a positive impact of galactooligosaccharides (GOS) on gut microbiota composition and activity, especially related to *Bifidobacterium*. However, the applied dosages of GOS are generally too high for application as supplements in the form of a capsule or tablet and data on the effect of lower dosages are limited.

Objective: The primary aim of the current study was to reveal the effect of low dosages (1.3 and 2 g) of GOS on *Bifidobacterium* in healthy women.

Method: Eighty-eight healthy women (40-70 y, BMI 18.5-30 kg/m2) were included in this randomized, parallel, double-blind study of 6 weeks. The participants were stratified for fiber intake, BMI and age and randomized to consume either 1.3 or 2.0g of GOS per day for 3 weeks after a control period of 3 weeks without any intervention. Fecal microbiota composition and food intake were determined by shotgun metagenomics sequencing and the TRAQQ App, respectively, at the start of the control period and at the start and end of the intervention period. Exploratively, self-perceived gut comfort, sleep quality and mental well-being were assessed on a weekly basis. Cluster analysis was performed based on baseline characteristics and study outcomes of participants.



Poster #38 cont.

Results: The relative abundance of *Bifidobacterium* in feces significantly increased after three weeks of daily consumption of both 1.3g (p<0.01) and 2.0g GOS (p<0.01). This was accompanied by a significant shift in the overall microbiota composition for the dosage of 2.0g GOS (p<0.01). Responder analysis showed a trend for an overall difference in initial fecal microbiota composition for responders and non-responders. We revealed clusters of participants on basis of diet, age and microbiota signatures.

Conclusion: Also low dosages of 2.0 and 1.3g, daily GOS consumption for 3 weeks increase the relative abundance of *Bifidobacterium* in feces of healthy women. This study supports the beneficial use of low dosages of GOS to support a healthy microbiome.

Poster #39 IAC

Microbiota modulatory effect of inulin-type fructans – current scientific data

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Abstract: Inulin and oligofructose are prebiotic dietary fibers which affect a limited number of gut bacteria and thereby increase host health. The lowest fructan dose previously shown to lead to increases in bifidobacteria was 3 g/d. This has also been confirmed in a meta-analysis with meta-regression. We aimed to demonstrate that 2.5 g/d oligofructose can lead to a significant increase in bifidobacteria thereby contributing to host health.

64 volunteers were randomized to consume 2.5 g/d Orafti® oligofructose or placebo for 14 days followed by a 4-week wash-out period and then crossed-over to the other intervention. Bifidobacterium spp. and affect (emotional state) were assessed at the beginning and end of each intervention period. Furthermore, digestive function was documented daily.

Enumeration of Bifidobacterium spp. showed that 2.5 g/d oligofructose led to significantly higher numbers compared to placebo (p = 0.016). Furthermore, stool frequency significantly increased during oligofructose compared to placebo (p = 0.0204) intake. With respect to affect, no significant changes were observed.

This study is the first to demonstrate a significant increase in Bifidobacterium after a very low dose of 2.5 g/d oligofructose compared to a no-fiber control. Increases in bifidobacteria corresponded to a significantly higher stool frequency. The study results add to the growing database on the microbiota modulatory and health effects of inulin type fructans.



Poster #40 IAC

Non-invasive continuous gut microbial fermentation measurement of inulin and resistant starch fibers for metabolic health

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Abstract:

Background: Previous research showed that one day supplementation of a specific fiber mix of inulin plus resistant starch showed specific effects on distal colonic fermentation and improved metabolic health parameters in lean men, though not in prediabetic overweight men. Our understanding regarding the gut microbiota host-metabolism axis is limited because it is not yet possible to study continuous real-time microbial fermentation in humans to date.

Aim: We aim to measure the switch from proteolytic to saccharolytic fermentation, as reflected by expired H2S, and H2 plus CH4 gases, respectively, after supplementation of the fiber mixture inulin+resistant starch over a 36-hour period. Changes in fermentation gas patterns in relation to gut microbiota composition and functionality, changes in energy expenditure and substrate metabolism, circulating hormones and metabolites will be studied.

Methods: This project will create and validate a novel continuous measurement system to study noninvasive time-resolved kinetics of fermentation and metabolism induced by foods or other factors. This system will comprise a whole-room calorimeter, with sensors that measure O2 and CO2, as well as microbial gas excretion i.e. H2S, H2, CH4 gas concentrations. This novel system will be validated with a double-blind, placebo-controlled, cross-over study with a two-week washout period between the two interventions. Twelve normoglycemic lean individuals and 12 individuals with prediabetes and/or insulin resistance and overweight and/or obesity will receive in a randomized order the dietary fiber mixture or placebo while residing in the chambers for 36-hours.

Perspective: Development and validation of this non-invasive continuous gut microbial fermentation measurement will allow us to measure real-time microbial fermentation of prebiotic inulin plus resistant starch fiber in humans for the first time. Continuous measurements will allow us to explore the time-resolved kinetics of fermentation and metabolism of food that reaches the gut microbiota faithfully. It will also provide a basis for novel personalized nutritional and lifestyle strategies and/or the development of functional foods and nutraceuticals to prevent gut, immune-related, and metabolic diseases.



Effect of quinoa processing on human gut microbiome through in vitro fermentation approach

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Abstract: Quinoa grain represents an excellent source of nutrition, including fiber and high quality and complete proteins. Little is known on the processing effect of quinoa on the human gut microbiome, therefore, we used an in vitro fermentation approach to assess changes in gut microbiome profile. A selected guinoa line was tested in raw, boiled, extruded, and baked (cookies) forms. Dietary fiber and total polyphenols were profiled; and in vitro gut microbiome fermentation were conducted from 10 healthy volunteers' microbiota with five time-points. The 16S rRNA amplicon sequencing was obtained on an Illumina MiSeg platform and data processed through the Mothur bioinformatic pipeline. Post-fermentation analysis revealed that boiled and extruded quinoa increased the total polyphenols significantly, whereas raw guinoa and 100% guinoa flour cookies exhibited significantly lower concentration of total polyphenol contents, while dietary fiber levels were consistently on the lower end in all products. Fecal fermentation trials with pre-digested guinoa substrates, derived from raw and processed samples, significantly increased the levels of beneficial lactic acid producing genera, Bifidobacterium, Lactobacillus, Pediococcus and Weissella. Raw and cooked guinoa all incurred a large increase of Bifidobacterium (15-20% relative abundance) in the first stage of the in vitro fermentation. In the second stage, the other lactic acid bacteria increased as well (total LAB relative abundance of 20-40%). Pediococcus and Weissella increased specifically with the raw guinoa, suggesting the presence of plant material that may be harder to ferment for the human gut microbiota. The bifidogenic effect was more marked and sustained with the boiled guinoa, while higher Lactobacillus levels were observed with the baked and extruded products. These findings indicate remarkable prebiotic properties of our quinoa line being somewhat maintained through a variety of popular cooking methods. Interestingly, the boiling method was found to optimize the prebiotic properties, suggesting that further studies with quinoa (and other grains) products should combine food chemistry and gut microbiota outcomes to understand which physiochemical properties predict the gut microbiota responses.



Cradle to Cravings: Priming Healthy Eating behaviour from early life to adulthood in mice using microbiota-targeted interventions

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Abstract: Early-life is a critical window in offspring development(1,2). An unhealthy diet during this period can negatively impact offspring metabolic function and eating behaviour later in life(1,3). Microbiota-targeted interventions hold promise for the treatment of aberrant functioning of metabolic status and eating behaviour(1,4-6). This study evaluates the impact of an early-life high-fat/high-sugar (HFHS) diet on eating behaviour later in life, and whether this can be attenuated by microbiota-targeted strategies.

Male and female C57BL/6J mice were exposed to either control or HFHS diet from birth for 5 weeks. Afterwards, all mice received control diet. Two groups with early-life HFHS diet were supplemented with a prebiotic (Fructo-Oligosaccharides+Galacto-Oligosaccharides, FOS+GOS) or a bacteria (B. longum APC1472) throughout the study. Meal pattern, saccharin and food preference were investigated at 6 and 11 weeks.

Five-week-old female and male pups exposed to early-life HFHS showed greater weight-gain compared to control pups with no microbiota-targeted intervention effects. Body weight was normalised after 1 and 2 weeks respectively, after switching to control diet. Early-life HFHS diet increased meal size, eating rate and total HFHS intake in 11-week-old females. Early-life HFHS increased preference towards palatable food at 6 and 11 weeks in both sexes whilst simultaneously increasing food grinding behaviour. APC1472 alleviated HFHS-induced alterations in food preference and food grinding behaviour in both sexes. APC1472 and FOS+GOS alleviated the alterations in meal pattern in females.

These findings demonstrate that an unhealthy diet in early-life has enduring effects on eating behaviour later in life in a sex-specific manner, which can be alleviated by microbiota-targeted strategies.

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Characterization of *Langra* mango peel powder and assessment of its prebiotic and antioxidant potential

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Abstract: A prebiotic is a substance that is selectively utilized by host microorganisms conferring health benefit. Commercially available prebiotics includes Inulin, FOS, and GOS. A new range promising candidates as prebiotics are polydextrose, resistant-starch, xylo-oligosaccharide, isomalto-oligosaccharide and non-fiber prebiotics including polyphenols and PUFA. Developing waste by-products into prebiotics is an interesting avenue to research. This study investigated the functional and prebiotic potential of dried Mango Peel Powder (MPP) of Langra cultivar (Varanasi's GI tagged, locally available). Proximate analysis revealed that MPP contains 6.45±0.20% moisture, 6.34±0.16% protein, 3.88±0.06% fat, 2.50±0.10% ash, 32.86±1.11% crude dietary fiber, and 47.97±2.52% carbohydrate. MPP displayed substantial antioxidant potential with 54.6% DPPH inhibitory activity, 15.67±0.15 mg GAE/g TPC, 8.88 mg QuE/g TFC, OHC of 1.47±0.02 g oil/g and a WHC of 4.7±0.02 g water/g. MPP could selectively stimulate the growth of two probiotic strains over enteric bacteria. It was revealed that a combination of MPP @5% with L. fermentum NCDC143 after 24h fermentation had the best in vitro prebiotic activity score of 3.35 ± 0.034 and 3.53 ± 0.060 against Escherichia coli ATCC 25922 and Enterococcus faecalis NCDC114, respectively. The percentage DPPH inhibition activity of MPP increased during fermentation with L. fermentum NCDC143, highlighting its role as a source of antioxidant polyphenols. Further studies are required to validate the prebiotic effect of Langra MPP through in-vivo or simulated in-vitro gut microbiota modulation after consumption of MPP or the extracted pure form of the principal component responsible for the selective stimulation of probiotic bacteria over Gram-negative pathogens.

Poster #44

Fucoidan from *Ascophyllum nodosum* and *Undaria pinnatifida* attenuate SARS-CoV-2 infection *in vitro* and *in vivo* by suppressing ACE2 and alleviating inflammation

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Poster #44 cont.

Abstract: The global healthcare challenge posed by COVID-19 pandemic necessitates the continuous exploration for novel antiviral agents. Fucoidans have demonstrated antiviral activity, yet the structureactivity relationship of fucoidans from *Ascophyllum nodosum* (FUCA) and *Undaria pinnatifida* (FUCU) against SARS-CoV-2 remains unclear. FUCA was characterized as a homopolymer with a hypothetical backbone structure of repeating (1+3) and (1+4) linked α -l-fucopyranose residues, whereas FUCU was a heteropolysaccharide consisting of Fuc1-3Gal1-6 repeats. Furthermore, FUCA demonstrated significantly higher anti-SARS-CoV-2 activity than FUCU in Caco-2-Nint cells (EC50: 48.66 vs 69.52 µg/mL), suggesting that the degree of branching rather than sulfate content affected the antiviral activity. Additionally, FUCA exhibited a dose-dependent inhibitory effect on ACE2, surpassing the inhibitory activity of FUCU. *In vitro*, both FUCA and FUCU treatments downregulated the expression of pro-inflammatory cytokines (IL-6, IFN- α , IFN- γ , and TNF- α) and anti-inflammatory cytokines (IL-10 and IFN- β) induced by viral infection. In hamsters, FUCA demonstrated greater effectiveness in attenuating lung and gastrointestinal injury and reducing ACE2 expression, compared to FUCU. Analysis of the 16S rRNA gene sequencing revealed that only FUCU partially alleviated the gut microbiota dysbiosis caused by SARS-CoV-2. Consequently, our study provides a scientific basis for considering fucoidans as poteintial prophylactic food components against SARS-CoV-2.

Poster #45

Ecological determinants of individualized effects of dietary fiber on the human gut microbiome

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Abstract:

Objective: Dietary fibers (DFs) contribute to human health and induce specific shifts in gut microbiome composition and functions. However, there is a substantial inter-individual variation in responses, and the determinants of this variability, as well as the consequences for health effects, are poorly understood. The objectives of this work were to characterize effects of distinct fibers on microbiome dynamics in a well-powered human trial, determine the ecological principles that drive responses, and elucidate whether individualized effects of fiber on the microbiome influence the physiological effects of fiber and can be predicted using baseline microbiome features.

Methods: In a six-week, parallel-arm, randomized controlled trial, adults with excess weight added 25 g (females) or 35 g (males) of one of three DF supplements to their usual diet daily: acacia gum (AG; n = 75), resistant starch type 4 (RS4; n = 75), or microcrystalline cellulose (MCC; non-fermentable; n = 45). A multiomics approach was applied to fecal samples collected at baseline, week 1, and week 6 and changes of microbiome composition and genomic functional capacity, metabolites (SCFAs and bile acids) and mechanistic and clinical host markers were assessed.

Results: MCC had no effect on fecal microbiota diversity and composition. The fermentable fibers decreased α-diversity and led to changes in the composition of the fecal microbiota in a subset of subjects (PERMANOVA R2: 1.8% (AG) and 1% (RS4), p < 0.01). Compositional shifts were highly specific to the chemical structure of the fiber, with AG increasing *Bifidobacterium longum* and *Faecalibacterium prausnitzii* while RS4 increased *Bifidobacterium adolescentis* and *Parabacteroides distasonis* (p < 0.001).



Poster #45 cont.

Responses were largely stable between week 1 and week 6 (45 - 80% of responders), but *Faecalibacterium prausnitzii* showed a transient response to AG with an increase at week 1 followed by a decrease at week 6 (40% of responders). Although statistically significant in the overall population, responses were highly individualized, with individual taxa showing a response in only 30-70% of participants.

Conclusions: AG and RS4 altered fecal microbiome diversity and composition and induced highly specific microbiome shifts with diverse individual and temporal patterns. Ongoing work is aimed at the characterization of the ecological drivers of responses and investigation of the question if individualized responses are predictable via the microbiome features and linked to the physiological effects of the fibers.

Poster #46

Sensory evaluation of high-fiber reformulated cereal-based foods aimed to restore gut microbiome functionality

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Abstract:

Objective: The Western diet is highly refined and undersupplies the gut microbiome with fermentable substrates, such as prebiotic fibres. Food reformulation can increase the fibre content of foods offering a potential tool to close the fibre gap in highly palatable Western style diets and improve microbiome functionality. However, the development of fibre-enriched food products is challenging due to the negative effects on palatability. The objective of this project is the development of common food products (scones, bread, and pasta) aiming to maximize the inclusion of fibres with varying physicochemical properties and evaluate their sensory acceptability in this single-blinded study.

Methods: Recipe formulations were designed to optimise the techno-functional properties and physiological benefits of fiber-enriched food products designed for a randomised crossover-controlled human nutritional trial. Soluble (beta-glucan and arabinoxylan) and insoluble isolated fibers (cellulose and resistant starch (RS)), including fibres that are considered candidate prebiotics (beta-glucan, arabinoxylan, and RS), were combined to substitute partial amounts of standard flours. Scones were prepared with formulations that replaced 47.5% of standard flours with isolated fibres (including 20% RS, 15% cellulose, 10% beta-glucan and 2.5% arabinoxylan) containing 13.5g of fibre/100g serving, with mid-dose recipes representing exactly half these replacement levels. Consumer acceptability and perception of health and nutrition related attributes was evaluated with twenty-six untrained consumers (16 female, 10 male) aged between 21-54y using a 10cm hedonic line scale for (a) low fibre/control (b) mid-dose fiber and (3) full-dose fiber scone formulations.

Results: No significant difference (p>0.05) was noted for the liking of aroma or perception of healthiness across all three scone formulations. Comparing the mid-dose to control scone formulation, fibre enrichment did not significantly affect the liking of flavour, texture, or overall acceptability. Higher fiber addition levels lead to a decrease in overall acceptability as noted in the full-dose scone formulation which was associated with an increase in perceived fibre content in comparison to the control.



Poster #46 cont.

No significant difference was however noted between the mid and full-dose scone formulations with respect to liking of appearance, texture, perceived fibre content, willingness to consume again or recommend to others. Experiments for the bread and pasta formulations are ongoing.

Conclusions: The findings highlight the potential of isolated fibres to enhance the nutritional quality of processed foods while maintaining consumer acceptability. The food products developed in this project have been included in a meal plan that will be tested in a human nutritional trial.

Poster #47

Methods for screening of fibre-based dietary prebiotics and in vitro testing of their metabolites across gut-brain-axis targets.

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Abstract: The microbiota-gut-brain axis has emerged as a potential new therapeutic target for the effective treatment of both metabolic [1] and central nervous system disorders [2]. Diet is believed to be a major driver of the composition and function of the microbiota, with dietary fibres known to have beneficial effects, such as the release of metabolites with promising bioactive functionality [3]. In this study, three selected fibre-based prebiotics - fructooligosaccharides (FOS), galactooligosaccharides (GOS), and resistant maltodextrin were used to assess the impacts of bacterial-derived metabolites on microbiota-gut-brain axis signalling. Firstly, an APC-developed in silico pipeline was used to screen the genetic capacity of available probiotic bacteria to digest selected prebiotics, while producing neuroactive molecules based on curated metabolic pathways. Next, the ability of selected probiotic bacteria to use the prebiotic substrates as a carbohydrate source was assessed using a prebiotic activity score. They were ranked based on the growth in media with the fibres relative to the growth in media with dextrose. This guided the selection of the most promising probiotic/prebiotic combinations for further investigation based on metabolic potential. Cell-free supernatants of the combination of four selected probiotic bacteria and three prebiotics were subsequently investigated in in vitro assays to probe mechanisms of action with a focus on targets in the gut-brain axis. Overall, this in silico and in vitro approach allows for functional screening of symbiotic combinations with the strongest metabolic potential and brain health benefits.

[1] https://doi.org/10.1179/1476830513Y.000000099

- [2] https://doi.org/10.1152/physrev.00018.2018
- [3] https://doi.org/10.1016/j.ebiom.2020.102968



Harnessing Xylanase Producing *Bacillus altitudinis* XYL17 Isolated from High-Altitude Regions of Sikkim Himalaya for Sustainable Xylooligosaccharides Production for potential Prebiotic Applications

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Abstract: The 2022 Global Hunger Index reveals a 16.3% prevalence of undernourishment in India, underscoring the impact of malnutrition on underdeveloped and developing nations. Minimizing undernourishment in the general population can be achieved by incorporating nutraceutical compounds derived from underutilized agricultural wastes. UN Sustainable Development Goals (SDGs) 2 and 12 aim for zero hunger and sustainable use of natural resources by promoting sustainable agricultural practices and utilizing agricultural byproducts, respectively. In the present study, highly active xylanase producing Bacillus altitudinis XYL17 was isolated from high-altitude soil of Sikkim Himalaya, India for the production of prebiotic xylooligosaccharides (XOS) from agrowastes. The xylanase demonstrates significantly high catalysis, with a recorded activity of 34000 U/mg protein upon optimized conditions. The whole-genome analysis of the bacteria revealed the sequence of the xylanase enzyme, which can aid in understanding the high catalytic ability and specificity of high-altitude biocatalysts. The biotransformation of xylan extracted from corn cob and rice straw by B. altitudinis XYL17 yielded essential XOS, encompassing xylopentaose, xylotetraose, xylotriose, and xylobiose. The addition of XOS led to enhanced growth of probiotic bacteria compared to growth in standard media. The present study proposes native bacteria catalyzed agrowastesderived XOS as affordable and eco-friendly nutraceuticals, contributing significantly to addressing nutrition deficits and achieving the Sustainable Development Goals (SDGs). Moreover, the sustainably derived biotransformed prebiotic products can be suggested as affordable and nutritious animal feed.



SECTION 4: Probiotic, Postbiotic and Microbial Interventions

Poster #49 SFA

Effects of Daily Probiotic Lactobacillus acidophilus Supplementation on Calcium Status, Bone Metabolism Biomarkers, and Bone Mineral Density Profiles in Postmenopausal Osteoporosis Women: A 12-Week Controlled and Randomized Clinical Study

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Abstract: Menopause poses a pivotal health challenge for women, correlating with an increased risk of bone fractures. Current osteoporosis treatments, with their associated adverse effects, prompt the exploration of alternative dietary interventions. In this 12-week controlled randomized clinical study, we investigated how probiotic Lactobacillus acidophilus impacts calcium status, bone metabolism biomarkers, and bone mineral density profiles in postmenopausal women with osteoporosis. 62 subjects meeting inclusion criteria were randomized into placebo (PL; n = 30) and probiotic (LA; n = 32) groups. Assessments included body composition, blood morphology, serum calcium, Dual-energy X-ray absorptiometry (DXA) bone mineral density, and bone turnover markers. Results within the LA group post-intervention revealed significant reductions in fat mass, body fat percentage, visceral fat, and trunk fat mass. White blood cell concentrations notably decreased in the LA group. Additionally, the LA group exhibited a lower gammaglutamyl transferase level than the PL group. Despite a significant decrease in serum calcium levels within the LA group, DXA bone mineral density profiles showed no differences. Negative correlations, especially between tartrate-resistant acid phosphatase and spine L1 and left femur, were identified. In conclusion, probiotic Lactobacillus acidophilus supplementation for 12 weeks influenced body composition, blood morphology, and serum calcium status in postmenopausal women. However, DXA bone mineral density profiles remained unchanged. Furthermore, our findings suggest that probiotic supplementation could serve as a viable alternative for managing menopausal osteoporosis.

Poster #50 removed



Poster #51 SFA

Probiotic lactic acid bacteria associated with fermented millet based milk beverage (Brukina) and effects on the gut microbiome

Authors and affiliations (<u>presenter underlined</u>): <u>Bless Hodasi</u> (University of Ghana, Accra, Ghana), Elmer Ametefe (University of Ghana, Accra, Ghana), Righteous Agoha (University of Ghana, Accra, Ghana)

Abstract: Our diet significantly influences our gut microbiome especially fermented foods which contain beneficial microorganisms known as probiotics. Brukina, a fermented beverage originating from Burkina Faso, but widely consumed in Ghana is produced from cooked millet and fermented cow milk. This study sought to characterize the lactic acid bacteria (LAB) present in Brukina and determine their impact on the gut microbiome of consumers.

FDA approved Brukina were obtained from supermarkets in Accra, Ghana. LAB load was determined. 16S rRNA amplicons from cultured LAB strains and DNA extracts from Brukina was sequenced. Acid and bile tolerance, adhesion capacity, antimicrobial activity and antibiotic susceptibility profile of the isolated lactic acid bacteria were determined. Human participants and animal models were fed with Brukina samples for four weeks. Fecal samples were collected at different time points thus day 0,3,7,14,21 and 28. DNA was extracted for 16S amplicon sequencing. Next generation sequencing was carried out followed by metagenomics analysis of the the sequence data.

LAB load ranged from 104 CFU/ml to 106 CFU/ml. 16S rRNA sequencing of genomic DNA identified the cultured LAB strains as Lactobacillus fermentum, Lactobacillus delbrueckii, Lactobacillus johnsonii, Lactobacillus prophage, and Lactobacillus taiwanensis. Direct DNA extraction from Brukina showed the presence of Limosilactobacillus fermentum, Enterobacter hormaechei, Alishewanella agri, Neobacillus fumarioli, Bacillus safensis and Faecalibaculum rodentia. The strains exhibited antimicrobial activity against four enteric pathogens. There was a notable increase in the abundance of Lactobacillus delbrueckii and Lactobacillus fermentum in the gastrointestinal tracts of human participants.


Poster #52 SFA

Modified bacteria producing human hormone as a novel potential therapeutic strategy for MASLD in mice

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Abstract: Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most prevalent liver disease worldwide. Obesity and gut disturbances, such as altered production of intestinal hormone fibroblast growth factor 19 (FGF19), are implicated in MASLD pathogenesis. Apart from diet and physical activity, there is no effective treatment against MASLD. In our study, we evaluated the effect of a modified bacteria producing human hormone analogue along with dietary change on liver and adipose tissue (AT).

MASLD was induced by feeding C57BL/6J mice with high fat-sugar diet for fourteen weeks. Subsequently, during the following seven weeks, all mice were switched to standard diet and the intervention group received single gelatine cubes with 109 CFU each of E.coli Nissle expressing aldafermin, FGF19 analogue (n=6); control groups received either 10^9 CFU/gelatine cube of non-modified E.coli Nissle (n=6) or gelatin cube with no treatment (n=6).

The effects of the intervention was evaluated by integrating transcriptomics with non-targeted metabolomics analysis in liver, and transcriptomics analysis in AT. This was complemented with histology, bioimaging analysis, and physiological data of body weight and plasma biomarkers.

EcNA alleviates MASLD: decreases hepatic steatosis, body weight, plasma aspartate aminotransferase, plasma cholesterol levels and downregulates pathways linked with oxidative stress and insulin resistance in liver. In AT an improvement in insulin sensitivity and energy metabolism was suggested. Upregulation of Bcl6 and Cnst expression in liver and AT supported amelioration of MASLD in both tissues.

Our results suggest the potential for using modified E.coli Nissle along with dietary changes for improving the recovery from MASLD.



Poster #53 SFA

Bacterial-Based Therapy with Akkermansia muciniphila to Modulate Tumor and Gut Microbiome and Activate T-Cells in Pancreatic Cancer

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Abstract: Providing adjuvants for immunotherapy for advanced and metastatic pancreatic adenocarcinoma (PDAC) is necessary for improved patient survival. Supplementation with Akkermansia muciniphila (AM), a bacterium routinely found enriched in immunotherapy responders, has the potential to modulate the immune system and increase the efficacy of immunotherapy. This study investigates the effects of oral AM supplementation in combination with anti-PD1 immunotherapy, in modifying the gut and tumor microbiome and T-cell activation in PDAC. Subcutaneous KPC tumor-bearing mice were treated with AM and anti-PD1, and tumor and spleen tissues were harvested for immune profiling by flow cytometry. To further investigate the effects of AM treatment on immunotherapy response, an inducible PDAC mouse model was used, and pancreas tissues were harvested for immune profiling after similar treatment with AM+anti-PD1. In the tumors of AM+PD1 treated KPC mice, there was a significant increase in the expression of T-cell activation markers, ICOS and PD1, on CD8+ T-cells. Systemically, there was less expression of ICOS and PDI on CD8+ T-cells, suggesting that these cells may be migrating from the spleen to the tumors in response to treatment. There were significant increases in T-cell activation in the pancreas and spleen of PDAC mice treated with AM+anti-PD1, coupled with decreased T-cell exhaustion in the pancreas. Additionally, to explore the direct effects of AM on tumor cell proliferation, KPC cells were treated in vitro with varying concentrations of AM cell free supernatant (CFS) in the growth media. KPC cell proliferation was significantly decreased at 15% and 20% CFS. Combining immunotherapy with an AM probiotic may enhance the anti-tumor immune response and directly affect tumor growth.

Poster #54 SFA

Modified Escherichia coli Nissle expressing IGF1 and FGF19 reduce liver fat accumulation and restore microbial equilibrium in a metabolic dysfunction-associated steatotic liver disease mice model

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Abstract:

Introduction: Endocrine dysregulation and intestinal microbiota unbalance are commonly associated with metabolic dysfunction-associated steatotic liver disease (MASLD). We aimed to investigate the effectiveness of modified probiotic Escherichia coli Nissle (EcN) 1917 expressing various hormones (IGF1, GLP-1, FGF19, Adiponectin) downregulated in MASLD as potential therapeutics.



Poster #54 cont. SFA

Method: Forty-one C57BL/6J mice underwent 14 weeks of high fat diet intervention for MASLD development. The mice were then separated into 7 groups and underwent 7 weeks of probiotic intervention while under control diet. Grouping: 1) without probiotic; 2) EcN without hormone expression; 3-6) EcN expressing IGFI, GLP-1, FGFI9, Adiponectin respectively; 7) liraglutide treatment. Liver fat was measured using MRI and Oil-Red-O staining of liver histological samples. 16S rRNA sequencing was used to assess the bacterial composition in the cecum, while untargeted metabolomics explored the overall host and bacterial metabolite profiles across plasma, liver, cecal content, and colon content.

Result: Mice receiving EcN expressing IGF1, GLP-1 and FGF19 were effective at reducing liver fat accumulation. Microbiota compositions were different between groups and microbial communities of mice receiving EcN expressing IGF1 and FGF19 had higher observed richness. Integrative analysis of bacterial and metabolomic pathway enrichment results revealed key metabolic pathways altered by our interventions with EcN expressing IGF1 and FGF19, contributing to their effectiveness.

Conclusion: EcN expressing IGF1 and FGF19 have the potential to reduce liver fat accumulation and restore microbial equilibrium. This may be a combined effect of hormones improving gut endocrine and immune function and EcN as a probiotic.

Poster #55 SFA

The impact of a L. brevis probiotic on brain and behavioural correlates of GABA in humans: A trial protocol

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Abstract: Certain bacteria residing in the human intestine produce compounds that can function as neurotransmitters in the host, such as Gamma Amino Butyric Acid (GABA). Little is known about the impact of neurotransmitters produced by the microbiota on brain and behavioural function in humans. The current project seeks to address this gap by altering the population of GABA producing bacteria in the human gut through a probiotic intervention, and by measuring multiple brain and behavioural indices of GABA. In a recent in-vitro study, we have demonstrated that L.brevis LB01 could produce significant quantities of GABA (Monteagudo-Mera et al., 2022).

Accordingly, the current placebo-controlled cross-over trial tested 60 male volunteers (age range: 18-50) on the impact of this probiotic on brain and behavioural function. All volunteers were invited to provide faecal, urine and blood samples, and undergo a neuroimaging and behavioural testing battery. Key outcome variables included the level of brain GABA as measured using Magnetic Resonance Spectroscopy, as well as proxy behavioural measures of available GABA. Volunteers enrolled on the study were invited to attend four study appointments and received a probiotic and placebo dietary intervention between visits, and maintained a record of their diet between the visits.

Analyses are currently underway and the initial outcomes of the trial will be presented at the conference.



Poster #56 SFA

Complex studies of Bifidobacterium adolescentis CCDM 368 surface polysaccharide BAP1 confirmed its structure and its immunomodulatory properties in preventing allergic reaction

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Abstract: Allergy diseases have become a common issue, particularly in developed countries, and the number of people affected by them increases yearly. However, the effectiveness of allergy treatments can be low in some patients or cause numerous side effects. Therefore, it is necessary to search for new therapeutic solutions.

BAP1 is a purified polysaccharide (PS) derived from the surface of the Bifidobacterium adolescentis CCDM 368 (Bad368). It is characterized by a strong induction of IFN- γ and inhibition of Th2-related, pro-allergic cytokines in cells isolated from ovalbumin (OVA)-sensitized mice. Thus, it was selected from Bad368 surface antigens as promising in the alleviation of allergy diseases. Further studies using flow cytometry showed high uptake of BAP1 by epithelial cells (99.9%) and its efficient transfer to dendritic cells (71.3%). To fully understand the function of BAP1, GC-MS and NMR analyses were used to determine the structure of the PS-creating unit. Studies revealed the presence of glucose, galactose, and rhamnose residues that build up a PS with the average molecular mass of 9.99 x 10^6 Da. Finally, to confirm the obtained results, in vivo experiments were performed. The tested PS was administered to mice in the mouse OVA-allergy model. A thorough investigation of serum, spleen, and lung samples confirmed in vitro results and indicated an important role of RORyt factor in BAP1 signalling.

Overall, BAP1 is a molecule with strong immunomodulatory properties and a well-known structure that shows promise in alleviation of allergy diseases and readiness for structure-function studies. However, further investigation is needed to fully confirm and understand the role of this PS.

Poster #57 SFA

Off-Target Effects can Impact the Ability of Bacteriocins to Control Clostridioides difficile in Complex Communities

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Abstract: Clostridioides difficile is one of the causative agents of nosocomial diarrhoea. As current antibiotic treatment failures and recurrence of infections are highly frequent, alternative strategies need to be developed for the handling of this disease. Lacticin 3147 and pediocin PA-1 are bacteriocins from different classes and activity spectra that include pathogenic C. difficile.



Poster #57 cont.

In this work we engineered a panel of Lactococcus lactis strains to produce either lacticin 3147, pediocin PA-1 or a combination of both bacteriocins resulting in higher activity against C. difficile. However, it is difficult to predict the indirect impact of these treatments in complex microbial communities. Hence, we assessed the effect of these L. lactis strains in a Simplified Human Intestinal Microbiota (SIHUMI-C) model. SIHUMI-C is a bacterial consortium of diverse human gut species including C. difficile. Each species can be individually tracked in a complex media by qPCR. The different L. lactis strains were added to SIHUMI-C, and samples were taken at intervals up to 48 h for genomic DNA extraction. The genome copy number of each SIHUMI-C member was evaluated using specific primers. Our results show an unexpected outcome, as the combined effect of both bacteriocins increases the levels of C. difficile in the consortium despite displaying higher inhibitory activity when tested individually against the same strain. These effects were analyzed considering antagonistic inter-species interactions within the SIHUMI-C community, providing a comprehensive insight into the possible mechanisms by which broad-spectrum antimicrobials could fail in controlling targeted species in complex communities.

Poster #58 SFA

Leveraging human clinical data to identify novel probiotic candidates to counter kidney stone disease

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Abstract: Gut microbiota alterations and dysregulated calcium handling in the body are hallmarks of kidney stone disease, and we have shown that metabolites produced by the gut microbiota directly enhance stone production. Probiotics could potentially improve outcomes of stone former (SF), but conflicting results from clinical studies make it difficult to identify promising strains. In this study, we aimed to identify potential probiotic candidates for stone disease by inquiring about the relationship between calcium homeostasis, the gut microbiota, and urolithiasis in a cross-sectional, single-center adult population. Thirty healthy controls (HCs) with no history of kidney stones and 31 calcium-containing SFs were recruited for the study. Serum, urine, and fecal samples were collected from participants along with relevant medical history. SFs had increased blood and urine calcium levels compared to HCs indicating abnormal calcium handling. 16S rRNA gene sequencing analysis showed that while the microbiota of cohorts was similar taxonomically, inferred functional alterations were consistent among SFs significantly differentiating them from HCs. In SFs, key gut microbiota bioenergetic functions were significantly correlated with serum and urine calcium, important clinical biomarkers of stone pathophysiology. Functional analysis revealed that lower free calcium levels in HCs were associated with improved metabolism of oxalate, a major constituent of 80% of stones, and sugars. Though insignificant, high calcium levels in SFs correlated to a loss of Bacteroides stercoris which has been shown to improve glucose metabolism and sensitivity and is likely to contain oxalate degrading genes. As such, B. stercoris may protect against stones and awaits testing in our models of urolithiasis.



Poster #59 SFA

A vaginal synthetic community of Lactobacillus crispatus, Lactobacillus jensensii and Limosilactobacillus substantiates co-existence in a module with probiotic potential

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Abstract: The vaginal microbiome of healthy women is mostly dominated by Lactobacillus spp. Yet, many women suffer from vaginal infections due to lactobacilli depletion. To prevent and treat these states, we need a deeper understanding of how lactobacilli dominate the vagina and interact with other microorganisms. Synthetic microbial communities (SynComs) enable studying community interactions, dynamics, and disturbances in more controlled conditions. These SynComs show probiotic potential, as single probiotic strains often fail to establish in the vaginal microbiome. In the Isala project, four modules of intercorrelated taxa were observed in vaginal microbiome samples of 3345 women. The L. crispatusmodule, containing also L. jensenii and Limosilactobacillus, is of interest as it showed to be most associated with health. To better understand and apply the architecture of this module, we performed top-down experiments, where swabs were cultivated over several days. These swabs evolved into a simpler community dominated by L. crispatus. Moreover, all three members of the L. crispatus-module stabilized, suggesting that the bacteria of the L. crispatus-module exert synergistic interactions mimicked in SynComs. Next, we manually assembled the module in a bottom-up approach. The fitness and stability of the members are evaluated with qPCR and metabolomics already unraveled putative cross-feeding. Preliminary in vitro results show enhanced inhibition of vaginal pathogens by a SynCom compared to single strains. These experiments are the first steps aiming to understand how lactic acid bacteria can positively interact in the vaginal ecosystem. As such, SynComs of the L. crispatus-module are promising probiotic communities and could greatly benefit the host.



Poster #60 IAC

A 10-strain probiotic mix decreases lipid accumulation by regulating fatty acid metabolism and food intake in *Caenorhabditis elegans*

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Presented by: <u>Denis Guyonnet</u>, Sanofi

Author Disclosures: Yishu Ding is Sanofi employee and may hold shares and/or stock options in the company. Renhui Zhang and Pei Zhao have nothing to disclose. Sanofi funded the study, which was performed by SunyBiotech, China.

Abstract:

Background: It is now well-acknowledged of the key role of gut microbiota in modulating metabolic health, that changing the gut microbiota composition potentially impact the progression of several metabolic diseases, including obesity and type 2 diabetes mellitus. Supplementation of certain strains of *Lactobacillus* and *Bifidobacterium* are shown to improve glucose homeostasis and reduce body weight and fat mass, while the mechanism of action remains inconclusive.

Objective: Taking *C. elegans* as a model, this study aims to explore the anti-lipid accumulation effect and potential working mechanisms of a 10-strain probiotic mix (*B. animalis subsp. lactis* HN019, *L. acidophilus* GLA-14, *B. animalis subsp. lactis* BI-04, *L. rhamnosus* HN001, *L. casei* LC-11, *B. bifidum* Bb-06, *L. gasseri* Lg-36, *Lactococcus lactis subsp. Lactis* LI-23, *L. plantarum* Lp-115, *Lacticaseibacillus paracasei* Lpc-37) in a commercialized product.

Methods: The 10-strain probiotic mix in different concentrations was mixed with OP50 and fed to *C. elegans* cultured on nematode growth medium (NGM) or glucose-NGM (gNGM). After a 65-hour culture, body length, width, pharyngeal pumping rate and lipid contents of the worm were examined. Gene expression were analyzed *via* RNAseq.

Results: Administration of 10-strain probiotic mix significantly reduced body width in gNGM *C. elegans*, while showed no impact on body width in NGM *C. elegans*. No change in body length was observed in either NGM or gNGM *C. elegans*. Oil red O staining revealed a significant lower level of lipid accumulation in NGM *C. elegans* treated with the probiotic mix in a dose-dependent manner, with the highest dosage inducing a dramatic reduction by 83% compared to the control group. A decreased content of triglyceride by 55% was also observed with the highest dosage. This anti-lipid accumulation effect was more pronounced in gNGM C. elegans with decreased lipid accumulation by 99% and triglyceride contents by 59% under the highest dosage compared to control worm. Pharyngeal pumping rates were decreased in both probiotic treated NGM and gNGM worms, suggesting an inhibited food intake. RNAseq analysis showed notable effect of probiotics on the expression of genes involved in multiple metabolic pathways, including downregulation of genes in fatty acid oxidation (e.g., acs-2). We also observed upregulated expression of DAF-7 and DAF-3 in the worm treated with the probiotic mix, suggesting the potential involvement of TGF- β signaling pathway in the lower food intake observed.

Conclusion: A 10-strain probiotic mix in a commercialized product significantly inhibited lipid accumulation via multiple pathways in C. elegans, including those regulating fatty acid metabolism and food intake. Our findings suggest the potential use of this probiotic mix in weight management.



Poster #61 IAC

Efficacy of a yeast postbiotic on cold/flu symptoms in healthy children: a randomized-controlled trial

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Abstract:

Background: Children attending school/daycare are at high risk of acute respiratory tract infections. EpiCor postbiotic, derived from yeast fermentate, has been demonstrated to support immune function in adults, reducing the incidence of cold/flu-like or allergy symptoms. As such, studies are warranted in children as available pharmaceutical options have unwanted side effects.

Methods: Two-hundred and fifty-six children aged 4-12 years attending school/daycare were randomized to either EpiCor or Placebo for 84 days during the 2022-2023 flu season in Ontario, Canada. The Canadian Acute Respiratory Illness and Flu Scale (CARIFS), completed daily by subjects' caregivers, was used to assess the incidence and severity of cold/flu symptoms and use of cold/flu medications. Adverse events were recorded.

Results: Total CARIFS severity scores, 'sore throat' and 'muscle aches or pains' symptom scores in the EpiCor group were significantly lower compared to Placebo during incidences of cold/flu (P≤0.05). Participants taking Placebo were 1.73 times more likely to use cold/flu medication compared to those receiving EpiCor (P=0.04). Incidence of cold/flu symptoms was not significantly different between groups. EpiCor was found to be safe and well-tolerated.

Conclusions: EpiCor supplementation resulted in significantly lower cold/flu symptom severity and less cold/flu medication usage than Placebo demonstrating a beneficial effect on immune function in children.



Poster #62 IAC

Beyond bacteria: Impact of yeast probiotic Saccharomyces cerevisiae on intestinal health of dogs

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Abstract:

Introduction: The study aimed to evaluate the effects of the dietary inclusion of yeast probiotic Saccharomyces cerevisiae (S. cerevisiae) on gut fermentative metabolites and fecal microbiota of dogs undergoing an abrupt dietary change.

Animals, materials and methods: Two diets were evaluated: with and without the inclusion of 0.12 g of probiotic (viable *S. cerevisiae*/animal/day). Diets were offered for 28 days to 16 adult beagle dogs, distributed in a completely randomized design (n=8). On day 0, 21, 23, 35 & 49, fecal samples were collected for ammonia analysis, pH, phenols, indoles, short-chain (SCFA) and branched-chain fatty acids (BCFA), total biogenic amines, and microbiota. Biogenic amines were analyzed by HPLC. Phenols, indoles, SCFA, and BCFA were analyzed by gas chromatography. Quantification of bacterial taxa was performed by qPCR and the dysbiosis index was calculated [1]. An analysis of variance (two-way ANOVA) was performed, using the function General Linear Model, to assess the effects of supplementation and interaction with the day of measurement. Fisher LSD with Bonferroni correction was applied as post hoc test. Beta diversity, using Bray–Curtis distances, and its plots were analyzed and generated using Past software 4.03. Analysis of similarity (ANOSIM) was used to evaluate the similarity of the microbiota and KO terms between groups at different time points. Differential abundance of KEGG Modules and KO terms between groups (at day 21 & 49) and diets was analyzed using linear discriminant analysis (LDA) effect size (LEfSe) on MicrobiomeAnalyst.

Results and discussion: The test group had lower (P<0.05) fecal pH and ammonia concentrations at day 23, lower (P<0.05) total biogenic amines production at day 21 & 49 and higher (P<0.05) fecal concentrations of butyrate regardless of the day, compared to control group. Moreover, the test group showed lower (P<0.05) dysbiosis index than control group regardless of the day and higher (P<0.05) relative abundance of *Bifidobacterium* (at days 35 & 49) compared to control group. Beta diversity demonstrated that the dietary supplementation of the probiotic resulted in modulation of the intestinal microbiota on day 49 (P<0.05) and a trend (P= 0.091) to change the microbiota on day 21 compared to the control group. The control group also showed upregulation in genes related to virulence factors, antibiotic resistance, and osmotic stress.

Conclusion: The inclusion of 0.12 g of *S. cerevisiae*/animal/day can modulate the intestinal microbiota and its metabolites, favoring eubiosis.

References: [1] Alshawaqfeh et al. (2017) FEMS Microbiol. Ecol Conflict of interest: The authors declare that the research received funding from Phileo by Lesaffre



Poster #63 IAC

BG-L47: A B. longum subsp. longum with a broad probiotic toolbox

Authors and affiliations (<u>presenter underlined</u>): <u>Ludwig Ermann Lundberg</u> ϕ 1,2, Manuel Mata Forsberg ϕ 3, Beatrice Marinacci ϕ 4, Gianfranco Grompone2, Rossella Grande4,5, Eva Sverremark-Ekström3 & Stefan Roos1,2

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Abstract: *Bifidobacterium longum* subsp. *longum* is a microbiota-stabilizing bacterial species in the human GI-tract, which colonizes in early infancy and persists throughout life. We previously showed that *B. longum* subsp. *longum* BG-L47 stimulates growth and bioactivity of *L. reuteri* DSM 17938 and its derived membrane vesicles and that the strain is safe for consumption in a human clinical study. Here we present BG-L47 as a stand-alone strain with several desirable probiotic properties including extensive fiber utilization and antimicrobial as well as immunomodulatory properties. Fiber degradation is a fundamental characteristic of bifidobacteria, and BG-L47 is equipped with glycoside hydrolases (GH's) relevant to both infant and adult diets. The strain efficiently metabolizes lacto-N-tetraose, a common human milk oligosaccharide, as well as many plant-derived glycans associated with an adult diet. The cellular localization of the GH's may be of importance for the ecological role of BG-L47 and how it contributes to the sharing of bioenergetic resources in the microbiota. The strain has sixteen predicted cell surface located GH's, including two membrane proteins, nine sortase-dependent proteins, three N-terminal membrane anchored proteins and two lipoproteins.

Another attributable probiotic trait crucial in the competition for resources and host access is antimicrobial activity. We assessed the Minimum Inhibitory Concentration (MIC) with broth microdilution and XTT metabolic assay, and the minimum bactericidal concentration (MBC) by CFU-counts. The supernatant of BG-L47 demonstrated inhibitory effects on the growth and metabolic activity of three clinically relevant pathogens, *E. coli* ATCC 25922, *S. aureus* ATCC 43300 and *P. aeruginosa* ATCC 27853 with a MIC of 20 µl against all three pathogens. The MBC values were 40 µl/100 µl against *E. coli* and *S. aureus*, and 20 µl/100 µl against *P. aeruginosa*.

Finally, we hypothesized that BG-L47 may be a potent immunomodulator. We evaluated the response of isolated monocytes exposed to BG-L47-derived extracellular membrane vesicles through RNA sequencing. 481 protein-coding genes were upregulated and 424 were downregulated. Notably, several of the downregulated genes belonged to antigen-presenting pathways, while upregulated genes included cytokines with regulatory and M2-polarizing capacity belonging to the IL-10 cytokine family. Additionally, 201 non-coding RNAs were differentially expressed. Conclusively, BG-L47 emerges as a potent strain of *B. longum* subsp. *longum* with the potential to persist in the intestine from infancy to adulthood, attributed to its broad fiber utilization capacity, antimicrobial properties and immunomodulatory potential.



Poster #64 IAC

Effects of the probiotics *Lactiplantibacillus plantarum* KABP011, KABP012 and KABP013 on serum bile acids and metabolic profile in healthy overweight subjects

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Abstract:

Objectives: Lactiplantibacillus plantarum KABP011, KABP012 and KABP013 (AB-LIFE®) has shown a beneficial effect in the management of lipid metabolism disorders in previous placebo-controlled studies (1,3), which is suggested to be mediated through the modulation of bile acid metabolism in the gut (4). The aim of this study was to investigate in vivo bile salt hydrolase activity of this probiotic formulation in humans.

Methods: A prospective, interventional, one-arm mechanistic study was conducted to evaluate the effect of a 4-week intervention with dose escalation in 20 healthy overweight individuals. Bile acid (BA) signatures, blood lipids and endocrine markers were measured in serum. The study was registered in Clinicaltrials.gov (NCT05378230).

Results: Probiotic intervention progressively decreased total conjugated BA in serum (baseline: 3.14 µmol/L [IQR 1.89;4.54], 28 days: 1.89 µmol/L [IQR 1.10;3.20]; p=0.004), the ratio of conjugated vs unconjugated BA (p=0.033) and FGF-19 concentrations (p=0.011). These effects were accompanied by a significant reduction of total cholesterol (p=0.038) and non-HDL cholesterol (p=0.035) levels. Fasting levels of circulating apolipoprotein(Apo) B100 and ApoB48 were also significantly reduced. Interestingly, probiotic intervention significantly decreased the concentration of small LDL cholesterol (LDL-C) particles (p=0.047) and increased the average LDL-C particle size (p=0.036). Functional testing indicated that LDL particles had a significantly lower susceptibility to oxidation, while HDL particles gained antioxidant capacity after the probiotic intake.

Conclusions: AB-LIFE® interferes with enterohepatic circulation of bile salts and improves plasma lipid profile in overweight subjects, reducing ApoB and increasing average LDL-C particle size.

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Poster #65 IAC

Acute physiological effects following *Bacillus subtilis* DE111® oral ingestion – a randomized, double blinded, placebo-controlled study in ileostomy participants.

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Abstract: A randomized, crossover, double-blind, placebo-controlled, single-site clinical trial was previously conducted with 11 ileostomy participants to investigate the germination and physiological activity of the spore-forming probiotic Bacillus subtilis DE111® in the small intestine. The protocol was approved by the Clinical Research Ethics Committee of Cork Teaching Hospitals (ECM 4 (d) 05/05/2020), registered on clinicaltrials.gov (NCT04489810) and followed the principles of the WMA Declaration of Helsinki and ICH-Good Clinical Practice guidelines. We demonstrated that DEIIII® can germinate in the small intestine as early as 4 hours after ingestion. In the current study, metabolomics, proteomics and sequencing technologies, enabled further analysis of these samples at this timepoint. Between groups, DE111® increased concentrations of the polyphenols trigonelline and 2,5-dihydroxybenzoic acid, orotic acid, the non-essential amino acid cystine and the lipokine 12,13-diHome, while reducing the neurotransmitter acetylcholine as compared to placebo. Within the *B. subtilis* DE111® treatment group the expression of leucocyte recruiting proteins, antimicrobial peptides and intestinal alkaline phosphatases was increased in the small intestine. Similarly, the expression of the proteins phosphodiesterase ENPP7, ceramidase ASAH2 and the adipokine Zn-alpha-2-glycoprotein involved in fatty acid and lipid metabolism were increased within the DE111® treatment group. Acute B. subtilis DE111® ingestion had limited detectable effect on the microbiome, with the main change being its increased presence. This is the first time such acute physiological findings have been demonstrated in the small intestine and support previous data suggesting a beneficial role of DEIII® in digestion, metabolism, and immune function that appears to begin within hours of consumption.

Poster #66 IAC

In vitro and in silico assessment of probiotic and functional properties of Bacillus subtilis DE111®

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Abstract: A previous study demonstrated the ability of the commercially available *B. subtilis* DEIII® to germinate within the human gastrointestinal tract as early as 3 hours following ingestion, but the mechanisms for its survival were unknown. Thus, this study explored the methods employed by this sporeforming probiotic to survive the digestive tract through a combination of metabolomic, enzymatic, and genomic analyses, as well as the potential health benefits conferred to the host.



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Genome mining revealed the presence of genes encoding proteins involved in acid and stress tolerance pathways, as well as those involved in adhesion. These included multi subunit ATPases, arginine deiminase (ADI) pathway genes (argBDR), stress (GroES/GroEL and DnaK/DnaJ) and extracellular polymeric substances (EPS) biosynthesis genes (pgsBCA). In vitro analysis revealed the broad antagonistic effects of DEIIII® towards pathogens of the skin, urinary tract, and gastro-intestinal tract, with in silico methods revealing anti-microbial bacteriocin genes in parallel. Genomic analysis further revealed the presence of enzyme-coding genes responsible for the synthesis of beneficial B-vitamins (thiamine, riboflavin, pyridoxin, biotin, and folate), vitamin K2, and seven amino acids including five essential amino acids (threonine, tryptophan, methionine, leucine, and lysine), as well as enzymes involved in the catabolism of dietary components (protease, lipases, and carbohyrolases). In vitro work confirmed an ability to degrade carbohydrates and lipids, as well as a high proteolytic capacity of DE111® against a milk substrate, leading to amino acid and short-chain fatty acid (SCFA) release. Antioxidant potential was observed both during the genetic analysis and via a laboratory total antioxidant capacity assay kit. Thus, the mechanisms by which DE111® is capable of surviving the harsh conditions encountered following ingestion and the potential benefits conferred therein have been investigated and confirmed, genetically and under laboratory conditions, supporting the use of DE111® as a nutrient supplement and its potential use in the preparation of functional foods.

Poster #67 IAC

Immunomodulatory and Antioxidant Properties of a Novel Potential Probiotic Bacillus clausii CSI08

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Abstract: Spore forming bacteria of the *Bacillus* genus have demonstrated potential as probiotics for human use. *Bacillus clausii* have been recognized as efficacious and safe agents for preventing and treating diarrhea in children and adults, with pronounced immunomodulatory properties during several *in vitro* and clinical studies. The novel strain of *B. clausii* CSI08 (Munispore®) was characterized for probiotic attributes such as resistance to gastric acid and bile salts, the ability to suppress the growth of human pathogens, the capacity to assimilate wide range of carbohydrates and to produce potentially beneficial enzymes. Both spores and vegetative cells of this strain were able to adhere to a mucous-producing intestinal cell line and to attenuate the LPS- and Poly I:C-triggered pro-inflammatory cytokine gene expression in HT-29 intestinal cell line. Vegetative cells of *B. clausii* CSI08 were also able to elicit a robust immune response in U937-derived macrophages. Furthermore, *B. clausii* CSI08 demonstrated cytoprotective effects in *in vitro* cell culture and *in vivo C. elegans* models of oxidative stress. Taken together these beneficial properties provide strong evidence for *B. clausii* CSI08 as a promising potential probiotic.



Poster #68 IAC

Bacillus megaterium Renuspore® as a potential probiotic for gut health and detoxification of unwanted dietary contaminants

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Abstract: While the rapid industrialization and urbanization of the last century have improved many facets of everyday life, human health has also been negatively impacted by the associated contamination. Bioaccumulation of these toxins in the environment result in gastrointestinal disorders, oxidative stress, DNA damage, inflammation, and chronic illnesses. Several pollutants that are highly toxic and difficult to degrade including heavy metals and xenobiotics such as flavorings, food additives, pesticides, and industrial chemicals are detectable in water and food. The use of probiotics is considered an economical and versatile tool for the detoxification of hazardous chemicals that are persistent in the environment and food chain, potentially for scavenging unwanted xenobiotics in the gut. In this study, the ability of Bacillus megaterium MIT411 (Renuspore®) to detoxify several environmental contaminants that can be found in the food chain was studied. The bacillus strain was additionally characterized for general probiotic properties including antimicrobial activity, dietary metabolism, and antioxidant activity. In silico studies revealed genes associated with carbohydrate, protein and lipid metabolism, xenobiotic chelation or degradation, and antioxidant properties. Bacillus megaterium MIT411 (Renuspore®) exhibited antimicrobial activity against opportunistic and zoonotic pathogens of the gut, the urinary tract, and the skin such as Escherichia coli, Salmonella enterica, Staphylococcus aureus, and Campylobacter jejuni and additionally demonstrated high levels of total antioxidant activities compared to L. rhamnosus GG. The metabolomic analysis revealed the proteolytic capability of Renuspore® toward milk proteins generating a diverse range amino acids and beneficial short-chain fatty acids (SCFAs). Moreover, Renuspore® effectively chelated the heavy metals, mercury and lead, without negatively impacting the beneficial minerals, iron, magnesium, or calcium, and degraded the environmental contaminants, nitrite, ammonia, and 4-Chloro-2-nitrophenol. These findings suggest that Renuspore® may play a beneficial role in supporting gut health metabolism and eliminating unwanted dietary contaminants.

Poster #69 IAC

Development of a multispecies probiotic formulation for use in recurrent urinary tract infections.

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Abstract: Recurrent urinary tract infections (rUTI) are a frequent and difficult to treat medical condition of especially premenopausal women worldwide. rUTIs significantly affect the quality of life of individual patients and represent a major financial burden to healthcare systems. Current treatments heavily rely on antibiotics, lacking innovation and evidence-based alternatives. The interplay between urinary, vaginal, and gut microbiota is increasingly recognized as a key factor in rUTI pathophysiology, opening avenues for



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microbiota-based interventions, specifically probiotics. In this study we aimed to develop a multispecies probiotic formulation for oral use against rUTI based on three main mechanisms of action; 1. inhibition of uropathogens that colonize the gastro-intestinal tract, 2. ability to produce acid to acidify the environment, also inhibiting pathogens to grow and 3. ability to survive the gastro-intestinal tract. Based on the above criteria 6 strains were selected as able to acidify the environment as well as survive their passage through the entire intestinal tract. Most importantly, these strains showed effectiveness in inhibit growth of uropathogens *Escherichia coli* (UPEC), *Klebsiella pneumonia, Proteus mirabilis* and *Enterococcus faecalis*. In addition, the selected strains showed efficacy to inhibit the vaginal pathogens *Candida albicans, Candida glabrata*, and *Gardnerella vaginalis*. Last, to enhance the formulation's effectiveness, functional ingredients including cranberry extract, D-mannose, and vitamin D were incorporated. In conclusion, we were able to design a new probiotic formulation that based on *in vitro* data is effective against rUTI. The formulation's incorporation of multiple strains and functional ingredients aligns with the variability of individual microbiomes, providing a versatile, promising approach for a diverse patient population for this prevalent and burdensome condition. This new probiotic formulation will be studied for its effectiveness in both an open label observational study and in a RCT aiming at supporting premenopausal

Poster #70

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

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



Antibiotic susceptibility profiles of lactobacilli isolated from artisanal Minas cheese

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Abstract: Lactobacilli are the lactic acid bacteria most commonly isolated from artisanal Minas cheese. Their extensive use by industry is related to their long history safe use in food. Despite the Qualified Presumption of Safety (QPS) and Generally Recognized as Safety (GRAS) status of lactobacilli, it is crucial to conduct studies to confirm safety at strain level, especially with respect to ensuring the absence of antimicrobial resistance. In this context, this study aimed to evaluate the antimicrobial susceptibility profiles of genetically non-redundant lactobacilli isolated from cheeses (n=26) produced in the Canastra and Serro regions, Minas Gerais State. For this, 126 lactobacilli colonies grown on MRS agar were selected and transferred to 3 mL of 0.85% saline solution resulting in a suspension at about 108 CFU/mL. Then, the cell suspensions were streaked onto Mueller Hinton agar plates using a sterile swab, and antibiotic discs of β lactams (penicillin G and ampicillin), aminoglycosides (gentamicin and streptomycin), macrolide (erythromycin), glycopeptides (vancomycin), sulphonamide (sulfamethoxazole/trimethoprim), chloramphenicol, and tetracycline were placed on top of the inoculated culture medium. Afterward, the plates were incubated at 35±2°C for 48 hours. The diameters of the inhibition zones (in mm) were measured using a digital calliper, and the results were interpreted according to the Bauer et al. (1966) and Santos et al. (2020). Staphylococcus aureus ATCC 25923 was used as a control. The profiles obtained showed 99 strains (78.6%) were resistant to vancomycin (an antibiotic to which many lactobacilli are naturally resistant), 56 (44.40%) to streptomycin, 17 (13.50%) to penicillin G, 13 (10.31%) to sulfamethoxazole/trimethoprim, 8 (6.35%) to tetracycline, 7 (5.55%) to gentamicin and erythromycin, 3 (2.40%) to ampicillin and 1 (0.79%) to chloramphenicol. Only one (0.79%) of the lactobacilli showed susceptibility to all antibiotics tested. Fifteen (11.9%) of lactobacilli demonstrated multidrug resistance. All isolates (100%) showed susceptibility to at least two antimicrobials from different classes. This study revealed the antibiotic resistance of lactobacilli isolated from artisanal cheeses; it can serve as a criterion for selecting lactobacilli strains to be used by the food industry as starter cultures or for further investigations to assess their probiotic potential.

Poster #72

In situ phytate degradation by *Bifidobacterium* spp. as a novel approach to tackle micronutrients deficiencies in early life.

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Poster #72 cont.

Abstract: Phytic acid is the main form of phosphorous storage in plant seeds. Due to its polyanionic nature, phytic acid chelates mineral cations such as iron, zinc, calcium and magnesium, both in food matrices and in the stomach, resulting in insoluble phytate salts. Monogastric animals, like humans, lack sufficient intestinal phytase enzymes to break down phytates, which pass undigested through the gastrointestinal tract, impeding the absorption minerals complexed to it. In low- and middle-income countries (LMICs), where complementary foods are mainly plant-based, such as cereal or pulse porridges, phytate significantly contributes to micronutrient deficiencies, especially in weaning children.

Phytase encoding genes have been identified in a variety of microorganisms (e.g., *Aspergillus niger, E.coli*), however only a few phytase degrading strains have been identified among *Bifidobacterium* spp., with *B. longum* subsp. *infantis* ATCC15697 being among the most active *Bifidobacterium* described. Being coloniser of the infant gut, Bifidobacteria could find valuable applications as probiotics for early life nutrition. Sufficient phytase activity in the gut could allow for *in situ* phytate degradation and offer an innovative approach to improve mineral absorption from plant-foods.

In this study, a collection of 50 *Bifidobacterium* spp. strains (species *B. longum*, *B. catenulatum*, *B. pseudocatenulatum*, *B. breve*, *B. bifidum*) was evaluated for phytase activity *in vitro*. Phytase producing strains were identified among members of the *B. longum* subsp. *longum*, *B. longum* subsp. *infantis* and *B. catenulatum* species, exhibiting comparable phytase activity to the reference strain *B. longum* subsp. *infantis* ATCC15697. The strains were sequenced and annotated, allowing bioinformatic identification of candidate phytase genes. Genes encoding candidate phytases were cloned and heterologously expressed in the host *B. breve* UCC2003, which lack phytase activity, and the resulting recombinant strains were assessed for their phytase activity. Genes encoding phytases were identified in *B. longum* subsp. *longum* NCIMB 8809, *B. longum* subsp. *infantis* MB0047, and *B. catenulatum* subsp. *kashiwanohense* APCKJ1 and are being caracterised. These findings deepen our understanding of phytase distribution and activity in the *Bifidobacterium* genus, laying the groundwork for tailored solutions to enhance micronutrient absorption from phytate-rich foods.

Poster #73

Conducting overviews, integrative reviews, systematic reviews and meta-analyses to determine health benefits of probiotics: Personal experiences.

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Abstract: The main intention of systematic reviews and meta-analyses are to establish recommendations for clinicians regarding the health benefits of probiotic consumption. After conducting several reviews and meta-analyses it was found that the health benefit of probiotic administration was strain-specific in many instances. This is in line with the recently published consensus statement on the recommendations to improve the quality of probiotic systematic reviews with meta-analyses. Another important challenge was the lack of strain information in clinical studies and the lack of using recent taxonomic updates in nomenclature. In the conducted meta-analysis on the effect of probiotics for preventing and treating infant regurgitation it was found that some clinical trials were more successful than others in showing a beneficial effect of probiotics. It was found that *Limosilactobacillus reuteri* DSM 17938 was most efficient. The conducted meta-analysis on the effectiveness of single-strain probiotic lactobacilli in reducing atopic dermatitis severity in children found that strains of *Limosilactobacillus reuteri* were more successful than strains of *Lactiplantibacillus plantarum*, *Lacticaseibacillus paracasei* or *Lacticaseibacillus rhamnosus*.



Poster #73 cont.

In the conducted meta-analysis on the effect of probiotics on maternal depression it was found that probiotics can improve maternal mental health, however, careful consideration must be given to correct strain selection. Clinical evidence in the conducted review showed that supplementation with various strains of Weizmannia coagulans (previously Bacillus coagulans) resulted in various statistically significant health effects in the probiotic groups, including relieving symptoms of irritable bowel syndrome, constipation, diarrhoea, and others. The overview of the antiviral effects of probiotics against rotavirus gastrointestinal infections in children found that the main probiotics that were effective were Saccharomyces cerevisiae var. boulardii, Lacticaseibacillus rhamnosus GG, and various multi-strain probiotics. The systematic review on the effectiveness of probiotics for the prevention of acute respiratorytract infections in older people also found that certain probiotic strains, such as Lactobacillus delbrueckii subsp. bulgaricus OLL1073R-1 and Bacillus subtilis CU1 were better than a placebo in lowering the incidence of acute upper respiratory tract infections in older people, whilst other probiotics, including Lacticaseibacillus rhamnosus GG did not exhibit statistically significant outcomes. Therefore, careful consideration to strain selection, probiotics quality, treatment time and patient age are important factors that need to be taken into consideration when comparing the effectiveness of various probiotics. Generalising conclusions is not evidence-based healthcare. Larger, robust, well-designed clinical studies with correct information on the probiotic strains used and addressing all factors are warranted to support the clinical evidence.

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Poster #75

Bridging the Gut-Brain Axis: Evaluating the Psychobiotic Potential of Yeasts through a Multifaceted Approach

Authors and affiliations (<u>presenter underlined</u>): <u>Amir Shazad</u>, PhD scholar, Libera Università di Bolzano, Italy, 39100 Email: <u>ashazad@unibz.it</u> PhD supervisor: Raffaella Di Cagno

Abstract: Mental disorders, notably anxiety and depression, have surged by 25% globally after COVID-19 pandemic. The microbiota-gut-brain axis, highlighting the connection between gut microbiota and neurological function, has gained significant attention for its potential in mental health interventions. Psychobiotics, which are microorganisms offering mental health benefits, represent a promising avenue for therapeutic exploration. While much research has focused on the psychobiotic potential of lactic acid bacteria, this study has highlighted the comparable capabilities of yeasts in modulating mental health. To elucidate this, ten yeast strains from diverse food sources were subjected to preliminary screening to assess growth adaptation, gastrointestinal transit resistance, β -glucosidase and phytase activity. The molecular characterization of the glutamic acid decarboxylase gene in all yeasts was conducted to investigate gamma-aminobutyric acid synthesis potential. High performing yeast strains underwent psychobiotic screening including phenotypic microarray analysis, short-chain fatty acids and neurotransmitters production. Results revealed diverse functional traits among yeast strains, with notable psychobiotic characteristics observed in selected strains. This multifaceted approach provides valuable insights into the psychobiotic potential of yeasts, highlighting their suitability for further exploration in mental health applications.

Poster #76

Early exposure of probiotics and gut microbiome in the TEDDY Study

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Poster #76 cont.

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Abstract: Objective: To study gut microbiota as a candidate mediator to explain the association between use of probiotics (<28days of age) and a lower risk of islet autoimmunity (IA) observed in The Environmental Determinants of Diabetes in the Young (TEDDY) study.

Methods: TEDDY is a prospective cohort study examining environmental risk factors of IA and Type 1 Diabetes (TID) in the US, Finland, Germany, and Sweden. Infant probiotic use from supplements was reported primarily in Finland and Sweden. In two nested case-control (NCC) studies examining IA and TID, the bacteria and viruses in monthly stool samples were determined from enrollment (age 3-4.5 months) till event time (mostly \geq 9 months). Controls were selected from the TEDDY cohort matching by study site, sex, and family history of TID. In this study, the controls from Finland (n=122) and Sweden (n=136) had their probiotic use by 28 days and between 28 and 90 days of age examined for impact on corresponding bacterial species between 3 and 9 months of age. In turn, bacterial species were examined in relation to frequent stool viruses. All associations were assessed by random effect models with logit link adjusting for NCC matching factors, age of stool collection and season of birth. Associations are reported as child specific odds ratios (OR with 95%CI).

Results: Probiotic use before 28 days of age and between 28 and 90 days of age were reported by 27% (33/122) and 9% (11/122) of Finnish children, and 4% (5/136) and 13% (17/136) of Swedish children, respectively. *Lactobacillus reuteri* was the most used probiotic species in Finland 71% (31/44) and Sweden 86% (19/22). Use of probiotic *L. reuteri* by 90 days significantly increased the odds of *L. reuteri* (>5 reads per million) in stools (OR=3.7, 95%CI=1.5–8.5, p=0.003). However, the introduction to *L. reuteri* in 28-90 days showed a declining impact with age when compared to children who received no probiotics by 90 days (interaction with age, p=0.01). *L. reuteri* correlated with lower odds of genus *Enterovirus* in stool (OR=0.42, 95%CI=0.21–0.84, p=0.01), in Finland (OR=0.35, 95%CI=0.13-0.97) and Sweden (OR=0.53, 95%CI=0.20-1.40).

Summary: Introduction to probiotic *L. reuteri* has a substantial impact on the respective species in the gut, which remained until 9 months of age if introduced before 28 days of age. As a reported risk component of IA, *Enterovirus* in stool may be the potential factor explaining the early probiotics correlation with a lower risk of IA.



Exploring the probiotic impact of orally administered lactic acid bacteria on the upper respiratory tract of children with otitis media

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Abstract:

Background: In children, otitis media (OM) drives antibiotic prescriptions, varying from 56% in the Netherlands to 95% in the USA, while for OM an upper limit of 20% prescriptions is advised by clinical guidelines. Microbiome studies suggest lactic acid bacteria (LAB) as intrinsic URT members, and their presence/abundance is linked to reduced otitis media with effusion (OME) in children. Moreover, meta-analyses propose LAB intake may alleviate upper respiratory tract (URT) infections like OME, but most studies focus on oral administration targeting the gut whereby effects are mediated via the gut-lung axis.

Methods: In an exploratory study, *Lacticaseibacillus rhamnosus* GG and *Bifidobacterium lactis* BB-12 (BB12) were orally administered in oil droplets in children (n = 38) with OME daily for 4 weeks prior to a tympanostomy. Samples from the nasopharynx, adenoids, right and left middle ear were collected. We monitored translocation of the applied probiotics in the droplets towards the URT via qPCR with species-specific primers and the microbiome of the different sampled niches via 16S rRNA gene sequencing.

Results: Orally administered *L. rhamnosus* GG was detected via qPCR in 50% of the nasopharynx samples (up to 10^5 CFU/sample) in the treated patients, while BB-12 could not be detected. No significant change was observed by 16S amplicon sequencing in the colonization by OM pathogens and LGG was found in the middle ear samples after the intervention, implicating the potential of LGG as a probiotic for OME.

Conclusion & future perspectives: Based on our findings, a new intervention with LGG and BB12 in cleft palate patients (n = 40) is currently planned, since these patients are prone to chronic OME. This planned study aims to longitudinally follow-up the microbiome in this patient group where the URT anatomy is seriously impacted and study the impact of the intervention on the URT microbiome. Besides, as clinical outcome measure, we will monitor the amount of OM episodes throughout the study.



Comparing the effects of probiotic *Bacillus subtilis* DE111 administration alone to combined bacteriophage and probiotic administration on metabolic markers and gut microbiota.

Authors and affiliations (<u>presenter underlined</u>): <u>Williams N, Lee S</u>, Ghanem N, Vazquez A, Wei Y, Johnson SA, Weir TL

Department of Food Science and Human Nutrition, Colorado State University, USA

Abstract:

Background: The gut microbiota is an important modulator of human health, making probiotic interventions an attractive option for supporting gastrointestinal (GI), cardiovascular, and immune system health. *Bacillus subtilis* is a spore-based probiotic that has shown great potential in improving mild-to-moderate gastrointestinal distress and reducing systemic inflammation. We hypothesized that the co-administration of *B. subtilis* DE111 with a commercial cocktail of *Escherichia coli*-targeting bacteriophages (PREforPRO) could exhibit enhanced beneficial effects over the probiotic administered alone.

Methods: Here we present results of a double-blinded, placebo-controlled parallel-arm probiotic intervention trial, where participants consumed (1) *B. subtilis*, (2) *B. subtilis* + PreforPro bacteriophages, or (3) a maltodextrin-based placebo for 6 weeks. The study population included healthy adults, some of whom had self-reported mild-to-moderate gastrointestinal distress. Stool and blood samples were collected at baseline and post-treatment. Primary outcomes included self-reported symptom severity and bowel habits captured via daily stool records using the Bristol Stool Scale. Secondary outcomes included composition of the gut microbiota and fecal SCFAs and blood markers of inflammation and gut barrier function (CRP, lipid profile, HbAlc, zonulin, and cytokines).

Results: Individuals on *B. subtilis* treatment alone had a reduced proportion of abnormal stools (g=0.33), and this effect was amplified when looking at only the subset of participants reporting abnormal bowel movements at baseline (g=.69). There was also a significant main effect for time related to the average number of weekly bowel movements reported (p<0.001), and significantly reduced total stools in both B. subtilis with and without bacteriophages at varying times after baseline. There were no significant changes in the gut microbiota composition, determined by alpha and beta diversity parameters, confirming previously published results suggesting no global microbiota disruption with the use of these supplements. Analysis of other secondary outcomes is ongoing.

Conclusions: Both interventions were safe and tolerable and can be used to support gastrointestinal health in individuals with mild GI distress. *B. subtilis* DE111 administered alone seems to have a greater influence on GI symptoms than when administered in combination with bacteriophages. Future subgroup analyses may be beneficial to establish who would experience the greatest benefits from consuming these supplements.

Breast-milk derived *Bifidobacterium longum* subsp. *infantis* CCFM1269 regulates intestinal Th1/Th2 immune balance in early life

Authors and affiliations (<u>presenter underlined</u>): <u>Bo Yang</u>^{1,2,3} *, Mengfan Ding^{1,2} , Bowen Li^{1,2} , R. Paul Ross^{3,4}, Catherine Stanton^{3,4,5}, Jianxin Zhao^{1,2,3} , Hao Zhang^{1,2,3} , Wei Chen^{1,2,3}

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Abstract:

Background and Rationale: *Bifidobacterium longum* subsp. *infantis*, a dominant colonizer of the infant gut, is closely associated with the incidence of infant diseases, particularly allergies and asthma, due to its impact on the balance of Th1/Th2 immune response.

Objectives: This study aimed to investigate the ability of eight different strains of *B. longum* subsp. *infantis* to regulate the Th1/Th2 balance and IgA secretion.

Methodology: The strains were administered orally to both female (n=8) and male (n=8) BALB/C dams, starting from 1 week to 3 weeks old. The levels of IgG2a, IgE, IL-4, IFN-γ, IgA, and sIgA were measured using an ELISA kit. RT-qPCR and Western blot analyses were performed to assess gene and protein expression. Additionally, 16S rRNA gene sequencing was conducted to examine the composition of the gut microbiota.

Results: The strains *B. longum* subsp. *infantis* 14MI and 18TI showed an increase in the colonic IgG2a/IgE ratio in male mice. CCFM1269 significantly increased the levels of colonic IFN-y and IgG2a, as well as the IgG2a/IgE ratio in female mice. It also significantly increased the IgG2a/IgE ratio and reduced the level of colonic IL-4 in male mice. Moreover, CCFM1269 was found to regulate the colonic JAK/STAT pathway in both male and female mice. CCFM1269, 14MI, and I10TI were able to increase colonic IgA levels in both females and males, while I8TI increased colonic IgA levels specifically in males. Additionally, CCFM1269 significantly increased colonic IgA levels specifically in males. Additionally, CCFM1269 significantly increased colonic slgA levels in females. CCFM1269, 14MI, 18TI, and I10TI also regulated IgA synthesis genes in the colon and Peyer's patch. Furthermore, I4MI, I5TI, and CCFM1269 increased the relative abundance of *Bifidobacterium* and *B. longum* subsp. *infantis* in both male and female mice, whereas I8TI only increased the relative abundance of *Bifidobacterium* and *B. longum* subsp. *infantis* in male mice. The diversity of slgA-coated bacteria in male mice was altered by I4MI, I5TI, I8TI, and CCFM1269, which also decreased the relative abundance of *Escherichia coli*.

Conclusions: These findings demonstrate that *B. longum* subsp. *infantis* can modulate the Th1/Th2 immune balance and promoting IgA levels, but its effects exhibit strain specificity. Moreover, the supplementation of different strains of *Bifidobacterium* in early infancy may lead to varying effects on IgA levels depending on dietary habits.





Daniel Merenstein, Georgetown University, ISAPP President

Dr. Daniel Merenstein, MD, is a Professor with tenure of Family Medicine at Georgetown University, where he also directs Family Medicine research. Dr. Merenstein has a secondary appointment in the undergraduate Department of Human Science, in the School of Health. Dr. Merenstein teaches two undergraduate classes, a research capstone and a seminar class on evaluating evidence based medical decisions. He has been funded by PCORI, NIH, USDA, foundations and industry. The primary goal of Dr. Merenstein's research is to provide answers to common clinical questions that lack evidence and improve patient care. Dr. Merenstein is a clinical trialist who has recruited over 2,000 participants for 10 probiotic trials since 2006. He is an expert on probiotics, on antibiotic stewardship in outpatient settings, and also conducts HIV research in a large women's cohort. He sees patients in clinic one day a week. Dan lives in Maryland with his wife and 4 boys.



Maria Marco, University of California, Davis, ISAPP Vice President

Dr. Maria Marco, PhD, is a Professor in the Department of Food Science and Technology at the University of California, Davis. She earned her bachelor's degree in microbiology at The Pennsylvania State University and her PhD in microbiology at the University of California, Berkeley. As a postdoc at NIZO food research in The Netherlands, she developed a love for lactic acid bacteria and the importance of these microorganisms in our foods and the digestive tract. Her postdoctoral studies led to the discovery that probiotics are metabolically active in the intestine and responsive to dietary intake. Dr. Marco started her lactic acid bacteria and gut health laboratory at UC Davis in 2008 and has built an internationallyrecognized, NIH, USDA, and NSF funded research program on probiotics, fermented foods, and dietary modulation of the gut microbiome. Dr. Marco also consults with and has received funding from international foundations and companies to investigate how certain microbes in foods or supplements may benefit health. She is active with science communication activities such as the EATLAC project and is the instructor for two food microbiology courses. Dr. Marco received the American Society for Microbiology Distinguished Lecturer award in 2012. Recently, she founded the ongoing Gordon Research Conference series on Lactic Acid Bacteria. Dr. Marco attended her first ISAPP meeting as a postdoc and participated as an invited expert before joining the ISAPP Board of Directors in 2019.



Gabriel Vinderola, Dairy Products Institute, Faculty of Chemical Engineering (UNL), ISAPP Secretary

Dr. Gabriel Vinderola, PhD, graduated from the Faculty of Chemical Engineering at the National University of Litoral (Santa Fe, Argentina) in 1997 and obtained his Ph.D. in Chemistry in 2002 at the same University. He is presently Principal Researcher at the Dairy Products Institute (CONICET-UNL) and Adjoint Professor at the Biotechnology and Food Technology Department of the Faculty of Chemical Engineering (National University of Litoral). He participated in the development of the first commercial cheese carrying probiotic bacteria from Latin America, released in the market in 1999. In 2011, he was awarded the prize in Food Technology for young scientists, by the National Academy of Natural, Physic and Exact Sciences from Argentina. His interests are technological and microbiological aspects of lactic acid bacteria, bifidobacteria, fermented foods, and probiotics. He has joined several research groups in Brazil, Canada, Spain, Italy, France, Germany, and Finland. He has co-edited the fifth (2019) and the sixth (2024) editions of the book Lactic Acid Bacteria: Microbiological and Functional Aspects. He is engaged in communication of science to the general audience.





Daniel Tancredi, University of California, Davis, ISAPP Treasurer

Prof. Daniel J. Tancredi, PhD, is Professor in Residence of Pediatrics in the University of California, Davis School of Medicine. He has over 25 years of experience and over 300 peerreviewed publications as a statistician collaborating on a variety of health-related research. A frequent collaborator on probiotic and prebiotic research, he has attended all but one ISAPP annual meeting since 2009 as an invited expert. In 2020, he joined the ISAPP Board of Directors. Colin Hill and Daniel co-host the ISAPP Podcast Series "Science, Microbes, and Health". On research teams, he develops and helps implement effective study designs and statistical analysis plans, especially in settings with clusters of longitudinal or otherwise correlated measurements, including cluster-randomized trials, surveys that use complex probability sampling techniques, and epidemiological research. He teaches statistics and critical appraisal of evidence to resident physicians; graduate students in biostatistics, epidemiology, and nursing; and professional scientists. Dan grew up in the American Midwest, in Kansas City, Missouri, and holds a bachelor's degree in behavioral science from the University of Chicago and masters and doctoral degrees in mathematics from the University of Illinois at Chicago. He lives in the small Northern California city of Davis, with his wife Laurel Beckett (UC Davis Distinguished Professor Emerita), their Samoyed dogs Simka and Milka, and near their two grandkids.



Colin Hill, APC Microbiome Ireland

Prof. Colin Hill, PhD, DSc, has a Ph.D in molecular microbiology and is a Professor in the School of Microbiology at University College Cork, Ireland. He is also a founding Principal Investigator in APC Microbiome Ireland, a large research centre devoted to the study of the role of the gut microbiota in health and disease. His main interests lie in the role of the microbiome in human and animal health. He is particularly interested in the effects of probiotics, bacteriocins, and bacteriophage. In 2005 Prof. Hill was awarded a D.Sc by the National University of Ireland in recognition of his contributions to research. In 2009 he was elected to the Royal Irish Academy and in 2010 he received the Metchnikoff Prize in Microbiology and was elected to the American Academy of Microbiology. He has published more than 600 papers and holds 25 patents. He was president of ISAPP from 2012-2015. More than 80 PhD students have been trained in his laboratory. <u>Google Scholar</u>.



Sarah Lebeer, University of Antwerp

Prof. Sarah Lebeer, PhD, is a research professor at the Department of Bioscience Engineering of the University of Antwerp, Belgium. She has studied bioscience engineering, with a specialisation in cell and gene technology/food & health, and obtained her Master at KU Leuven (Belgium). In 2008, she obtained a PhD degree with a topic on the mode of action of gastro-intestinal probiotics in inflammatory bowel diseases and a scholarship in the team of Prof. Jos Vanderleyden (KU Leuven). After a postdoc on the interaction between lactobacilli, viruses, and mucosal immunology, in November 2011, she was offered a tenure-track position at the University of Antwerp. Since then, she is leading the Laboratory for Applied Microbiology and Biotechnology. In 2020, she was awarded an ERC Starting Grant that enables her to gain more in-depth knowledge of the evolutionary history and ecology of lactobacilli. This rationale was also an important driving force to revise the Lactobacillus genus taxonomy with a large international consortium. Within the ERC project, Sarah has also launched the Isala citizen-science project to gain new insights into the role of vaginal lactobacilli for women's health. Since 2018, Sarah is an academic board member of the International Scientific Association on Probiotics and Prebiotics. Communicating about beneficial microbes and probiotics for experts and laymen is an important inspiration for her daily work.





Geoffrey Preidis, Baylor College of Medicine and Texas Children's Hospital, USA

Dr. Geoffrey A. Preidis, M.D., Ph.D., Associate Professor of Pediatrics at Baylor College of Medicine in Houston, Texas, is a board-certified Pediatric Gastroenterologist with basic and translational research expertise in nutrition-sensitive mechanisms that regulate intestinal and liver physiology, growth, and development. Based at Texas Children's Hospital, which contains the largest neonatal intensive care unit in the United States, Dr. Preidis explores how the intestinal microbiome develops in extreme prematurity and how probiotics might be used to reduce the risk of necrotizing enterocolitis, sepsis, feeding intolerance, growth failure, and death in this vulnerable population. His biomedical research career began during undergraduate studies at Harvard University. He developed an interest in Neonatal Gastroenterology while in the Medical Scientist Training Program at Baylor College of Medicine. He completed internship and residency training in Pediatrics, as well as fellowship training in Pediatric Gastroenterology, Hepatology & Nutrition, at Baylor College of Medicine.

Eamonn Quigley, Houston Methodist Hospital and Weill Cornell Medical College

Dr. Eamonn M M Quigley MD FRCP FACP MACG FRCPI MWGO is David M Underwood Chair of Medicine in Digestive Disorders and Chief of the Division of Gastroenterology and Hepatology at Houston Methodist Hospital. A native of Cork, Ireland, he graduated in medicine from University College Cork. He trained in internal medicine in Glasgow, completed a two-year research fellowship at the Mayo Clinic, and training in gastroenterology in Manchester, UK. He joined the University of Nebraska Medical Center in 1986 where he rose to become Chief of Gastroenterology and Hepatology. Returning to Cork in 1998 he served as Dean of the Medical School and a PI at the Alimentary Pharmabiotic Center. He served as president of the American College of Gastroenterology and the WGO and as editor-in-chief of the American Journal of Gastroenterology. Interests include IBS, gastrointestinal motility and the role of gut microbiota in health and disease. He has authored over 1000 publications and has received awards and honorary titles world-wide. Married for over 40 years to Dr Una O'Sullivan they have 4 children and 5 grandchildren. Interests outside of medicine include literature, music and sport and rugby, in particular; Dr Quigley remains a passionate supporter of Munster and Irish rugby.



Seppo Salminen, University of Turku, ISAPP Past President

Prof Seppo Salminen, PhD, is a Professor at the Faculty of Medicine and director of the Functional Foods Forum, University of Turku, Finland. He has been visiting professor at RMIT University, Melbourne, Australia, and BOKU University, Vienna Austria. His main research interests are probiotics, prebiotics, and intestinal microbiota modulation as well as functional foods and health and regulatory issues in novel foods and health claims. He has been active in the International Life Sciences Institute Europe, the International Dairy Federation, and the International Scientific Association for Probiotics and Prebiotics (past President and current Board Member). He received his MS at Washington State University (USA) in 1978, MSc from the University of Helsinki in 1979, and PhD from the University of Surrey (United Kingdom) in 1982. He has around 500 journal articles and several textbooks and book chapters and he has received several international awards including the ISF-Institute Pasteur Metchnikoff Price, Swiss Price on Modern Nutrition and the Grand Prix du Yoplait.



Karen Scott, Rowett Institute, University of Aberdeen

Prof. Karen Scott, PhD, is a Professor of Research in the Gut Microbiology Group at the Rowett Institute, University of Aberdeen. She leads a research team investigating the (molecular) mechanisms by which key members of the gut microbiota interact with the diet and host, at different life-stages. The fermentation products of gut bacteria contribute to gut health and are differentially expressed on different substrates, including prebiotics. In vitro bacterial growth studies utilising our large culture collection of gut anaerobes (in pure culture, mixed culture, fermentor systems, and also with human cells) and bioinformatic analyses illustrate niche-specific processes and bacterial interactions, identifying strains with potential for use as probiotic/live biotherapeutic products. She has numerous highly cited publications and has attracted multiple research council and commercially funded grants.





Kelly Swanson, University of Illinois

Prof. Kelly Swanson, PhD, is Director of the Division of Nutritional Sciences and the Kraft Heinz Company Endowed Professor in Human Nutrition at the University of Illinois Urbana-Champaign. His laboratory studies the effects of nutritional intervention on health outcomes, identifying how nutrients impact host physiology and gut microbiota, with primary emphasis on gastrointestinal health and obesity in dogs, cats, humans, and rodent models. Much of his lab's work has focused on dietary fibers, prebiotics, probiotics, synbiotics, and postbiotics. Over the past 2 decades, he has established an internationally recognized research program, highlighted by approximately \$26 million in research support, over 160 invited lectures at scientific and professional meetings, 260 peer-reviewed journal articles, and 17 research and teaching awards. He has trained nearly 50 graduate students and post-doctoral fellows, hosted over 15 international visiting scholars, and mentored 40 undergraduate research projects. In addition to research, Kelly teaches 3-4 classes each year to undergraduate and graduate students and has been named to the university's 'List of Teachers Ranked as Excellent by Their Students' over 30 times. He also serves on advisory boards for many companies in the human and pet food industries as well as non-profit organizations, including the Institute for the Advancement of Food and Nutrition Sciences (IAFNS).



Hania Szajewska, The Medical University of Warsaw, Department of Paediatrics

Prof. Hania Szajewska, MD, PhD, is Professor and Chair of the Department of Paediatrics at the Medical University of Warsaw. Among her various functions, she served as the Editor-in-Chief of the Journal of Pediatric Gastroenterology and Nutrition; a member of the Council, and then as the General Secretary of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN); the Secretary of the ESPGHAN Committee on Nutrition. Most recently, she joined the Board of Directors of the International Scientific Association for Probiotics and Prebiotics (ISAPP). Prof. Szajewska has broad interests in paediatric nutrition but her research focuses on the effects of early nutritional interventions on later outcome (especially food allergy); and the gut microbiota modifications such as with various biotics (probiotics, prebiotics, synbiotics, postbiotics). She is or has been actively involved in several European Union-funded research projects. She is an enthusiastic advocate for the practice of evidence-based medicine. She has co-authored more than 350 publications. In 2020 and 2021, Prof. Szajewska has been ranked as one of the world's top 2% most-cited researchers.



Kristin Verbeke, KU Leuven

Prof. Kristin Verbeke, Pharm, PhD, graduated from the KU Leuven, Belgium as a pharmacist in 1991. She obtained a PhD in Pharmaceutical Sciences at the Laboratory of Radiopharmaceutical Chemistry in 1995 and subsequently spend a postdoctoral period in developing radioactively labelled compounds. In 2002, she was appointed at the department of gastroenterology of the Medical Faculty of the Leuven University where she became involved in the use of stable isotope labelled compounds to evaluate gastrointestinal functions. Within the University Hospitals Leuven, she is responsible for the clinical application of diagnostic 13C- and H2-breath tests. Her current research interest specifically addresses the microbial bacterial metabolism in the human colon. Her team has developed several analytical techniques based on mass spectrometry and stable isotope or radioisotope technologies to evaluate several aspects of intestinal metabolism and function in humans (transit time, intestinal permeability, carbohydrate fermentation, protein fermentation, metabolome analysis). Collaborative research has allowed showing an aberrant bacterial metabolism in patient groups with end stage renal failure, inflammatory bowel diseases, irritable bowel disorders and alcohol abuse. These collaborations all have resulted in high quality peer-reviewed papers. In addition, she showed the impact of dietary interventions (modulation of macronutrient composition, pre- or probiotic interventions) on the microbial metabolism and its impact on health. As a PI, she acquired grant support from the university and different funding bodies and successfully completed these projects. Similarly, she supervised several PhD projects that all resulted in the achievement of a PhD degree. Her research resulted in over 200 full research papers. Together with colleague Prof. J. Delcour, she was the beneficiary of the W.K. Kellogg Chair in Cereal Sciences and Nutrition (2010-2020). She is the president of the Belgian Nutrition Society, the vice-chair of the Leuven Food Science and Nutrition Center, and the co-chair of the Prebiotic task force at ILSI Europe. Furthermore, Kristin Verbeke is the editor of the journal Gut Microbiome and member of the editorial board of Gastrointestinal Disorders.





Anisha Wijeyesekera, University of Reading

Dr. Anisha Wijeyesekera, PhD, is a Lecturer in Human Microbiome Studies and Director of Postgraduate Research Studies, in the Department of Food and Nutritional Sciences at the University of Reading, UK. Her research interfaces analytical chemistry with microbiology and nutrition, for functional assessment of the gut microbiota. Dr. Wijeyesekera's research includes in vitro as well as in vivo human studies. She applies metabolic profiling approaches to generate metabolic readouts of the gut microbiota using in vitro gut model systems, as well as molecular phenotyping of human biological samples for better insight into host-gut microbiota interactions. The ultimate aim of her research is to identify potential targets for therapeutic modulation through dietary intervention (in particular, prebiotics and probiotics), and capturing the impact of such interventions on gut and overall health. Dr. Wijeyesekera's research portfolio includes projects funded by research councils, charities/societies, and the food industry. Dr. Wijeyesekera joined the ISAPP Board of Directors in 2021. She is also a member of the Academic Board for the Royal Society of Chemistry's Community of Analytical Measurement Sciences, and the London Metabolomics Network Committee.



Marla Cunningham, ISAPP Executive Director

Marla Cunningham is the Executive Director of ISAPP. Prior to 2023, Marla was an industry scientist within the ISAPP community and brings over 20 years of experience in the probiotic, prebiotic, and natural products industry, working across innovation, clinical research, product development, regulatory compliance, and education. She trained as a healthcare practitioner in complementary medicine and has written and presented extensively for clinical audiences on the implementation of biotic-related science. Marla has broad scientific literacy across the fields of biotic substances, microbiome, gut health, nutrition, and human physiology, and brings a passion for harnessing science to deliver beneficial changes in health for our community and our planet.

ISAPP is pleased to announce the following Board Member positions for 2024-2025

Maria Marco, ISAPP President Sarah Lebeer, ISAPP Vice President Anisha Wijeyesekera, ISAPP Secretary Kelly Swanson, ISAPP Treasurer Daniel Merenstein, Past ISAPP President





Mariya Petrova, Senior IAC Representative 2023-2024

Dr. Mariya Petrova, PhD, is an internationally recognized expert in the field of human microbiota and probiotics. Mariya holds a PhD degree in Bioscience Engineering from KU Leuven, Belgium, and has worked closely with probiotic and microbiome organizations. In 2020 Mariya started working as Senior Scientific Educator and Senior Scientist at Winclove Probiotics. She is responsible for sharing her knowledge with health professionals, scientists, and business partners and bridging the gap between science and commerce. For the last two years she also served as ISAPP Industry Advisory Committee (IAC) representative to facilitate communication, represent industry viewpoints and promote collaboration between ISAPP board and industry members. Finally, she is also an independent consultant who leads her own consultancy, Microbiome Insights and Probiotics, working with various stakeholders.



Shalome Bassett, Junior IAC Representative 2023-2024

Dr. Shalome Bassett, PhD, is currently Programme Leader Scientific Affairs and Principal Scientist at Fonterra. She is a molecular microbiologist based at Fonterra's Research and Development Centre in Palmerston North, New Zealand, and an Affiliated Researcher at the Riddet Institute, Massey University. She has a diverse background in microbial research, having worked for over 30 years in various research, science management and technology development roles across academia, government, and the food industry. Over the past six years, she has established and led Fonterra's Probiotics Discovery and Kowbucha™ Research Programmes, where she's been responsible for developing new commercial probiotic strains for human health as well as a viable on-farm solution for reducing methane in cattle, respectively. In the past, she's also dabbled in various gut pathogens, plant microbiology (with PhD research on symbiotic fungal endophytes of forages) and the development of vaccines against bovine tuberculosis. Her current interests lie in the interactions between probiotic strains, diet and genetic background, and the gut microbiome for improved health outcomes. Shalome loves being able to build new collaborations and relationships and to communicate science to a wide range of people in a way that can interest and excite them. She has served as Junior IAC advisor to the ISAPP Board for the past year and moves into the Senior IAC representative position for 2024-2025.





Cathy Lordan, PhD, Teagasc and APC Microbiome Ireland, SFA President

Current Research: My research is based on human milk oligosaccharides (HMOs), found in breast milk, and their impact on the early life gut microbiota, with a specific focus on Bifidobacterium. I am looking at how HMOs impact both composition and functional potential of the infant microbial community, including the metabolites they produce as a result of HMO availability.



Brendan Daisley, PhD, University of Guelph, SFA Vice President

Research Interests: I am interested in how probiotic science can be applied to favourably impact human, wildlife, and overall planetary health. A major focus of my current work is to elucidate mechanisms by which beneficial strains of bacteria can directly and/or indirectly improve immunity, detoxification of pesticides, and the nutritional status of honey bees. Through this work, I hope to contribute to a sustainable future by developing a framework of how microbial-based solutions can be used to support the health of important pollinator insects that are critical to the global food supply.



Breanna Metras, University of Illinois Urbana Champaign, SFA Treasurer

Research Interests: I am interested in how fermented foods can affect the host gut microbiome and how current federal regulations impact fermented food production. A major focus of my current work is better understanding the differences between grain and commercially produced kefir. My research has consisted of microbial plating, animal models, and in vitro fermentation to determine how compositional and microbial differences in kefir may affect host health. Through this work, I hope to contribute evidence that shows improved regulations in labeling and characterizing fermented foods are needed so they might retain authenticity when produced large scale.



David Hourigan, APC Microbiome Ireland, SFA Secretary

Research Interests: My research is centered around the role of bacteriocins, which are small ribosomally encoded antimicrobial peptides produced by bacteria, and their subsequent role within the microbiome. My research focuses on exploiting their effectiveness as selective antibacterial agents to curate the microbiota to tackle AMR and climate change. Interests include functional, ecological, and evolutionary genomics, microbial ecology, and bacterial competition.



Sarah Ahannach, PhD, University of Antwerp, Belgium, SFA Director of Outreach and Community Engagement

Research interests: I am interested in the deeper understanding of women's microbiome and how microbial management and cutting-edge microbiome analyses can improve women's health and safety. A major focus of my work is studying the inner workings of the vaginal ecosystem (Isala project), vitamin-producing bacteria, and applying microbiome analysis to forensics with a focus on sexual assault cases (GeneDoe project). I hope to contribute to knowledge on the stability and dynamics of the female microbiome by presenting novel understandings for future intervention studies to unravel underlying mechanisms; the development of probiotics and biotherapeutics; and for developing novel tools that could be used in diagnostics and criminal investigations.



Patricia Sanz Morales, University of Reading, UK, SFA Director of Communications and Development

Current research: I am interested in understanding the prebiotic effects of human milk oligosaccharides (HMOs) in adult gut microbiomes, with a particular focus on Irritable Bowel Syndrome (IBS). I combine in vitro and in vivo approaches to assess the influence of HMOs on the gut microbiome through metabolic and microbial profiling, and subsequent impact on human health. I hope to provide some clarity on the potential of HMOs as therapy option in IBS through this work.

