THE 9TH BENEFICIAL MICROBES CONFERENCE

ABSTRACTS OF LECTURES & POSTERS

PRE- AND PROBIOTICS FOR LIFELONG Human AND Animal HEALTH

14-16 NOVEMBER 2022

AMSTERDAM

THE NETHERLANDS

www.BeneficialMicrobes2022.org

CONTENTS

THE LIST OF PARTICIPANTS CAN BE FOUND AT:

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ey to the abstracts of lectures and posters:

- abstracts of lectures and posters are grouped separately
- lectures are grouped according to the daily programme
- posters are grouped in an alphabetical order according to the presenting author

BENEFICIAL MICROBES CONFERENCE SERIES

9th BENEFICIAL MICROBES CONFERENCE

The **9th Beneficial Microbes Conference** will provide an overview of the latest scientific results and future developments related to beneficial microbes, including prebiotics, probiotics and postbiotics, and their importance to human and animal health across the lifespan.

The conference is unique in its kind. There are up to 50+ invited speakers, representing a great line-up of scientists with a strong track record in their respective field of research. Topics include beneficial microbes across the lifespan, from early life to healthy ageing; the gut-brain axis; personal care; renewable-based prebiotics; non-digestible carbohydrates in the gut microbiome (in co-operation with ILSI Europe); animal health and nutrition; and more.

The conference promotes the creation of new initiatives for the customised application of prebiotics, probiotics and postbiotics in food, feed, and healthcare in a networking environment. The conference topics are intended to meet the needs of researchers, food, feed, and healthcare professionals who want to be updated on the advances in prebiotics, probiotics and postbiotics research. Interested attendees include basic and translational research scientists, nutritionists, dietitians, physicians, students, policy makers, and key opinion leaders.

CONFERENCE CHAIR

Prof. Koen Venema	Maastricht University and Beneficial Microbes Consultancy, the Netherlands

ADVISORY BOARD

Dr Frédérique Chaucheyras-Durand	Lallemand, France
Dr Maria Carmen Collado	Department of Food Biotechnology, Spanish National Research Council, Spain
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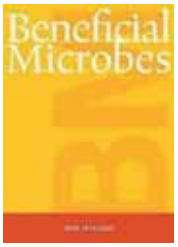

PROGRAMME AT A GLANCE

MONDAY 14 NOVEMBER 2022

13:00 – 13:15	Opening of the **9th Beneficial Microbes Conference**
13:15 – 14:15	Plenary session *Beneficial microbes – new and unexpected insights*
14:15 – 15:15	Plenary session *Postbiotics, definition and scope – a panel debate*
15:15 – 15:45	Networking break & poster viewing
15:45 – 17:25	Plenary session *Beneficial microbes and early life*
17:25 – 18:00	Sponsor pitches and Speed presentations *Short presentations by our sponsors and by selected poster presenters*
18:00 – 18:45	Poster viewing, drinks & snacks

TUESDAY 15 NOVEMBER 2022

08:45 – 10:30	Session 1 *Beneficial microbes in animal health and nutrition – Part 1*	Session 2 *Beneficial microbes and human health – Part 1*
10:30 – 11:00	Networking break & poster viewing	
11:00 – 12:45	Session 3 *Bacteriophage-bacteria interactions in the gut*	Session 4 *Beneficial microbes in personal care*
12:30 – 14:00	Lunch beak & poster viewing	
14:00 – 15:45	Session 5 *Beneficial microbes and the gut-brain axis*	Session 6 *Towards renewable-based prebiotics*
15:45 – 16:15	Networking break & poster viewing	
16:15 – 17:35	Session 7 *Beneficial microbes in animal health and nutrition – Part 2*	Session 8 *Beneficial microbes and human health – Part 2*

WEDNESDAY 16 NOVEMBER 2022

08:45 – 10:30	Plenary session *Structure and function of non-digestible carbohydrates in the gut microbiome* in co-operation with ILSI Europe
10:30 – 11:00	Networking break & poster viewing
11:00 – 12:00	Plenary session *Beneficial microbes and healthy ageing*
12:20 – 12:30	Top five lessons learned
12:30	Closing of the **9th Beneficial Microbes Conference**

MONDAY 14 NOVEMBER 2022

13:00 Introduction to the **9th Beneficial Microbes Conference**
Prof. Koen Venema, conference chair

PLENARY SESSION
BENEFICIAL MICROBES – NEW AND UNEXPECTED INSIGHTS

Chair: Prof. Koen Venema, Centre for Healthy Eating and Food Innovation, Maastricht University, the Netherlands

13:15 *Role of the lung microbiome in CNS autoimmunity*
Dr Francesca Odoardi, Institute for Neuroimmunology and Multiple Sclerosis Research, University Medical Center Göttingen, Germany

13:35 *Genetics and exposome factors shaping the Dutch microbiome landscape*
Prof. Alexandra Zhernakova, Department of Genetics, University Medical Center Groningen, the Netherlands

13:55 *Polyunsaturated fatty acids as prebiotics and the concept of the 'correct prebiotic diet': innovation or confirmation?*
Dr Lara Costantini, Department of Ecological and Biological Sciences, Tuscia University, Italy

PLENARY SESSION
POSTBIOTICS, DEFINITON AND SCOPE – A PANEL DEBATE
A panel debate on the International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics, and its caveats.

Chair: Prof. Koen Venema, Maastricht University, the Netherlands

14:15 *The rationale behind ISAPP's definition of postbiotics*
Dr Gabriel Vinderola, Instituto de Lactología Industrial (CONICET-UNL), National University of Litoral, Argentina

14:30 *Looking for 'solid' definitions*
Prof. Lorenzo Morelli, Department for Sustainable Food Processes, Università Cattolica del Sacro Cuore, Italy

14:45 Panel debate between the panellists and the audience
Panellists:
- Dr Gabriel Vinderola, University of Litoral, Argentina
- Prof. Seppo Salminen, Functional Foods Forum, University of Turku, Finland
- Prof. Lorenzo Morelli, Università Cattolica del Sacro Cuore, Italy
- Dr Guus Roeselers, Danone Nutricia Research, the Netherlands

15:15 – 15:45 **Networking break & poster viewing**

PLENARY SESSION
BENEFICIAL MICROBES AND EARLY LIFE

Chair: Dr Guus Roeselers, Danone Nutricia Research, the Netherlands

15:45 *On the origin of species – host, dietary and environmental factors shaping the early-life microbiome*
Dr John Penders, Department of Medical Microbiology, Maastricht University Medical Center, the Netherlands

16:05 *The ying and yang of microbiome signatures in early life*
Prof. Lindsay Hall, Quadram Institute, UK

16:25 *The respiratory microbiome in early life: key to infection protection*
Prof. Debby Bogaert, Centre for Inflammation Research, The University of Edinburgh, UK

16:45 *Using probiotics for maternal depression*
Prof. Sabina Fijan, Faculty of Health Sciences, University of Maribor, Slovenia

17:05 *The gut microbiota and the maternal immune response in pregnant mice; effect of antibiotics and probiotics*
Dr Marijke Faas, Department of Pathology and Medical Biology, University of Groningen and University Medical Center Groningen, the Netherlands

PLENARY SESSION
SPONSOR PITCHES AND SPEED PRESENTATIONS
Short presentations (5-minutes) by our sponsors and by selected poster presenters to inspire the audience to visit their booths and posters, respectively.

17:25 – 18:00

Chair: Dr Guus Roeselers, Danone Nutricia Research, the Netherlands

Sponsor pitches

- BaseClear, the Netherlands
- ILSI Europe, Belgium

Speed presentations

- *Longitudinal gut mycobiota changes in Japanese infants during first three years of life*
  Riko Mishima, Kyushu University, Japan
- *Short chain fatty acid inhibition of bacterial plasmid conjugation in broth and chicken ceca explants*
  Logan C. Ott, Iowa State University, USA
- *The role of galacto-oligosaccharides in the recovery from dysbiosis in patients on long-term atypical antipsychotic treatment*
  Nienke de Bles, Leiden University Medical Center, Leiden, the Netherlands
- *Study protocol: effects of butyrate on affective patterns, microbiome composition and depressive symptoms in young adults – a randomized clinical trial*
  Vera Korenblik, Amsterdam University Medical Center, the Netherlands
- *Lacticaseibacillus rhamnosus HA-114: an innovative probiotic strain to support weight management efforts through the gut-brain axis*
  Dr Mélanie Le Barz, Lallemand Health Solutions, Canada
- *A predictive model for microbiome-dependent response to dietary fibres bases on in vitro biological data predicts microbiota response to dietary intervention*
  Clémentine Thabuis, Roquette, France

18:00 – 18:45
Poster viewing, drinks & snacks

TUESDAY 15 NOVEMBER 2022

SESSION 1
BENEFICIAL MICROBES IN ANIMAL HEALTH AND NUTRITION – PART 1

Chair: Dr Frédérique Chaucheyras-Durand, Lallemand, France

08:45 *Evolution of the microbiome in female dogs around parturition and in early lactation, and impact of live yeast Saccharomyces boulardii CNCM I-1079*
Quentin Garrigues, École Nationale Vétérinaire de Toulouse, France

09:05 *Gut neuroimmune axis and the microbiota: elucidating crosstalk to control bacterial infections in poultry*
Dr Melha Mellata, Department of Food Science and Human Nutrition, Iowa State University, USA

09:25 *Plant extracts: gut microbiota and beyond*
Naiana Einhardt Manzke, Animal Health Concepts, the Netherlands

09:45 *King of the Hill – modulating the rainbow trout microbiome*
Dr Kasper Rømer Villumsen, Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark

10:05 *Probiotics impact on Tenebrio molitor performance, microbial composition and pathogen infection*
Carlotta Savio, INRAE, France

10:30 **Networking break & poster viewing**

SESSION 2
BENEFICIAL MICROBES AND HUMAN HEALTH – PART 1

Chair: Dr Emily Hollister, Diversigen Inc., USA

08:45 *Impact of a maternal supplementation with prebiotics on the microbiota, the immune system and allergy development in offspring*
Dr Carole Brosseau, Biopolymères Interactions Assemblages, INRAE, France

09:05 *Immunomodulation by biotics: preventive effect against rotavirus infection*
Prof. Francisco J. Pérez Cano, Department of Biochemistry and Physiology, University of Barcelona, Spain

09:25 *How we can treat irritable bowel syndrome and obesity by modulating the gut microbiota*
Dr Goran Hauser, Department of Gastroenterology, University of Rijeka, Croatia

09:45 *Clostridium butyricum MIYAIRI588® strain, as a potential live biotherapeutic product – stimulation of cancer immunotherapy*
Prof. Shigeru Kamiya, Department of Infectious Diseases, Kyorin University School of Medicine, Japan

10:05 Submitted abstracts:

- *Effect of gut microbiota of children with autism spectrum disorder on behaviour and ASD-related biological markers in germ-free mice*
  Léa Roussin, M.Sc., Mycalis Institute, INRAE, France
- *Pre- and probiotics to relieve constipation-related complaints in irritable bowel syndrome*
  Maartje van den Belt, M.Sc., Food and Biobased Research, Wageningen University & Research, the Netherlands

10:30 **Networking break & poster viewing**

TUESDAY 15 NOVEMBER 2022

SESSION 3
BACTERIOPHAGE-BACTERIA INTERACTIONS IN THE GUT

Chair: Dr Sabrina Green, Department of Biosystems, KU Leuven, Belgium

11:00 *Bacteriophages in the human gut microbiome: opportunities or threats?*
Dr Evelien Adriaenssens, Quadram Institute, UK

11:20 *Mucosal interactions as a bridge between past and future prophylactic phage therapy*
Dr Gabriel Magno de Freitas Almeida, The Arctic University of Norway, Norway and University of Jyväskylä, Finland

11:40 *Development of phage-based feed additives to enhance fed cattle health and performance*
Dr Jacques Mathieu, Rice University, USA

12:00 *Manufacturing and formulation or bacteriophages for oral dosage forms*
Dr Danish Malik, Department of Chemical Engineering, Loughborough University, UK

12:20 *Phage therapy for the gut and beyond*
Dr Sabrina Green, Department of Biosystems, KU Leuven, Belgium

12:45 **Lunch break & poster viewing**

SESSION 4
BENEFICIAL MICROBES IN PERSONAL CARE

Chair: Dr Thomas D. Leser, Human Health Innovation, Chr. Hansen A/S, Denmark

11:00 *Preclinical model to evaluate the beneficialty of cosmetic ingredients for skin microbiota*
Maria João Carvalho, Center for Biotechnology and Fine Chemistry, Universidade Católica Portuguesa, Portugal

11:20 *Probiotic supplement for personal care*
Laura Huuskonen, M.Sc., Global Health and Nutrition Science, IFF Health & Biosciences, Finland

11:40 *Nitrate as a prebiotic and nitrate-reducing bacteria as probiotics for the oral microbiome*
Dr Bob Rosier, Department of Health and Genomics, FISABIO Foundation, Spain

12:00 *The potential of precision probiotic Hafnia alvei HA4597 to support weight loss*
Nina Vinot, TargEDys, France

12:20 Submitted abstracts:

- *The skin microbiome in atopic dermatitis patients in Belgium and how to modulate it with probiotics*
  Lize Delanghe, M.Sc., University of Antwerp, Belgium
- *Effect of 10-12 months supplementation with the probiotic Lactobacillus rhamnosus, LGG® (DSM33156) and L. paracasei subsp. paracasei, L. CASEI 431® (DSM33451) and arginine on caries increment in healthy children: A randomized, parallel-grouped, placebo-controlled clinical trial*
  Camilla Juhl Pørksen, M.Sc., University of Copenhagen, Denmark

12:45 **Lunch break & poster viewing**

TUESDAY 15 NOVEMBER 2022

SESSION 5
BENEFICIAL MICROBES AND THE GUT-BRAIN AXIS

Chair: Prof. Michiel Kleerebezem, Host-Microbe Interactomics, Wageningen University & Research, the Netherlands

14:00 *An apple a day keeps the psychiatrist away*
Prof. Iris Sommer, Research Institute Brain and Cognition, University Medical Center Groningen, the Netherlands

14:20 *The microbiome and cognition domains*
Prof. José-Manuel Fernández-Real, Department of Medical Sciences, University of Giron, Spain

14:40 *Anxiolytic effects of a daily prebiotic in healthy young female volunteers*
Dr Kathrin Cohen Kadosh, School of Psychology, University of Surrey, UK

15:00 *Efforts to understand the impact of the gut microbiota on human neurobiology*
Dr Alejandro Arias Vásquez, Donders Institute for Brain, Cognition and Behaviour, Radboudumc, the Netherlands

15:20 *Overcoming the brain barrier: a challenge for bacteria?*
Prof. Roosmarijn Vandenbroucke, Department of Biomedical Molecular Biology, Ghent University, Belgium

15:45 **Networking break & poster viewing**

SESSION 6
TOWARDS RENEWABLE-BASED PREBIOTICS

Chair: Dr Guus Roeselers, Danone Nutricia Research, the Netherlands

14:00 *Prebiotic tea phenolics: gut microbial conversions, renewable sourcing, and sustainable extraction*
Dr Wouter de Bruijn, Department of Agrotechnology and Food Sciences, Wageningen University & Research, the Netherlands

14:20 *Cello-oligosaccharides: potential prebiotics and their biosynthesis from renewable carbohydrate-based resources*
Dr Chao Zhong, Institute of Biotechnology and Biochemical Engineering, Graz University of Technology, Austria

14:40 *Turning waste/by-products into novel (personalized) prebiotics that modulate the gut microbiota*
Prof. Koen Venema, Centre for Healthy Eating and Food Innovation, Maastricht University, the Netherlands

15:00 *Biomass as a renewable source of prebiotics*
Prof. James Clark, Circa Renewable Chemistry Institute, Green Chemistry Centre of Excellence, University of York, UK

15:20 *New opportunities to design next-generation prebiotics from renewable sources: from sustainability to precision nutrition?*
Dr Lorena Ruiz, Department of Microbiology and Biochemistry of Dairy Products, Instituto de Productos Lácteos de Asturias, Spain

15:45 **Networking break & poster viewing**

TUESDAY 15 NOVEMBER 2022

SESSION 7
BENEFICIAL MICROBES IN ANIMAL HEALTH AND NUTRITION – PART 2

Chair: Prof. Richard Ducatelle, Department of Pathology, Bacteriology and Poultry Diseases, Ghent University, Belgium

16:15 *Beneficial impact of Saccharomyces boulardii I-1079 probiotic on functionally important gut anaerobes, experimental proofs*
Dr Frédérique Chaucheyras-Durand, Lallemand SAS and Clermont Auvergne University, INRAE, France

16:35 *Abnormal diet, abnormal behaviour – reviewing the role of diet and the microbiota-gut-brain axis in tail biting pigs*
Dr Cecilie Kobek-Kjeldager, Department of Animal Science, Aarhus University, Denmark

16:55 *Novel molecular mechanisms from wildlife probiotics to inform immunomodulatory microbiome therapies*
Dr Jorge Gutierrez-Merino, Department of Nutritional Sciences, University of Surrey, UK

17:15 *Probiotics for honeybees (Apis mellifera L.)*
Dr Adriana Nowak, Department of Environmental Biotechnology, Lodz University of Technology, Poland

SESSION 8
BENEFICIAL MICROBES AND HUMAN HEALTH – PART 2

Chair: Dr Jiro Nakayama, Department of Bioscience and Biotechnology, Kyushu University, Japan

16:15 *Probiotic Bifidobacterium in the prevention of cognitive impairment – clinical evidence and mechanism studies*
Dr Jinzhong Xiao, Next Generation Science Institute, Morinaga Milk, Japan

16:35 *Advanced analysis for identification of key microbial biomarkers in microbiome data from clinical trials*
Dr Eline Klaassens, BaseClear, the Netherlands

16:55 *Bacterial probiotic and postbiotics from Lactobacillus helveticus HA122 and L. plantarum HA-119 allow reinforcing gut barrier function in a zebrafish model*
Dr Emmanuelle Apper, Lallemand SAS, France

17:15 Submitted abstracts:

- *Lacticaseibacillus casei isolate from the human respiratory tract as potential probiotic against cystic fibrosis*
  Eline Cauwenberghs, M.Sc., Department of Bioscience Engineering, University of Antwerp, Belgium
- *Targeting the gut-brain axis in autism: machine learning analysis of the gut microbiome shows association with autism spectrum disorder*
  Lucía Peralta Marzal, Department of Pharmacology, Utrecht University, the Netherlands

WEDNESDAY 16 NOVEMBER 2022

PLENARY SESSION
STRUCTURE AND FUNCTION OF NON-DIGESTIBLE CARBOHYDRATES IN THE GUT MICROBIOME (in co-operation with ILSI Europe)
This session will focus on what we know and what we need to know about the structure-function relationship in dietary carbohydrates.

Chair: Dr Alexandra Meynier, Mondelēz International, France

08:45 Introduction by the chair

08:55 *Structure-function relationships in dietary carbohydrates: what do we know and what do we need to know?*
Prof. Robert Rastall, Department of Food and Nutritional Sciences, University of Reading, UK

09:15 *Systemic effects of prebiotics*
Prof. Koen Venema, Centre for Healthy Eating and Food Innovation, University of Maastricht, the Netherlands

09:35 *Modelling prebiotic activity*
Dr Maria Wiese, Department of Microbiology and Systems Biology, TNO, the Netherlands

09:55 *Future prebiotics: enabling technologies*
Dr F. Javier Moreno, Institute of Food Science Research (CSIC-UAM), Spain

10:15 Q&A

10:30 **Networking break & poster viewing**

PLENARY SESSION
BENEFICIAL MICROBES AND HEALTHY AGEING

Chair: Prof. Michiel Kleerebezem, Host-Microbe Interactomics, Wageningen University & Research, the Netherlands

11:00 *Microbiome and neurological disorders: Alzheimer's and ageing*
Prof.Satya Prakash, Department of Biomedical Engineering, McGill University, Canada

11:20 *Nutritional strategies for health promotion in elderly; present and future of the 'biotics' family*
Dr Miguel Gueimonde, Department of Microbiology and Biochemistry of Dairy Products, Instituto de Productos Lácteos de Asturias (IPLA-CSIC), Spain

11:40 *Targeting the gut to prevent or counteract age-related metabolic disturbances and pathologies: combining old and new concepts in nutrition*
Dr Isabelle Savary-Auzeloux, Unité Nutrition Humaine, Université Clermont Auvergne, INRAE, France

12:00 *Beneficial microbes, should it get personal?*
Dr Patrick Veiga, INRAE, France

12:20 Top five lessons learned
Prof. Koen Venema, conference chair

12:30 Closing of the **9th Beneficial Microbes Conference**

Take your packed lunched to eat along the way!

HOW CAN WE HELP YOU?

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The Microbial Genomics Experts

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Ingredient Discovery, Characterisation & Approval

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From discovery of novel microbial strains to regulatory approval

02

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REASONS TO JOIN US

- Access to a multidisciplinary network of leading scientists from academia, government, risk assessment bodies and industry
- Collaborate with international organisations like WHO, FAO and the European Commission to build the scientific basis for public health
- Contribute to scientific discussions and documents on a pre-competitive basis, that are widely recognised as highly credible, reliable and relevant
- Provides collective, cost-effective funding to build timely science in areas of public health interest

LEARN MORE ABOUT OUR TASK FORCES

The Prebiotics Task Force aims to improve the understanding of mechanisms of prebiotics linking them to individual health benefits and to identify and explore the health benefits of prebiotic fermentation.

The Probiotics Task Force aims to increase scientific understanding, awareness of the real impact of probiotics direct/indirect benefits on health.

The Health Benefits Assessment of Food Task Force aims at investigating specific components in the diet to assess their benefits on health and wellness beyond basic nutrition.

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LECTURE ABSTRACTS

ROLE OF THE LUNG MICROBIOME IN CNS AUTOIMMUNITY

Francesca Odoardi[1], L. Hosang[1], R.C. Canals[1], F.J. van der Flier[1], J. Hollensteiner[2], R. Daniel[2] and A. Flügel[1]

[1] Institute for Neuroimmunology and Multiple Sclerosis Research, University Medical Center Göttingen, Germany
[2] Department of Genomic and Applied Microbiology, University of Göttingen, Germany

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In order to supply the organism with oxygen, the lungs are connected to the environment via a large exchange surface, which houses a special microbial flora. This pulmonary microbiota contributes to the regulation of local immune responses in physiological and pathological processes but their role in controlling immune reactions outside the lung is unknown. By working on animal models of multiple sclerosis, we detect an unexpected link between the lung microbiome and brain immune reactivity. The lung microbiome seems to regulate the immune responsiveness of the CNS by acting on the microglia, the CNS resident immune cells. Indeed, by shifting the microbiota towards lipopolysaccharide-enriched phyla by local treatment with neomycin induced a type I interferon-primed state in brain-resident microglial cells. As consequence, their responsiveness towards autoimmune-dominated type II interferon stimulation was impaired, leading to decreased pro-inflammatory response, immune cell recruitment and clinical signs of CNS autoimmunity. Our data demonstrate the existence of a lung-brain axis controlling the susceptibility to autoimmune disease development.

GENETICS AND EXPOSOME FACTORS SHAPING THE DUTCH MICROBIOME LANDSCAPE

Alexandra Zhernakova

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The gut microbiome is a complex organ that is highly dynamic and is influenced by both environment and host factors. In a large microbiome study in Dutch population cohort ‘Lifelines’ (10,000 participants), we determined the effect of environment, shared household, and individual genetics on the composition of gut microbiome.

Environment has a major effect on interindividual differences in microbiome. In particular, individual diet, medication, diseases, and exposures to smoking, pets and pollutants are influencing the gut ecosystem. Individuals in the same household have more similar microbiomes, which is largely explained by the shared environment. Host genetics is affecting a subset of heritable bacteria and can be linked to functional variations in bacterial genes and gene clusters. Using the wide-scale analysis of antibodies against gut bacteria we identified host genetic variants that determine the individual immune reactions to commensal bacteria. Integrated analysis of diet, lifestyle, genetics and gut microbiome and human metabolites identifies complex interaction of various factors relevant for human health.

POLYUNSATURATED FATTY ACIDS AS PREBIOTICS AND THE CONCEPT OF THE 'CORRECT PREBIOTIC DIET': INNOVATION OR CONFIRMATION?

Lara Costantini

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The omega-3 polyunsaturated fatty acids (PUFAs) are important dietary component of the Mediterranean diet, which has been declared by UNESCO as intangible cultural heritage of humanity. Among omega-3 PUFAs, eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6) are the two main bioactive forms in humans and they are found in animal origin foods, such as fish. The PUFAs are considered ‘candidate prebiotics’ due to the ability to be metabolized by the gut microbiota but the role of the resulting metabolites in the host is less known. Recent partial evidence shows that these metabolites can have important health effects in the host, reinforcing the concept of the prebiotic action of PUFAs. For example, an increase of iso-valerate and iso-butyrate was found after PUFAs supplementation, the same short-chain fatty acids found after inulin supplementation. Anyway, data have shown that a single carbon source feeding leads to a significant loss of beneficial bacteria indicating that this nutritional strategy may not be the prebiotic one. Bacteria, like all living organisms, cannot benefit from a single nutritional molecule, whether it be fibres or fatty acids. The understanding of what can be defined ‘the correct prebiotic diet’ of our symbiotic microbes, and their biochemical-metabolic processes – as the basis of their healthy composition and healthy interactions with the host – should be the new frontier in the field of the gut microbiota research.

PLENARY SESSION
POSTBIOTICS, DEFINITION AND SCOPE – A PANEL DEBATE
A panel debate on the International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics, and its caveats.

THE RATIONALE BEHIND ISAPP'S DEFINITION OF POSTBIOTICS

Gabriel Vinderola

Instituto de Lactología Industrial (INLAIN, UNL-CONICET), Facultad de Ingeniería Química, Universidad Nacional del Litoral, Argentina

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In the last 20 years at least, the scientific community has proposed a diversity of terms, such as non-viable probiotics, paraprobiotics, ghostbiotics, heat-inactivated probiotics or, most commonly, postbiotics, to refer to inanimate microorganisms and/or their components that confer health benefits. In this session, the characteristics of different definitions of 'postbiotics' that have emerged over past years will be discussed. In 2021, the International Scientific Association for Probiotics and Prebiotics (ISAPP) defined a postbiotic as "a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host". This definition of postbiotic requires that the whole, or components, of inactivated microbes be present, with or without metabolic end products. The definition proposed by ISAPP is comprehensive enough to allow the development of postbiotics from different microorganisms, to be applied in different body sites, encouraging innovation in a promising area for any regulatory category and for companion or production animals, and plant or human health. From a technological perspective, the inactivation process (heating, radiation, high pressure) may be of relevance for the functionality.

The ISAPP definition of postbiotic focuses on inanimate microbial cells as we believe this approach is the best use of the term 'postbiotic' (meaning 'after life', not 'from life') and further embraces innovation in a growing and evolving scientific concept that encompasses the potential health- conferring benefits of dead and/or inactivated cells. We did not see value in generating a term for molecules, which typically are already well- defined and named. We recognize that microbial metabolites are important, albeit not essential, components of postbiotics, and our definition encompasses their inclusion. If specific metabolites are not identified, then the term cell- free supernatant is sufficient. We remain convinced that the complex preparations with dead and/or inactivated cells that provide a health benefit are well described by the term 'postbiotics'.

This field is in its infancy, and we feel we have an opportunity now to coalesce around a more encompassing term. First definitions are not necessarily the best – consider the first definition published for probiotics in 1965 was "substances secreted by one microorganism that stimulate another microorganism". In the case of postbiotics, we felt that available definitions were not sufficient and we proposed an alternative that encompasses current science. The final decision on the most appropriate and useful definition will rest with the scientific community and regulatory authorities.

LOOKING FOR ‘SOLID’ DEFINITIONS

Lorenzo Morelli

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After a little more than 20 years since the FAO/WHO definition of ‘probiotics’ a plethora of new ‘XXXbiotics’ definitions have been formulated by marketing people as well as by scientists. However, to propose a definition of a food component beneficial for health is not an easy task and requires to be supported by solid scientific data, to be compliant with the regulations, to be understood by consumers. Items such as quality and quantity of the active ingredient and shelf-life stability are mandatory items to be included into the definition. If we use as a refence the FAO/WHO guidelines defining probiotics, we may notice that:

- *Quality*: characterization of the ‘active ingredient’ is a mandatory requirement and it is discussed in several sections of the FAO/WHO documents. Strains must be characterized by means of the most updated techniques in order to fully identify the active substance. It is also “strongly urged that for the sake of full disclosure, probiotic strains be deposited in an internationally recognized culture collection.”
- *Quantity and shelf-life*: the number of viable cells is another mandatory requirement. The ‘adequate amount’ is present in the definition itself. Consultation also state “There is a need to accurately enumerate the probiotic bacteria in food products in order to include them on the label. The label should state the viable concentration of each probiotic present at the end of shelf life.”

All the above point out that a definition needs to be qualified by means of measurable parameters, clearly identified and measurable by means of analytical techniques recognized by the scientific world and accepted by regulatory bodies. While there are no doubts (even if the body of knowledge is still at the beginning) that bacterial metabolites as well as whole, unviable cells, can exert beneficial actions, identification of the bacterial ‘component(s)’ responsible for this action is still pending. However, we cannot assume that the whole, unviable bacterial cell remains stable during the shelf life and therefore, for each product containing unviable microbial cells it is necessary to identify a bacterial component related to the observed beneficial effect and to develop analytical assays able to measure the amount of intact active ingredients. Otherwise, it seems extremely difficult to provide consumers with high quality, shelf-stable products.

PLENARY SESSION
BENEFICIAL MICROBES AND EARLY LIFE

ON THE ORIGIN OF SPECIES – HOST, DIETARY AND ENVIRONMENTAL FACTORS SHAPING THE EARLY-LIFE MICROBIOME

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The gut microbiota is established in the neonatal period and further matures throughout infancy and childhood. The microbiota assembly and maturations play a pivotal role in the development of the mucosal tissue, immune maturation, and host metabolism. Perturbations in microbiota assembly and maturation during this critical time-window can therefore have profound effects on future health and susceptibility to non-communicable diseases. Over the past decades considerable advances have been made in our understanding of the microbiota maturation during infancy, nevertheless a large part of inter-individual variation in microbiota composition remains unexplained. This indicates that either important deterministic factors of microbiota assembly and maturation might have so far been overlooked or that other ecological processes such as stochastic effects or historical contingency play a major role. Here, we will provide an overview of the major deterministic factors identified so far and present latest findings of additional host, dietary and environmental influences of infant microbiota development.

Within the KOALA Birth Cohort Study, we have examined the impact of human milk oligosaccharide (HMO) profiles in maternal milk on the infant gut microbiota composition at the age of one month. Despite, major differences in HMO profiles, driven by maternal Lewis and secretor status, effects on infant microbiota were modest. However, some individual HMOs showed clear effects on the abundance of specific microbial taxa. Next to infant feeding, also the timing and introduction of complementary foods plays a major role in microbiota maturation as shown by preliminary results of our LucKi Gut Study. Next to the environmental selection by diet, nourishing the growth of specific microbes, also host factors can have a profound effect on microbiota development. One such example is the bi-directional interactions of bile acids and the microbiota. Using neonatal mice models, we showed major age-dependent microbial and metabolic shifts and identified bile acids as potent drivers of early intestinal microbiota maturation. Oral gavage of specific bile acids to neonatal mice confirmed the acceleration of postnatal microbiota maturation.

Together these results show the complexity of early life microbiota variation and provide specific leads to manipulate the microbiota in a critical period. To further enhance our understanding, there is a clear need on studies that take the various ecological phenomena into account, but also focus on unravelling other so far unknown deterministic factors that might contribute to the variation and acquisition of the microbiota, and thereby the vital biological processes in life.

THE YING AND YANG OF MICROBIOME SIGNATURES IN EARLY LIFE

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Initial colonisation of the gut by pioneer bacterial species is the first key step for host well-being. The process of initial gut microbiota colonisation in preterm babies is radically interrupted due to a variety of factors including mode of delivery and antibiotics. This aberrant colonisation of premature infants appears pivotal to the development of a number of diseases, including necrotising enterocolitis (NEC). I will discuss how probiotic supplementation (with the right diet) represents a powerful opportunity for strategically manipulating the preterm microbiota and how certain microbiota members may either act as commensals or acquire virulence traits that are linked to NEC. I will also touch on how non-invasive faecal profiling of microbial and immune biomarkers can be used to identify infants that are at risk of developing NEC.

THE RESPIRATORY MICROBIOME IN EARLY LIFE: KEY TO INFECTION PROTECTION

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Respiratory tract infections are a major global health concern, accounting for high morbidity and mortality, especially in young children. Traditionally, it is thought that bacterial respiratory tract infections, including otitis media and pneumonia, are caused by a limited number of pathogens, including viruses such as respiratory syncytial virus, and influenza viruses, and bacteria such as *S. pneumoniae* and *H. influenzae*. However, over recent years it has become apparent that the upper respiratory tract (URT) microbiome has a direct role in health and disease, including susceptibility to and severity of respiratory infections.

Analogous to the gut microbiome, evidence is accumulating that the respiratory microbial community may elicit protection against respiratory infections through direct colonization resistance, and immunomodulatory properties. Recently, we and others have obtained evidence for the existence of different respiratory microbiota profiles, that are related to stability of microbiota and susceptibility to and severity of respiratory infections in early life. Consecutively, the early life microbiome development is also related to respiratory health outcome in childhood.

In this presentation, we will review the current body of evidence regarding potentially protective microbes and their function. I will also present the latest evidence on microbiome development in early life in our infant cohort studies, and how this early window of life may be utilized for future intervention strategies.

USING PROBIOTICS FOR MATERNAL DEPRESSION: RESULTS OF A SYSTEMATIC REVIEW AND META-ANALYSIS

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The mother and infant form a deep bond, and their interactions can be mediated by various biological factors, such as mother and infant characteristics, including mood, depression, maternal behaviour style, age, biological risk, disability, temperament, and irritable infant, for example, due to colic and other health and disease conditions. One of the possible mechanisms influencing maternal mental health is the manipulation of the gut-brain axis by consuming probiotic supplements. According to the consensus statement by the International Scientific Association for Probiotics and Prebiotics, probiotics are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host”. Probiotics can also have an indirect influence on maternal mental health via the modulation of the infant microbiome and consequently improve the infant’s health and thus, indirectly lead to an improvement in maternal mood.

A systematic review evaluated the efficacy of probiotics on maternal mental health by searching for randomised controlled trials via international databases: Cochrane Library, PubMed, Scopus, ScienceDirect, and Web of Science until January 2022. A meta-analysis was performed using the Cochrane Collaboration methodology where possible. We found seven clinical trials that included the word probiotics and addressed maternal depression and/or anxiety. Of these, five trials investigated the influence of maternal probiotic supplementation on the gut-brain axis. Two of these trials found an impact on depressive symptoms in pregnant and postpartum women. One included supplementation with fish oil and *Lacticaseibacillus rhamnosus* HN001 and *Bifidobacterium animalis* subsp. *lactis* 420, and the other included supplementation with *L. rhamnosus* HN001. The remaining two trials investigated the indirect influence of *Limosilactobacillus reuteri* DSM 17938 on maternal depression via supplementation of probiotics and subsequent influence on the crying of colicky infants. One of these trials found an impact on maternal depression. Meta-analysis of two studies of pregnant and postnatal women and two studies of infants consuming probiotics on the outcome of the Edinburgh Postnatal Depression Scale for mothers showed no statistical difference.

The findings indicate that maternal depression is very complex and is influenced by various bidirectional factors. One of the factors that can improve maternal mental health is probiotics; however, careful consideration must be given to correct strain selection as strain-specific effectiveness. Further well-designed, robust clinical studies are warranted and should include probiotic supplementation after birth and careful consideration should be given to measuring outcomes in a reliable way.

THE GUT MICROBIOTA AND THE MATERNAL IMMUNE RESPONSE IN PREGNANT MICE; EFFECT OF ANTIBIOTICS AND PROBIOTICS

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During pregnancy, the maternal gut microbiome changes. These changes in the maternal microbiome are important for a healthy pregnancy since they help adapt the maternal immune response to pregnancy. The adaptation of the immune response to pregnancy is important to protect the semi-allogeneic foetus. Maladaptations in the maternal immune response during pregnancy may result in complications for both the mother and foetus. We hypothesize that maternal gut dysbiosis will result in aberrant adaptations of the maternal immune response and thus affect pregnancy outcome. We also hypothesize that treatment with probiotics will improve the maternal microbiome and therefore also improve the maternal immune response and improve pregnancy outcome. Data to support these hypotheses will be shown in the presentation.

PLENARY SESSION
SPONSOR PITCHES & SPEED PRESENTATIONS
Short presentations (5-minutes) by our sponsors and by selected poster presenters to inspire the audience to visit their booths and posters, respectively.

Sponsor pitches

- BaseClear, the Netherlands
- ILSI Europe, Belgium

Speed presentations

The abstracts can be found in the section 'Poster abstracts (pages 72-126).

P9
The role of galacto-oligosaccharides in the recovery from dysbiosis in patients on long-term atypical antipsychotic treatment
Nienke de Bles
Leiden University Medical Center, Leiden, the Netherlands

P22
Study protocol: effects of butyrate on affective patterns, microbiome composition and depressive symptoms in young adults – a randomized clinical trial
Vera Korenblik
Amsterdam University Medical Center, the Netherlands

P23
Lacticaseibacillus rhamnosus HA-114: an innovative probiotic strain to support weight management efforts through the gut-brain axis
Mélanie Le Barz
Lallemand Health Solutions, Canada

P30
Longitudinal gut mycobiota changes in Japanese infants during first three years of life
Riko Mishima
Kyushu University, Japan

P33
Short chain fatty acid inhibition of bacterial plasmid conjugation in broth and chicken ceca explants
Logan C. Ott
Iowa State University, USA

P43
A predictive model for microbiome-dependent response to dietary fibres bases on *in vitro* biological data predicts microbiota response to dietary intervention
Clémentine Thabuis
Roquette, France

SESSION 1
BENEFICIAL MICROBES IN ANIMAL HEALTH AND NUTRITION – PART 1

EVOLUTION OF THE GUT MICROBIOME IN FEMALE DOGS AROUND PARTURITION AND IN EARLY LACTATION, AND IMPACT OF LIVE YEAST *SACCHAROMYCES BOULARDII* CNCM I-1079

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During and after pregnancy, bitches experience physiologic and metabolic changes which may also impact their microbiota composition, as observed in other species. Maternal microbiome plays critical roles in maternal and infant health over parturition, but it remains elusive in canine species. We aimed to analyse shifts in faecal microbiota composition during pregnancy and lactation and evaluate if the use of a probiotic could impact gut microbiota and improve reproductive performances in bitches. The study was carried out within one breeding kennel, on 36 dams of 9 different large-sized breeds. Dams were separated into two groups, control and live yeast *Saccharomyces boulardii* CNCM I-1079 (SB) groups, respectively (n=18/group), randomized according to their breeds, parity, age, body condition score and faecal score. Supplementation was administrated to the SB group from the 28th day of gestation till 56th day after parturition. Number of puppies born and stillborn, their birth weight and growth rate were monitored. In addition, maternal faecal samples were collected via rectal swabs on day 28 and 56 of gestation, and day 1, 28 and 56 after parturition. Illumina MiSeq (Illumina, San Diego, CA, USA) sequencing was performed using the V3-V4 regions of the 16S rRNA gene amplicon after DNA was extracted from swabs.

Physiological status of bitches strongly impacted their faecal microbiota. Indeed, bacterial richness decreased over gestation before increasing back after parturition. This evolution might be linked to the evolution of hormones (progesterone) and pro-inflammatory molecules' concentration. Beta diversity allowed to highlight 3 bacterial profiles: one during gestation, one after birth and one during lactation. The abundance of some key bacteria of milk microbiota (bifidobacteria and lactobacilli) almost disappeared at parturition, suggesting a potential bacterial entero-mammary pathway. The live yeast supplementation also altered the alpha-diversity of the faecal microbiota just before parturition, and impacted the composition one day after parturition, by promoting the relative abundance of some bacteria, notably *Faecalibacterium prausnitzii*, *Clostridium hiranonis*, *Dorea* sp., and *Bacteroides plebeius*. The SB group gave birth to significantly less low-body weight puppies than the control group ($p<0.01$), and puppies of SB dams showed significantly less variation in their growth than control ones.

This study revealed major modifications of the bacterial composition around parturition in the bitch, as in other mammals. Those bacterial modifications might be linked to concomitant hormonal, immune and energetic metabolisms changes. It also demonstrated that a supplementation of bitches during gestation and lactation with *S. boulardii* CNCM-1079 is a promising nutritional strategy to support growth and health of puppies while modifying the maternal faecal microbiota around parturition. Comparison of the maternal microbiota with the one of their offspring would allow to further understand the impact of maternal microbiota on early microbial colonization of the puppy's gut, and to evaluate the impact of live yeast in this context.

GUT NEUROIMMUNE AXIS AND THE MICROBIOTA: ELUCIDATING CROSSTALK TO CONTROL BACTERIAL INFECTIONS IN POULTRY

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The crosstalk between neurochemicals and the gut microbiota is an emerging topic in the field of neuromicrobiology that is being explored in relation to the health and well-being of both humans and animals. However, little is known about this interaction in food-producing animals, such as chickens. The poultry gut microbiota is important in host health and performance and serves as a major reservoir for *Enterobacteriaceae,* which includes pathogens such as *Salmonella* and *Escherichia coli*. These bacteria are rapidly becoming resistant to the last resort antibiotics; thus, they are an emerging health and food safety concern.

My talk will present our recent research on the role of neurochemicals in the gut of chickens. Our findings elucidate the link between neurochemicals and the gut microbiota, and the impact of the environment and live prophylactics on this interaction. Finally, our research shows the possibility of altering the intestinal immunological response via neurochemical and metabolic pathways to improve bacterial resistance, especially against immunotolerant bacteria such as *Salmonella*. Our findings provide evidence that targeting the neuroimmunological axis can be an effective strategy to minimize *Salmonella* persistence in poultry to improve food safety and should be further explored.

PLANT EXTRACTS: GUT MICROBIOTA AND BEYOND

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With a growing demand for safe and sustainable alternatives to antimicrobials in animal production, functional feed ingredients such as plant extracts have been evaluated for their potential as antibacterial, antioxidant, and their impact on the gut microbiota. Plant extracts are natural metabolic of plants, which are considered for some specialists to be the plants' true hormones, fluids of the immune system, contributing to the removal of pests and attracting pollinating agents [1]. Though, extraction methods, plant maturity, and harvesting time may influence the effectiveness of the plant extracts and, to overcome these variabilities, nature identical compounds have become more popular in the animal industry [2,3]. In the small intestine, some plant extracts can have a selective antimicrobial effect, resulting in a shift in the microbial ecology in favour of lactic acid producing bacteria and reducing the number of pathogenic bacteria, which offers an alternative to antibiotics while maintain gut health and performance [4,5]. Maastricht University developed the TNO, an *in vitro* model of the colon (TIM-2) for pigs [6], which allowed us to study pigs' microbiota composition and short-chain fatty acids (SCFA) production when blends of plant extracts (BPE) were applied with different levels of protein.

This experiment was performed in duplicate, and the TIM-2 was inoculated with an adult pig microbiota. The treatments were as follow: (i) SIEM (simulated ileal efflux medium), standard medium as a control; (ii) BPE-1; (iii) BPE-2; (iv) high protein (simulated by adding additional casein); (v) casein + BPE-1; and (vi) casein + BPE-2. The addition of both BPE led to the modulation of the gut microbiota of pigs and major differences in taxa modulation were found when the level of protein was increased. The inclusion of both BPEs led to a slightly reduction of the SCFA production comparing to the control group, which may be related to the changes in the microbiota. The addition of protein to the SIEM had a direct impact on the results and further studies should be done using a complete diet to understand the effects of the BPEs in the presence of other nutrients, which would simulate a more realistic environment for those bacteria. In conclusion, plant extracts seem to have the ability of modulate gut microbiota and it could be seen has a potential for improving animal health and production.

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KING OF THE HILL – MODULATING THE RAINBOW TROUT MICROBIOME

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For several decades, aquaculture has undergone near exponential growth worldwide. With increasing production intensities aiming to meet the projected requirements for a growing population, a continued optimization of rearing and growth conditions must be considered a key component moving forward. Given their potential for optimization of feed utilization, supplementation of fish feed with pre-, pro- or synbiotic additives have been investigated for their potential in this field. While a number of studies have looked into their potential, only one probiotic supplement is currently authorized for use within the EU. As such, the playing field is potentially open for competition, alternatives and improvements.

As part of a broader effort to reduce outbreaks of bacterial infections, and thus the use of antimicrobials, in aquaculture, we have worked on improving our current knowledge of the effects of pre-, pro-, and synbiotic additives to fish feeds. Working with salmonid fishes, and rainbow trout in particular, we and our collaborators have conducted larger feed trials, established and characterized *in vivo* infection models and thus investigated effects of a range of supplements, either alone or in combination, on various feed performance parameters, disease resilience and composition of the intestinal microbiome. Here I will try to summarize the work done so far, address who is the king of the hill, and how firm the grip on that throne is in terms of the trout microbiome, and briefly introduce the next step in our process.

PROBIOTICS IMPACT ON *TENEBRIO MOLITOR* PERFORMANCE, MICROBIAL COMPOSITION AND PATHOGEN INFECTION

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The yellow mealworm *Tenebrio molitor* is an insect model for infection and immunity studies and is mass-produced as feed and food. The industrial rearing of *T. molitor* on agricultural by-products may expose larvae and adults to entomopathogens used as biocontrol agents, like bacterial spores/toxins of *Bacillus thuringiensis* or conidia of the fungus *Metarhizium brunneum*, that could impact the performance of *T. molitor*. Therefore, as for other animal livestock's, the possible benefits of addition of active or tantalized probiotic bacteria to the feed are investigated by providing two probiotic stains to *T. molitor* larvae in optimal rearing system conditions and when the insect is exposed to *B. thuringiensis*, and *M. brunneum* alone or to both entomopathogens. Alongside infections, growth and survival performance and the insect microbiota composition was analysed by 16S rRNA sequencing to measure the impact of probiotics on *T. molitor* and the microbial composition. The study is focused on the hypothesis that (i) *M. brunneum* and *B. thuringiensis* have different mechanisms of infection, therefore dose and timing of pathogen exposure will influence the outcome; (ii) the presence of probiotics may help the insect to cope with the infection by improving immunity, by presenting a shorter period for pathogen clearance and by expressing higher performance. Preliminary results show evidence of positive effects of adding a vital probiotics strain to the feed on *T. molitor* performance and survival, suggesting a role of probiotic metabolites in maintaining host health.

SESSION 2
BENEFICIAL MICROBES AND HUMAN HEALTH – PART 1

IMPACT OF A MATERNAL SUPPLEMENTATION WITH PREBIOTIC ON THE MICROBIOTA, THE IMMUNE SYSTEM AND ALLERGY DEVELOPMENT IN OFFSPRING

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Allergies are a major public health issue. No treatment nor effective preventive strategy were established so far. They are linked to the dysfunction of three biological actors: the microbiota, the immune system and the epithelial barriers. These actors set up during the first 1000 days of life. Allergic symptoms may be observed within the first months of life. According to the DoHad concept, our environment and especially our diet during this period will drive the establishment of these systems and the occurrence of allergy. In this context, pregnancy represents an optimal window of intervention in the regulation of the allergic process. Among food, prebiotics are interesting in this context. They are substrates selectively used by host microorganisms that provide health benefits. They can act on the microbiota by increasing the bifidogenic bacteria, strengthen the epithelial barriers and act on the immune system to set up a tolerogenic immune environment.

The aim of our research was to characterize in mice the effects of GOS/inulin prebiotic administered during gestation on the transfer of microbial and immune factors from mother to pup and its protective effect on the occurrence of food allergy.We demonstrated that the intake of prebiotic during gestation modulates the composition and the functionality of the maternal microbiota in favour of strains beneficial for health (Firmicutes, Bacteroidetes). A significant increase in short-chain fatty acids was observed in the stools of mothers supplemented with prebiotics and in their amniotic fluid. These modulations induced a specific microbial imprint in adult offspring. Supplementation with prebiotics during gestation significantly increased the frequency of regulatory B and T lymphocytes in the placenta. These regulatory B cells were found in the intestine and bone marrow of the foetus and were maintained into adulthood. Finally, the transfer of a microbial imprint and a tolerogenic environment through prebiotic supplementation protected offspring from the development of food allergy. Currently, our objective is to validate this preventive strategy in the PREGRALL clinical study, which consists of supplementing allergic mothers with GOS/inulin during their pregnancy in order to reduce the risk of allergies occurrence in children. Our work will allow to provide new nutritional recommendations for pregnant women to reduce these pathologies.

IMMUNOMODULATION BY BIOTICS: PREVENTIVE EFFECT AGAINST ROTAVIRUS INFECTION

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Infants are born with an immature immune system, frequently unable to cope with all threads they encounter in early life. Maternal breast milk contains bioactive compounds that help infants to acquire immunity either by the transfer of passive immunity or by promoting maturation and development of their immune system. Among these bioactive compounds, human milk oligosaccharides (HMOs) have been shown to have prebiotic properties and several bacteria from breast milk have been isolated and used as probiotics. In addition, postbiotic products can be also found in breast milk. Nowadays many mothers feed their infants with infant formula for different reasons, although it is well known that breast milk is the gold standard in infant nutrition during the first 6 months of life. Therefore, it is of interest to test specific biotics already present or similar to those found in breast milk in order to provide the infant with the best nutrition and protection against diseases. Moreover, it is also interesting to know if administration of probiotics to the mother can also benefit their offspring. Our research during the last years has aimed to establish the immunomodulatory activity of biotics (probiotics, prebiotics and postbiotics) at preclinical level in the context of health and infection in early life.

Firstly, the modulatory activity of 2'-fucosyllactose, the most abundant HMO found in breast milk, but also other prebiotics used in infant formulas such as a mixture of short chain galacto-oligosaccharides and long chain fructo-oligosaccharides at a 9:1 ratio, and their combination have been tested. This type of products differentially modulates the immune development influencing gut growth, microbiota composition and functionality and some immune biomarkers of maturation. Postbiotics supplemented during this period are also able to modulate immunedevelopment. Then, the modulatory activity of these products was tested in the context of rotavirus (RV) infection, one of the most common diarrheal diseases in infancy. The above prebiotics, but also postbiotics and several probiotics from different origins (*Lactobacillus* and *Bifidobacterium* strains), including one from breast milk, evidenced amelioration of severity and incidence of RV-induced diarrhea in suckling rats. They used different mechanisms and whereas ones seemed to strengthen the intestinal barrier function and therefore to limit the neonatal anti-viral response, others evidenced an intestinal trophic effect and RV blocking activity. Some combinations kept the activities found for the different compounds separately and showed additive effects in several variables. Finally, supplementing the mother during gestation and suckling with particular strains of probiotic bacteria modulated breast milk composition, maternal immunity, and that of their offspring. Additionally, the existence of an entero-mammary route has been demonstrated and a positive impact in their offspring RV-associated diarrhea too.

Although some evidence of their mechanisms of action is revealed, further studies are needed to better understand their function and to recommend their inclusion in infant formulas or use them as supplements.

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HOW WE CAN TREAT IRRITABLE BOWEL SYNDROME AND OBESITY BY MODULATING THE GUT MICROBIOTA

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Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder affecting between 5 and 10% of the general population, characterised by recurrent abdominal pain associated with altered stool shape and frequency. The pathophysiology of IBS is incompletely understood, but several peripheral and central mechanisms are known to be involved. The microbiome influences most of the physiological processes associated with the pathophysiology of IBS. Some studies show that patients with IBS have a different gastrointestinal microbiome (GIT) than healthy controls. The microbial diversity of the intestinal microbiota of patients with IBS is reduced compared to the microbiota of healthy controls. The majority of IBS patients have an increased proportion of *Firmicutes* and a lower proportion of *Bacteroidetes*. However, data on the abundance of specific species within these 2 microbial phyla are not consistent. Within the *Firmicutes* class, levels of beneficial bacteria such as *Lactobacillus* spp. and *Bifidobacterium* spp. have been reported to be decreased, increased or unchanged in patients with IBS. Some studies show that patients with obesity have a different GIT than healthy, lean controls. Obese individuals also have more *Firmicutes* and fewer *Bacteroidetes* than the lean individuals. When obese volunteers followed a low-fat or low-carbohydrate diet for a year and lost up to 25% of their body weight, the proportion of *Firmicutes* in their colon decreased and that of *Bacteroidetes* increased. However, the levels of both types of bacteria never reached the levels of the group that was lean at the start of the diet. Meta-analysis of 27 randomized control trials (RCTs) included in the meta-analysis, 23 observed positive effects on weight loss.

Taking probiotics or synbiotics could lead to significant weight loss, either while maintaining usual lifestyle habits or in combination with energy restriction and/or increased physical activity for an average of 12 weeks. Specific strains of the *Lactobacillus* and *Bifidobacterium* genera were most commonly used and showed the best results in reducing body weight. Both probiotics and synbiotics have the potential to support weight loss in overweight and obese populations. The prevalence of IBS in patients with obesity is about 30% and increases proportionally with BMI. There are few RCTs with probiotics in this subpopulation.

Points to consider in IBS/obesity clinical trials and probiotics:

- selection of the optimal IBS population;
- endpoint – the expectations of the regulatory body and the nature of the study population;
- global endpoint (includes pain and changes in bowel habits, but also less studied but very important symptoms, such as bloating, fullness and fatigue)';
- EMA recommends two co-primary endpoints as the primary endpoint, namely "patient's global assessment of symptoms" and assessment of abdominal discomfort/pain;
- FDA recommends a primary endpoint that measures the effect of treatment on two important signs and symptoms of IBS, i.e., abnormal defecation and abdominal pain.

CLOSTRIDIUM BUTYRICUM MIYAIRI588® STRAIN, AS A POTENTIAL LIVE BIOTHERAPEUTIC PRODUCT – STIMULATION OF CANCER IMMUNOTHERAPY

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Clostridium butyricum MIYAIRI588® strain (CBM588) is one of Japanese probiotics for medicine approved by the Japanese government. *C. butyricum* is one of the indigenous intestinal microbiota and detected in 10-20 % of the Japanese population. The effects of CBM588 on infectious diseases have been reported. The effect of CBM588 on *Clostridioides difficile* was examined by the experiment using germ-free mice. In gnotobiotic mice co-administered with *C. difficile* VPI10463 strain and CBM588, no serious pathological changes were observed. Recently, it has been reported that oral administration of CBM588 protected antibiotic-treated mice against *C. difficile* infection (CDI). It was also shown that CBM588 suppressed CDI through the induction of neutrophils, Th1 and Th17 cells. In a clinical study for children treated with antibiotics, it was indicated that CBM588 was effective for prevention of antibiotic-associated diarrhoea.

Live biotherapeutic product (LBP) is defined as a biological product that contains live organisms, such as bacteria, and is applicable to the prevention, treatment or cure of a disease or condition of human beings. Recently, it has been reported that CBM588, one of the LBPs, was effective in cancer patients treated with immune checkpoint inhibitor (ICI). Progression free survival was significantly longer in the patients with metastatic renal cell carcinoma receiving ICIs (nivolumab and ipilimumab) with CBM588 than without (12.7 months vs. 2.5 months). In addition, there was a statistically significant increase in *Bifidobacterium* spp. in the patients receiving CBM588 and responding to the treatment. It was also shown that the levels of various cytokines, including IL-10, IL-12, GM-CSF, MIP-beta and MCP-1, were increased in the patients treated with ICIs and CBM588. It was indicated that CBM588 has a positive impact on therapeutic efficacy of ICIs in patients with malignancies, including metastatic renal cell carcinoma. In further studies, the mechanism of action and the effects on the microbiota and immune cells need to be elucidated.

EFFECT OF GUT MICROBIOTA OF CHILDREN WITH AUTISM SPECTRUM DISORDER ON BEHAVIOR AND ASD-RELATED BIOLOGICAL MARKERS IN GERM-FREE MICE

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The involvement of the microbiota-gut-brain axis has recently been taken into account in the study of the pathophysiology of autism spectrum disorders (ASD). Preclinical studies have shown that interventions on the gut microbiota, such as probiotic treatments or microbiota transplantations, can modulate behaviour in animal models of ASD. The germ-free mouse is a potential model, as it shows impairments in social behaviour and increased repetitive behaviours compared to conventional mice. Therefore, our project aimed to test the effect of faecal transplantation from children with ASD on behaviour and several biological markers in germ-free mice. We hypothesized that behavioural phenotypes impaired in germ-free mice would not be improved, or even be worsened, by faecal microbiota from children with ASD, with or without gastro-intestinal (GI) symptoms as a co-morbidity (two distinct 'ASD' groups) compared with mice receiving the microbiota from their neurotypical siblings (two distinct 'Siblings' groups). We chose to perform the same experiment on two strains of mice: BALB/c and C57BL/6, as they show distinct emotivity levels, and have analysed ASD related behaviours in the transplanted animals (i.e., social behaviour, repetitive behaviour, anxiety and cognition (spatial memory)). In addition, we are analysing microbiota composition and fermentation activity, as well as markers of inflammation, permeability, and the serotoninergic system.

Transplantation of faecal microbiota from children with ASD into both strains of mice was not accompanied by behavioural changes, with the exception of alterations in spatial memory in C57BL/6. Despite this, the composition of the microbiota that implanted into our four groups of BALB/c mice was distinct between groups, both in diversity and composition, with differences up to the phylum level. Plus, short chain fatty acid profile also differed between groups indicating a difference of fermentation activity of the microbiota. The differences in the microbiota are accompanied by a reduction of the number of serotoninergic neurons in the raphe nuclei and of serotonin positive cells in the ileum in both "ASD" groups compared to their respective 'Siblings' groups. For C57BL/6 mice, these analyses are currently ongoing.

These preliminary results show that the microbiota of children with ASD, can cause some behavioural and biochemical differences in germ-free recipient mice. The current stage of our study is to complete all ongoing analyses and determine whether correlations exist between those differences and the microbial composition and activity of the transferred microbiota.

PRE- AND PROBIOTICS TO RELIEVE CONSTIPATION-RELATED COMPLAINTS IN IRRITABLE BOWEL SYNDROME

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Irritable Bowel Syndrome (IBS) is a disease that affects a large number of people. To date, no adequate treatment is available. This is partially due to the heterogeneity of the patients and the complicated pathology in which not all mechanisms are understood. Dietary interventions are one promising route to relieve IBS-related complaints, such as constipation. The objectives of the NUTRIC study were to determine the effects of a 4-week intervention with either a prebiotic supplement or a probiotic supplement on stool pattern (including stool frequency, consistency, and volume), gastrointestinal (GI) complaints, and quality of life in IBS patients suffering from constipation (IBS-C).

In total, 180 IBS-C patients were included in a randomized double-blinded placebo-controlled human intervention study. The study consisted of two periods. First a 4-week observation period (week 1-4), which was similar for all three parallel arms (n=60 per arm), followed by a 4-week intervention period (week 5-8) in which study participants received one of three dietary supplements: prebiotic acacia fibre (Inavea™), probiotic *Bifidobacterium lactis* BLa80, or placebo supplement (maltodextrin). At the start and at the end of both study periods, study participants completed several online questionnaires on their IBS-related complaints (IBS-SSS, PAC-SYM), their Quality of Life (PAC-QOL, HADS), and their habitual dietary intake (FFQ). During both study periods, study participants also completed short daily questionnaires via an EMA app on their phone (LifeData LLC, Marion, IN, USA) asking for their stool pattern, gastrointestinal (GI) complaints, and supplement compliance. Prebiotics supplementation significantly improved stool frequency as compared to the placebo treatment, both when assessed on a daily basis and when assessed as the difference (delta) between the observational period and the intervention period (p=0.04 and p=0.02, respectively). This increase in stool frequency was also clinically significant with an increase of >1 stools per week. Probiotics supplementation showed a trend towards improved stool frequency over time as compared to the placebo treatment (P=0.08). Probiotics supplementation furthermore showed a significant decrease in symptom severity, as assessed with the IBS-SSS questionnaire (p=0.02). In conclusion, daily dietary supplementation with prebiotic and probiotic supplements may significantly relieve IBS-related complaints by increasing the stool frequency and decreasing symptom severity, respectively.

SESSION 3
BACTERIOPHAGE-BACTERIA INTERACTIONS IN THE GUT

BACTERIOPHAGES IN THE HUMAN GUT MICROBIOME: OPPORTUNITIES OR THREATS?

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Bacteriophages have long been an underexplored component in microbiome studies, but no more. Interest in phages has boomed and research groups all over the world, have shown the importance of phages in a wide range of environments. The advances in sequencing technology and bioinformatics are now enabling us to reconstruct whole phage genomes and allowing us to investigate the phage diversity at the strain level. Phages are a major component of the human gut virome, but for the majority of individual phages, we do not know how and if they contribute to human health. In this talk, I will discuss the challenges associated with analysing the human gut virome and provide an example of what happens to the healthy human microbiome after phage treatment using model system of the human colon.

MUCOSAL INTERACTIONS AS A BRIDGE BETWEEN PAST AND FUTURE PROPHYLACTIC PHAGE THERAPY

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The clinical use of bacteriophages (phages) to treat bacterial infections is not a new concept. The development of phage therapy in the early 20th century was heralded as a breakthrough in medical care and phage use became routine in many countries until disappearing almost completely during the 1940s-50s as antibiotics took over. The use of phages as prophylactic agents has been tested with success, but so far no mechanism has been proposed to support this approach. The bacteriophage adherence to mucus (BAM) model proposes an ancient symbiosis between phages and metazoans, in which phages attached to mucosal layers protect animals against invading bacteria. Given the importance of mucosal surfaces for pathogenic bacteria (point of entry to the metazoan host), for phages (increased encounter rates with invading bacteria) and for the metazoan itself (phage-derived protective layer), the tripartite interactions in mucus are relevant but under-studied at the present.

In this talk, I will discuss our latest advances on phage-bacteria interactions in mucosal conditions. Inspired by the BAM model we have shown that a mucus-associated phage can prevent bacterial disease. In this system mucins have a double role: adhesion to them keeps the phage for days inside the mucosa, while mucin exposure increases bacterial virulence and at the same time its susceptibility to the mucosal phage. Then we followed up with the investigation of how the mucosal environment affects the development of phage resistance in bacteria, discovering that mucin exposure induces CRISPR-Cas activity. Our main model has been *Flavobacterium columnare*, but by studying other phage-bacteria pairs we found out that the model is also relevant for human-associated bacteria. Taken together these findings points out to an important but overlooked role of the influence of biological interactions in mucosal conditions to basic research and to clinics. From one side, considering the effect of mucosal signals in phage-bacteria dynamics can lead to a better understanding of evolutionary processes and tripartite interactions. From a clinical perspective, exploring the mechanisms behind these interactions can improve current phage therapy and allow the justification and development of prophylactic phage therapy approaches against dangerous bacterial targets.

DEVELOPMENT OF PHAGE-BASED FEED ADDITIVES TO ENHANCE FED CATTLE HEALTH AND PERFORMANCE

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As antibiotic use in animal agriculture accounts for a large proportion of global consumption, identifying alternative treatment or management strategies is critical for mitigating the proliferation of antibiotic-resistant bacteria (ARB). In the USA, the antibiotics tylosin and monensin are commonly provided to feedlot cattle as feed additives. The rationale for providing tylosin is to reduce the occurrence and severity of bovine liver abscesses by inhibiting the primary etiological agent, *Fusobacterium necrophorum*. Monensin has been shown to increase cattle feed efficiency by disrupting ion gradients in a diverse range of bacteria. Thus, each of these antibiotics provide substantial economic benefit to the fed cattle industry. However, macrolide antibiotics, such as tylosin, are important for human health, and monensin, while previously considered unimportant for human health, is now suspected of promoting cross-resistance to vancomycin. As an alternative to these two antibiotics, our group is developing bacteriophage-based feed additives capable of inhibiting the growth of *F. necrophorum* as well as select ruminal species shown to be negatively correlated with cattle feed efficiency. As highly ubiquitous and efficient bacterial predators, bacteriophages (phages) are ideal microbiome engineering tools for selectively modulating bacterial species abundances within the bovine rumen.

Using selective enrichments, we isolated and characterized over fifteen novel *F. necrophorum* phages belonging to at least six distinct groups. Most phages displayed a fairly broad intraspecific host range, and were able to infect multiple contemporary *F. necrophorum* strains isolated from geographically disparate US cattle. All phages were found to be temperate, with dsDNA genomes ranging from approximately 36 kbp to 114 kbp. Bacterial genome sequencing and prophage induction revealed almost all contemporary *F. necrophorum* isolates harboured one or more active prophages. Despite being temperate, φBB37 was able to inhibit the growth of the high leukotoxin-producing strain *F. necrophorum* 8L1 for over 40 h *in vitro*, while phage cocktails were effective for up to 60 h. Most phages displayed good productivity (> 10^9 PFU/mL) after minimal optimization and show little yield variation during scale up. Efforts to generate virulent mutants using random mutagenesis have been unsuccessful but are ongoing.

Cattle phenotypes, such as feed efficiency, are likely impacted by many different ruminal species and could require novel strategies to steer microbiome function towards a desired state. As proof-of-concept, we focused initial efforts on two species which were the most amenable to phage-mediated biocontrol based on ease of phage isolation and their demonstrated impact on feed efficiency. Surprisingly, and against conventional wisdom, we found a higher abundance and prevalence of *Fusobacterium varium* and associated phages in cattle rumen relative to *F. necrophorum* and suspect it may play a detrimental role in feed efficiency as a hyper-ammonia-producing bacteria. Both lytic and temperate *F. varium* phages were isolated from most rumen fluid samples. In general, these phages have fairly broad host ranges and produce high-titer lysates (up to 10^{11} PFU/ml). However, the average magnitude and duration of inhibition was less than that observed for *F. necrophorum* phages. *Streptococcus bovis* complex species have also been associated with decreased cattle feed efficiency and was more amenable to phage isolation than other ruminal species. We isolated five novel *S. bovis* phages and found φCSJ3-A to be highly effective at inhibiting host growth for up to 30 h. These phages appear to be lytic on the basis of genomic characterization and lysogeny assays. However, using a small library of contemporary isolates, we found their host ranges to be fairly strain-specific. This limits their use for predefined cocktails unless host-range expansion protocols can be successfully employed. Overall, our results highlight the potential for replacing antibiotics with phage-based feed additives, though improved methods for generating random deletion mutants or engineering non-model phages would likely accelerate the development of phages for gut and rumen microbiome engineering.

MANUFACTURING AND FORMULATION OR BACTERIOPHAGES FOR ORAL DOSAGE FORMS

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The talk will cover both upstream manufacturing of the phage drug substance (DS) using aerated stirred tank fermenters and their downstream purification to remove contaminating host cell proteins, host cell DNA and endotoxin etc. Formulation considerations for the DS as liquids and solid dosage forms will be discussed. Microencapsulation approaches using spray drying and microfluidic droplet generation systems using pH responsive triggered release formulations for gastrointestinal delivery of phages will be presented.

PHAGE THERAPY FOR THE GUT AND BEYOND

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The human gastrointestinal tract (GIT) is a reservoir of extraintestinal pathogenic *E. coli* or ExPEC – a major cause of UTI, bacteremia and sepsis. The multidrug-resistant (MDR) sequence type (ST)131 is the ExPEC clone responsible for a majority of infections and the dissemination of drug resistance worldwide. Antibiotics may no longer be viable as a therapy for these MDR infections. Also, antibiotics are not selective killers and can alter the microbiota setting the stage for overgrowth of these strains or other opportunistic pathogens. New treatments are needed to cure MDR infections and prevent the dissemination of drug resistance. Bacteriophages or phages are bacterial viruses that kill specific bacteria and can thus be used to kill MDR ExPEC within the GIT without altering the intestinal microbiota. In order to gain a greater understanding of phage killing within the gut microbiome, we modelled ExPEC infections *ex-vivo* using intestinal contents from mice. Using our model, we show that ExPEC-specific phages do not alter the diversity of the intestinal microbiome. Also, our studies have shown that despite efficient killing of ExPEC strains in conventional media or in blood infections in mice, within the intestinal environment, some phages encounter barriers to productive infection. Using a combination of biochemical approaches and the ‘caecal media’ described above, we determined the inhibitory component is intestinal mucus. Using a selective screening process of environmental samples, we identified a unique mucophilic phage capable of showing enhanced infective capacity only in the presence of mucus. Interestingly, this phage was capable of removing ExPEC from the mouse cecum, suggesting intestinal editing of harmful pathobionts may require specialize mucus-loving phages.

A thorough understanding of the intestinal barriers phage encounter is needed in order to implement phage therapy as a viable treatment for MDR bacterial infection and prevent the spread of resistance.

SESSION 4
BENEFICIAL MICROBES IN PERSONAL CARE

PRECLINICAL MODEL TO EVALUATE THE BENEFICIALTY OF COSMETIC INGREDIENTS FOR SKIN MICROBIOTA

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The cosmetic industry is currently looking for ingredients that can maintain and restore the skin microbial community, such as pre-, pro- and postbiotics, since its equilibrium is associated with healthy and younger skins, acting as anti-aging shields. In this work, a preclinical model will be presented using human volunteer microbiota samples for the evaluation of cosmetic ingredients on the skin microbiota. The model was validated with a new ingredient based on an extract rich in phenolic compounds and with a known prebiotic/postbiotic benchmark. Briefly, skin microbiota samples were collected from the face of 12 female volunteers with ages between 25 to 35 years-old without diagnosed skin diseases. Samples were divided in two conditions: control and ingredient, which were incubated overnight. After incubation, microbial DNA was extracted and quantitative real-time PCR (qPCR) was used with universal and specific primers to quantify the bacterial and fungal load of specific genera and species. Further, samples were evaluated by next-generation sequencing (NGS), where 16S rRNA gene and ITS region amplicons were sequenced to obtain the profile of skin microbiota.

The present model allowed to compare the relative abundance of specific skin microbiota genera and species present in samples, when in the presence of the ingredients, and the baseline (without ingredient), especially of important commensal such as *Staphylococcus epidermidis* and *Cutibacterium acnes*. When testing the phenolic compounds ingredient, qPCR analysis did not show significant differences in the main genera evaluated, namely *Staphylococcus*, *Cutibacterium*, *Corynebacterium* and *Malassezia*, when comparing the control and ingredient groups. *S. epidermidis* and *C. acnes* demonstrated an increased abundance in the presence of the ingredient, but the ratio of these species remains undisturbed. These results were supported by NGS. Concerning the benchmark, the results obtained with the model were well correlated with the *in vivo* data presented by the brand technical datasheet, concluding that the model can be used to screen these types of ingredients with a high level of trust.

PROBIOTIC SUPPLEMENT FOR PERSONAL CARE

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The skin consists of an epidermis, that acts as a mediator of the permeability barrier to the skin, and of a dermis below it, which, among other things, is composed of connective tissue, giving the skin its ability to hold structure and resist external forces. In the connective tissue, dermal fibroblasts are responsible for secreting the major structural protein, type I collagen, among various other extracellular matrix (ECM) molecules. During aging, the production of ECM proteins is reduced and synthesis of ECM degrading proteinases is increased, leading to imbalanced homeostasis and wrinkle formation. The term gut-skin axis refers to the two-way dialogue between gut and skin through microbiota, microbial metabolites, and immune system.

There is an increasing number of studies exploring oral probiotics for skin diseases. Skin aging is an inevitable process, and it is exacerbated by challenging external conditions. *Bifidobacterium animalis* ssp. *lactis* Bl-04, a probiotic, has been previously noticed to have beneficial influences on gut bacteria and immune system modulation. Here, the effects of the *B. lactis* Bl-04 on skin structure were explored for the first time. Exposing a monolayer of human dermal fibroblasts *in vitro* to postbiotics, metabolites produced by bacteria, Bl-04 postbiotics were shown to support collagen metabolism in the skin. A randomized, placebo-controlled, triple-blinded clinical trial was conducted to explore further the efficacy of probiotic Bl-04 supplementation (1.75×10^9 colony forming units) on healthy Korean female volunteers (n=148) aged 33-60 years with wrinkles and dry skin. The trial lasted 12 weeks and was conducted during the dry winter season towards the spring. Consumption of Bl-04 was found to alleviate facial wrinkling at 4 weeks, especially in the younger population, as quantified by objective imaging methods. Towards the end of the intervention, when weather conditions became more favourable for the skin, the effects on wrinkle parameters did not differ significantly. Besides the environmental circumstances and participant age, it was noted that skin condition and lifestyle factors affected the measured parameters.

B. lactis Bl-04 was discovered to benefit the skin *in vitro* and in human clinical trial. There are several challenges when screening *in vitro* the gut-mediated systemic effects of ingested products for skin benefits requiring complex *in vitro* cell or tissue models and balancing between the extend of the screening. Furthermore, for additional elucidation of the role of an oral supplement for skin beauty purposes, it may not be feasible to conduct animal studies due to strong ethical considerations in the cosmetics industry. Therefore, the continuous development of cell and tissue models in this area would benefit the research related to gut-skin axis, as well.

NITRATE AS A PREBIOTIC AND NITRATE-REDUCING BACTERIA AS PROBIOTICS FOR THE ORAL MICROBIOME

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Salivary glands concentrate plasma nitrate into saliva, leading to high nitrate concentrations that can reach 5-8 mM after a nitrate-rich vegetable meal. Whereas human cells cannot reduce nitrate to nitrite effectively, certain oral bacteria can. This leads to an increase in systemic nitrite that can improve cardiometabolic health through nitric oxide availability. Nitrate is also an ecological factor that can induce rapid changes in structure and function of polymicrobial communities, but the effects on the oral microbiota had not been clarified. To test this, an *in vitro* study was set up to determine the effect of nitrate on oral communities grown from saliva of 12 healthy individuals. In a second study, 53 nitrate-reducing isolates were obtained and the effect of six probiotic candidates from the genus *Rothia* was tested in the same *in vitro* model. Finally, we studied the effects of beetroot extracts on oral acidification after sugar rinsing in 24 individuals and reviewed the literature for other studies testing the effect of other nitrate-rich vegetables on the oral microbiota. Supernatants or saliva samples were taken for nitrate, nitrite, ammonium, lactate, and pH measurements. Additionally, the bacterial composition of *in vitro* biofilms and salivary pellets were determined using 16S rRNA gene Illumina sequencing. We showed that nitrate stimulates the growth of the beneficial genera *Rothia* and *Neisseria* in our *in vitro* model (an observation supported by clinical studies), while potentially decreasing caries-, halitosis- and periodontal disease-associated bacteria. Additionally, nitrate limited or prevented lactic acid (organic acid involved in caries development) accumulation and pH drops during sugar fermentation by the oral microbiota. A selection of *Rothia* isolates further increased lactate usage and nitrate reduction capacities of oral communities, being of potential benefit for dental health and systemic health, respectively. We propose that nitrate could be used as a prebiotic and nitrate reducing isolates as potential probiotics to treat or prevent oral dysbiosis and improve general oral health.

THE POTENTIAL OF PRECISION PROBIOTIC *HAFNIA ALVEI* HA4597 TO SUPPORT WEIGHT LOSS

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Stemming from 20 years of research with the French National Institute for Medical Research (Inserm), TargEDys introduces the discovery journey of a probiotic mechanism of action based on a metabolite produced by the bacterium *Hafnia alvei* HA4597, which presents a homology of sequence and conformation to a key satiety hormone. This protein, caseinolytic peptidase B (ClpB) is a molecular mimetic of alpha-MSH and was shown to activate the hormone's receptors, MCR, and stimulate the anorexigenic pathway, supporting weight loss through the regulation of appetite. From the discovery of this alpha-MSH analogue to the mechanistic confirmation, preclinical proof of concept in mice models of obesity, and human efficacy in over 230 overweight adults, TargEDys showcases this first example of a molecular mimetic in the probiotics sector.

This target-based approach can be replicated for many targets, as shown by the second example of technology developed that is based on oxytocin mimetics for mental well-being.

THE SKIN MICROBIOME IN ATOPIC DERMATITIS PATIENTS IN BELGIUM AND HOW TO MODULATE IT WITH PROBIOTICS

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Atopic dermatitis (AD) is an inflammatory skin condition, affecting 20% of children in Western countries. Evidence is growing that AD is associated with reduced microbial diversity and higher abundance of *Staphylococcus aureus.* This role for microbes in the pathogenesis of AD shows potential for probiotics. Selected probiotics should ideally inhibit pathogens, restore the skin microbiome composition and/or barrier function and execute anti-inflammatory effects. To the best of our knowledge, such multi-acting probiotic strategies have not yet been explored for AD in humans.

First, the microbiome of 54 healthy controls was mapped with metagenomic shotgun sequencing at two timepoints with a 12-week interval. Correlations with lifestyle and environmental factors were made by extensive questionnaires. In parallel, the skin microbiome of 29 patients with mild and 6 patients with moderate-to-severe AD was compared with the healthy controls. Subsequently, the impact of a probiotic cream with live lactobacilli was studied in a proof-of-concept (POC) split-body, placebo-controlled clinical trial in 7 patients with mild AD. Over 12 weeks, AD symptoms were scored and the skin microbiome was studied.Longitudinal analysis of the healthy skin microbiome showed that adolescents and adults have a more stable beta-diversity compared to children under 12 years old. Shotgun sequencing also showed a reduction in *Corynebacerium*, *Streptococcus* and *Roseomonas* spp. on AD lesions. The beneficial role of these taxa is underexplored, in part because they are not ‘generally recognized as safe’. Therefore, we decided to select lactobacilli as potential beneficial taxa, they are generally safe, closely related to *Streptococcus,* do occur on the skin, can inhibit *S. aureus* and also produce lactic acid. In the POC clinical trial, the probiotic cream was safe and well tolerated. AD symptom scoring showed a large heterogeneity, with no significant differences observed for the treated zones. However, the lactobacilli were highly detected in the probiotic arm and did reduce the relative abundance of *S. aureus*.

Taken together, our study revealed new insights in potential beneficial taxa on the skin such as lactic acid bacteria *Streptococcus* and *Lactobacillales*. Particularly, lactobacilli formulated in a probiotic cream could be applied in a safe and detectable way on the skin and positively impacted the microbiome of AD patients. This study thus shows potential for probiotics in the treatment of AD. However, the heterogeneity of AD also learnt us that an adapted study design is necessary to enable evaluation of the clinical performance of probiotics in mild AD.

EFFECT OF 10-12 MONTHS SUPPLEMENTATION WITH THE PROBIOTIC *LACTOBACILLUS RHAMNOSUS*, LGG® (DSM33156) AND *L. PARACASEI* SUBSP. *PARACASEI*, L. CASEI 431® (DSM33451) AND ARGININE ON CARIES INCREMENT IN HEALTHY CHILDREN: A RANDOMIZED, PARALLEL-GROUPED, PLACEBO-CONTROLLED CLINICAL TRIAL

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The aim of this study was to investigate the efficacy of the probiotic *Lactobacillus rhamnosus*, LGG® (DSM33156) and *L. paracasei* subsp. *paracasei*, L. CASEI 431® (DSM33451) and arginine on dental caries increment in healthy Danish children aged 5-9 years. The investigation employed a randomized, double blinded, parallel-grouped, placebo-controlled clinical trial. A total of 371 children from nine public schools in four municipalities in Denmark were screened and 28 patients were excluded due to screening failures. Thus, 343 children were included in the study. After informed parental consent, the subjects were randomly assigned to: Intervention group (n=172) who received a lozenge containing 2 billion CFU in total of the two strains LGG® and L. CASEI 431® (50%:50%) and arginine 2% and Placebo group (n=171) who received an identical lozenge without probiotics and arginine. Participants were instructed to take one lozenge a day, encouraged to brush their teeth twice a day and to avoid food containing probiotics. Primary canines and molars, and permanent first molars were examined clinically and radiographically at baseline and follow-up using a modified dmf-s/DMF-S (decayed/missing/filled surfaces on primary/permanent teeth) scoring system where d was defined by ICDAS (International Caries Detection and Assessment System). ICDAS scores ranged from 0 (sound surface) to 6 (surface with extensive dentin cavity). Thereafter, the ordinary ∆dmf-s (primary teeth) was calculated where d contained ICDAS scores 3 to 6. The modified dmf-s/DMF-S and ∆dmf-s were computed to analyse the efficacy of the lozenge. All statistical tests were assessed using unadjusted non-parametric Mann-Whitney U test with a nominal two-sided significance level of 5%. Compliance was assessed by weekly online surveys and by counting excess lozenges. The intervention period was 10-12 months. For the analyses, 288 children (intervention group 141 and placebo group 147) were included and 55 patients were excluded due to dropout. The mean modified dmf-s/DMF-S increased with 2.3 or with 0.04 when divided with the number of surface in the intervention group and with 4.6 or 0.06/surface in the placebo group from baseline to follow-up (p=0.31). The mean ∆dmf-s increased with 0.01 dmf-s or 0.002/surface in the intervention group and 0.49 dmf-s or 0.01/surface in the placebo group (p=0.007). No product related side-effects were reported.

In conlusion, daily consumption of a lozenge containing probiotic strains LGG® and L. CASEI 431® and arginine for 10-12 months showed a statistically significant reduction in dental caries using ∆dmf-s, but not with modified dmf-s/DMF-S.

SESSION 5
BENEFICIAL MICROBES AND THE GUT-BRAIN AXIS

AN APPLE A DAY KEEPS THE PSYCHIATRIST AWAY

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In the NoGutsNoGlory (NGNG) study, we try to disentangle the complex knot between psychiatric symptoms, nutritional habits, medication, microbiome, and gastro-intestinal complaints. We investigated gastro-intestinal complains in 120 patients with severe psychiatric disorders (schizophrenia and bipolar disorder). We found many significant correlations, but strongest associations were found between gastro-intestinal complaints and depression. When we also take nutritional habits into account, a lack in fibre content was strongly associated with psychiatric complaints, again most pronounced for depression. We designed the Brain anti-inflammatory nutrition (Brain menu), which is based on the Shivappa index and combined with fish oil and fibre-rich ingredients, which we will provide to 120 patients with psychiatric and neurological disorders. So far, the first 20 patients have been included. They were motivated and adhered well to the Brain menu. Another part of the NGNG project is the development of innervated gut organoids, which was successful. In the next stage, these organoids will be tested with different bacterial strains and probiotic products.

THE MICROBIOME AND COGNITION DOMAINS

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Diverse studies hinted at bacterial microbiome dysbiosis as having a substantial impact on the pathophysiology and development of obesity and type 2 diabetes. The gut microorganism ecology interacts with metabolic impairment and could play a role in other systemic traits of those prevalent metabolic diseases. Recent research has revealed a link between gut bacteria composition and functionality with several cognitive domains. Immediate and short-term memory, inhibitory control and depressive traits are substantially associated with the gut bacterial ecology. In addition, the presence of some bacteriophages, such as *Caudovirales*, in acid lactic bacteria run in parallel to enhanced executive function and immediate memory in *Drosophila*, mice, and humans.

ANXIOLYTIC EFFECTS OF A DAILY PREBIOTIC IN HEALTHY YOUNG FEMALE VOLUNTEERS

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Research in both human and animal models has highlighted the important role that gut microbiota play in regulating the brain and subsequent behaviour, particularly within the context of mental health problems, such as anxiety. These models have also suggested that modifying microbial ecology therapeutically via the intake of psychobiotics (e.g., prebiotics), may reduce symptoms of anxiety- a finding which has much potential for future interventions and therapy. The transferability of these animal models to humans however is less clear. Here I will present the results from a 4-week double blind, placebo-controlled prebiotic supplement intervention study which used emotion regulation abilities as a model for mental health to understand how changes in gut microbiome diversity relate to psychological functioning in a sample of female participants. In this study, we focused on multiple indices of well-being and diet (self-report measures and behavioural testing), and we also obtained stool samples from each participant for microbiome sequencing. Our results showed that in addition to a significant increase in beneficial Bifidobacterium in the gut microbiome, the prebiotics intervention reduced self-reported high trait anxiety and reduced negative attentional bias. Moreover, we found that participants in the prebiotics group changed their nutritional intake by reducing sugar and carbohydrate intake. Together, the results highlight the feasibility of using a dietary intervention to improve outcomes at both the gut microbiome and behavioural level and bring us closer in translating animal research to the human model.

EFFORTS TO UNDERSTAND THE IMPACT OF THE GUT MICROBIOTA ON HUMAN NEUROBIOLOGY

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A majority of the studies investigating the microbiota-gut-brain axis in humans focus on behavioural measures, including clinical diagnoses and questionnaires, providing evidence for a link between the gut microbiota composition and cognitive and emotional functioning. Our work focus on how the variation of the human bacteriome – in interaction with the host's genome – has an effect on human neurobiology, cognitive functioning and brain structure and function. I will discuss our approach to detect these signals and a selection of our current findings. I will focus on the microbial signals associated with comorbid psychiatric diseases and neurodevelopmental disorders showcasing analytical approaches, including Randomized Lasso stability-selection and a meta-analysis of the association between gut microbiota with ADHD. I will also describe exploratory evidence for multivariate associative patterns between the gut microbiota and brain network connectivity in healthy humans and why is this important to understand the effects the gut microbiota, via the gut brain axis, have on human brain homeostasis.

OVERCOMING THE BRAIN BARRIER: A CHALLENGE FOR BACTERIA?

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The gut microbiota represents a diverse and dynamic population of microorganisms that can influence the health of the host, including the development of neurological disorders such as Alzheimer's disease. However, the mechanisms behind the interplay between our gut and brain is still elusive. In this talk, I will focus on bacteria-derived metabolites and outer membrane vesicles (OMVs) as central players in the gut-brain axis despite the presence of tight barriers between blood and brain. Understanding the mechanisms behind this gut-brain crosstalk will provide us new insights that may pave the way for novel therapeutic strategies to treat neurological disorders.

SESSION 6
TOWARDS RENEWABLE-BASED PREBIOTICS

PREBIOTIC TEA PHENOLICS: GUT MICROBIAL CONVERSIONS, RENEWABLE SOURCING, AND SUSTAINABLE EXTRACTION

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Tea is one of the most consumed beverages in the world and is commonly associated with various health benefits. These health benefits have been ascribed to the phenolics, primarily catechins and their oxidation products, which are present in high quantities in tea. However, due to the relatively low bioavailability of these phenolics in the small intestine, they reach the colon where they can (i) be metabolised by gut microbiota and (ii) modulate the gut microbiota composition. Thus, tea may actually confer a significant part of its health benefits via the reciprocal interactions of tea phenolics with the gut microbiota, rather than via direct absorption of these bioactive compounds. Tea phenolics can even be considered to be prebiotics, as the health benefits they confer via modulation of gut microbiota composition and via formation of health-promoting microbial metabolites is in many ways analogous to the prebiotic action of conventional oligosaccharide prebiotics.

Therefore, it is of interest to explore strategies to increase tea phenolic intake, for example through supplementation or food fortification. These compounds should be obtained from a renewable source and old tea leaves, which form an agricultural waste stream, are a promising raw material in this respect. Based on our analyses, we conclude that old tea leaves still contain approximately 9.5% ([w/w] dry weight) of phenolic compounds, including 4.3% ([w/w] dry weight) of catechins. In addition, it is essential to find sustainable alternatives to the energy-intensive and non-selective approaches that are currently used to extract phenolics from tea leaves and similar raw materials. Our results showed that pulsed-electric field (PEF) pre-treatment enhances the effectiveness of phenolic extraction from tea leaves, while potentially being up to a 1000-fold more energy-efficient than drying via heating. In conclusion, old tea leaves are a renewable source of tea phenolics, which confer prebiotic effects, and these compounds can be effectively extracted from tea leaves after energy-efficient PEF pre-treatment.

CELLO-OLIGOSACCHARIDES: POTENTIAL PREBIOTICS AND THEIR BIOSYNTHESIS FROM RENEWABLE CARBOHYDRATE-BASED RESOURCES

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Nowadays, the rapidly growing evidence on the dynamic composition of the gut microbiota in relation to human health and development strongly motivates the search for new prebiotic non-digestible oligosaccharides (NDOs) with selective agency in promoting the growth of microbiota species. Short-chain cello-oligosaccharides (COS) with degree of polymerization (DP) less than 6 are promising as novel NDOs since they are not digested by the human arsenal of digestive glycoside hydrolases, and now have drawn significant interests from the food industry. Generally, the routes for COS production are based upon the depolymerization of cellulose or bottom-up synthesis. However, COS production in high yield and with proper DP control remain challenging. Technology fit for the bulk production of soluble COS is currently lacking.

Here, through the use of glycoside phosphorylases (cascade), an efficient approach to the bottom-up synthesis of COS from renewable carbohydrate resources (sucrose, glucose) are presented [1-4]. Process intensification gave a soluble COS production in ~100 g/l titer and in 82% yield from sucrose (0.5 M). Purified COS (≥ 95% purity) was examined for growth promotion of probiotic strains. Referenced against established NDOs prebiotics (trans-galacto-oligosaccharides, inulin) and cellobiose, COS showed up to 4.1-fold stimulation of cell density for *Clostridium butyricum*, *Lactococcus lactis*, *Lactobacillus paracasei*, and *Lactobacillus rhamnosus*, but were less efficient with *Bifidobacterium* sp. Our study thus demonstrates an efficient enzymatic production of the COS and reveals them as selectively functional carbohydrates with significant prebiotic potential.

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TURNING WASTE/BY-PRODUCTS INTO NOVEL (PERSONALIZED) PREBIOTICS THAT MODULATE THE GUT MICROBIOTA

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The gut microbiota plays a role in many diseases and disorders because it interacts with the host at multiple levels, amongst which the immunological, metabolic, and neurological levels. Although it is difficult to speak about a normal microbiota because the composition is defined by many factors and differs between individuals, an abnormal microbiota composition – or dysbiosis – has been observed in many instances. Whether this is the chicken (cause) or the egg (effect) is not always clear. However, various microbial taxa have been linked to health, while others have been observed to be higher in abundance in disease. The gut microbiota composition is very much defined by diet. There are a few well-defined prebiotics that specifically modulate certain beneficial taxa in the gut microbiota such as *Bifidobacterium* species, but with the discovery of more and more other 'beneficial microbes®', such as *Faecalibacterium prausnitzii*, *Eubacterium hallii*, *Akkermansia muciniphila*, or *Dysosmobacter welbionis*, other, novel prebiotics are required that specifically target these bacterial taxa.

Using the validated, sophisticated, dynamic *in vitro* model of the colon (TIM-2), we have screened numerous waste-streams or by-products of fruit- and vegetable-processing, to valorise these into functional foods or prebiotics that modulate the gut microbiota composition in general, or in a targeted manner, respectively. Moreover, by analysing the fibres present in these side-streams we aim to establish structure-function-relationship between the fibres present and their gut microbiota modulatory capacity. This includes studying the glycosylhydrolase enzymes (GHs) present in selected 'beneficial microbes®'. Creating a catalogue of GHs in the microbiota of individuals could ultimately lead to the development of personalized prebiotics, from waste or by-products in a circular, sustainable, and renewable manner. The presentation will highlight some of the results obtained with the TIM-2 system with respect to targeted stimulation of certain taxa, related to structure of certain (novel) prebiotics, and includes the assessment of the production of the health-beneficial metabolites, the short-chain fatty acids (SCFA). The use of a standardized microbial inoculum for TIM-2 allows for this selective screening of fibres because each experiment starts with the same microbiota composition, while allowing for testing the effect of the novel substrates under physiological conditions that closely mimic *in vivo*, including absorption of SCFA. In other models, these metabolites accumulate and lead to un-physiological changes in microbiota composition and activity. Therefore, TIM-2, which nears its 25th year of existence, is a great tool to develop these novel prebiotics from waste or by-products.

BIOMASS AS A RENEWABLE SOURCE OF PREBIOTICS

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Xylo-oligosaccharides (XOS) produced from biomass offer a plethora of excellent physicochemical and physiological properties to be used as natural prebiotic nutraceuticals. One of the most promising technologies for accessing these oligomers which combines good yields and low environmental footprint is the microwave processing of biomass [1]. The approach offers an additive-free alternative to the use of bio- (slow but with few side-reactions) or chemo- (quick but likely to lead to impurities) catalysts. The type of biomass and the operating conditions are important factors in influencing the quality and quantity of the XOS products although there will inevitably be a compromise between the two.

From an economic perspective, the production of XOS can be very cost-effective with costs using this microwave approach being estimated at less then € 10/kg. Microwaves are becoming a well recognised energy efficient method for the deconstruction of complex biomass [2]. However, the capex costs of microwave reactors cannot be ignored. An holistic and better economic model is to seek to associate an XOS production facility with the more common chemical valorisation of the cellulose component of biomass. The use of microwaves to valorise different primary components of biomass is gaining increasing attention [3,4].

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NEW OPPORTUNITIES TO DESIGN NEXT-GENERATION PREBIOTICS FROM RENEWABLE SOURCES: FROM SUSTAINABILITY TO PRECISION NUTRITION?

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Worldwide, one-third of food produced for human consumption is lost or wasted mainly during primary production and food processing, causing significant environmental and economic impacts, and affecting food security in a context of global population growth. Hence, contributing to zero food waste production schemes is becoming a priority to meet Sustainable Development Goals. Particularly, vegetable and agrifood wastes are lost at higher rates due to their high moisture content that make them prone to microbial degradation. However, these wastes are rich sources of compounds with promising biotechnological and health promoting applications such as a wide and diverse range of non-digestible carbohydrates, enzymes or biopolymers, among others. Therefore, these wastes can be integrated into circular economy models through extracting added-value compounds for other applications.

Among the valorisable ingredients present in agri-food wastes, the presence of a wide array of carbohydrate fibres including resistant starch, inulin, cellulose, hemicellulose, pectin, and alginates, is very remarkable. Some of these carbohydrate families include emergent prebiotics and can be metabolized by specific commensal bacteria in the large intestine, including representative species of next-generation probiotics, leading to the production of metabolites, such as short chain fatty acids (SFCA) that exert important functions on human physiology. Fine physico-chemical, structural and functional analyses of agrifood waste-derived pectin and arabinoxylan structures have demonstrated specific microbial modulation patterns associated to particular physico-chemical properties through *in vitro* fermentation models. Integration of this wealth of data through computational modelling could be used for hypothesis formulation and elucidation of the potential mechanism of action of these compounds. These approaches can diversify the opportunities to valorise agri-food residues through formulating of novel prebiotics for targeted particular population groups.

SESSION 7
BENEFICIAL MICROBES IN ANIMAL HEALTH AND NUTRITION – PART 2

BENEFICIAL IMPACT OF *SACCHAROMYCES BOULARDII* I-1079 PROBIOTIC ON FUNCTIONALLY IMPORTANT GUT ANAEROBES: EXPERIMENTAL PROOFS

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It is well recognized that gut microbiota plays a crucial role in nutrition and health of all mammals, both humans and animals. Beneficial interactions between gut microbial communities and their hosts, leading to gut homeostasis, are greatly dependent on the physico-chemical and nutritional conditions of the intestinal environment. Indeed, a range of functionally important bacterial species have been reported as being quite sensitive to gut environmental conditions, e.g., pH, oxygen levels, nutrient availability, or vitamins and co-factors supply. In certain periods of life, such as in early life, around parturition, or under nutritional or environmental stresses, the gastro-intestinal tract microbiota can be quite unstable, leading to uncomplete nutrient digestion and feed valorisation, but also to poor immune status, threatening animal wellbeing and health.

Feeding strategies aiming to enhance gut functions through beneficial impacts on microbiota have been developed in the livestock sector and among those, the supplementation with live yeast *Saccharomyces boulardii* CNCM I-1079 (SB) has consistently shown to promote anaerobic species in the gut, notably in early life in pre-weaned piglets or calves, or in adult animals encountering stressful situations such as parturition, heat stress, or pathogen challenge. A particular impact has been observed on *Faecalibacterium prausnitzii*, whose relative abundance, measured by 16S metataxonomic analysis of faecal contents, has been increased in supplemented animals in several trials in piglets, calves, bitches, or broilers. *F. prausnitzii* is recognized to be in animals but also in humans an active member of the gut community involved in SCFA production, in particular butyrate, and production of anti-inflammatory compounds promoting gut health. In young calves, the genus *Akkermansia,* described for its role in immune system maturation, has been shown to be also positively impacted by SB supplementation. In the same study, lactic acid-producing and -utilizing taxa, respectively *Lactobacillales* and *Negativicutes*, have been significantly enriched in the faces of supplemented calves, suggesting a better adaptation to milk digestion. Nutritional impacts are thus measured as well with SB. Furthermore, the bacterial genus *Fibrobacter*, known to play a key role in dietary structural carbohydrates degradation was found in higher proportions in the faeces of young piglets supplemented with SB, and in adult heat-stressed pigs, higher levels of the keystone anaerobic species *Ruminococcus bromii* at thermoneutrality has been suggested to be one of the causes for higher energy retention observed under thermal stress in SB supplemented pigs. By modifying the gut environment through pH and redox control, modulating resident microbiota composition, or supplying growth factors such as acetate, SB could positively affect growth and metabolic activity of those bacteria which are generally described as being very sensitive to changes in ecological conditions of their biotope. SB has also been proved to be an efficient strategy to alleviate gut dysbiosis by limiting the decrease in alpha-diversity often observed in challenging situations. For instance, SB supplementation altered the alpha-diversity of the faecal microbiota of bitches just before parturition, and in poultry SB has been applied with success in a model of *Campylobacter* challenge, with a reduction of the prevalence of the pathogen together with a higher alpha-diversity and promotion of important bacterial taxa such as *Faecalibacterium*, *Lactobacillus* spp. and *Lactobacillus reuteri*. In most of the studies reported here, the positive effect of *S. boulardii* CNCM I-1079 on gut microbiota has been translated into ameliorated feed conversion rate and/or growth, which is obviously an important objective for the farmer. Concomitant health benefits have been measured through decrease in therapeutic treatments against digestive and/or respiratory disorders and add significant financial return to the producer which strengthens the recommendation to use this probiotic on-farm.

ABNORMAL DIET, ABNORMAL BEHAVIOUR – REVIEWING THE ROLE OF DIET AND THE MICROBIOTA-GUT-BRAIN AXIS IN TAIL BITING PIGS

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Tail biting is a multifactorial, abnormal, damaging behaviour in growing pigs that is detrimental to the health and welfare as well as costly for the farmer. It occurs as a consequence of a build-up of stressors experienced by the pig including diet-related risk factors (e.g., composition, quality, amount consumed, form and accessibility). Based on a literature review [1], we will argue how the diet of commercial pigs can be an underlying cause for abnormal behaviour via the microbiota-gut-brain axis (MGBA). While the diet of commercial pigs is fine-tuned to increase production efficiency, it is abnormal when considering the dietary pattern pigs evolve to consume in nature. Recent advances in the field of the MGBA present pathways through which a diet low in dietary fibre, high in digestible protein and offered in a competitive environment - as is common in commercial pig production - can lead to reduced stress resilience and changes in behaviour via dysbiosis of the gut microbiota. While research in the MGBA in farm animals is still scarce, the first studies link gut microbiota and damaging behaviour in pigs and poultry. We propose that much more is to learn from cross-sectional work linking diet and gut health with behaviour and welfare in order to achieve a more sustainable animal production. In the future, knowledge about the MGBA may be used to develop dietary interventions that support a beneficial microbial community to improve stress resilience and mitigate damaging behaviour in farm animals.

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NOVEL MOLECULAR MECHANISMS BY WILDLIFE PROBIOTICS TO INFORM IMMUNO-MODULATORY MICROBIOME THERAPIES

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The discovery of the human (and animal) microbiome has revolutionized the field of medicine and biological sciences. To date, we have access to endless data informing of the microbial diversity present in different organs and body systems, and how this diversity correlates with many medical conditions. However, we are still unsure which of the many commensal microbes that reside the host are the main drivers that restore or protect health from disease. Much littler the information is concerning the molecules that these key commensals possess to interact with the immune system. This important question has been addressed by our research team as we are trying to reveal the molecules that beneficial bacteria utilize to induce host beneficial responses. Our recent investigations have shown that certain species of lactobacilli that were isolated from wildlife, significantly activate the production of type I interferon (IFN-I) cytokines in macrophages. IFN-I cytokines are essential to confer protection against microbial infections and auto-immune disorders. For the first time, we have proved that this IFN-I activation is predominantly driven by cGAS, a molecule that activates the cytosolic sensor STING upon the recognition of bacterial DNA. Furthermore, we have observed that lactobacilli encode some surface proteins with the potential to interact with macrophages for subsequent phagocytosis via non-opsonic scavenger receptors. Therefore, we are focused on determining the role that these surface proteins play as a port of entry in macrophages and characterize the IFN-I-mediated intracellular signalling initiated by cGAS. Elucidating these unknown mechanisms will be important to inform on how specific molecules of commensals modulate or stimulate host responses that, in unhealthy individuals, are exacerbated or inhibited. Overall, our studies will provide a better understanding on the molecular crosstalk between the microbiome and mammalian cells, paving the way for major therapeutic discoveries.

PROBIOTICS FOR HONEYBEES (*Apis mellifera* L.)

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The sudden death of honeybee colonies (colony collapse disorder, CCD) is a global problem. There are several factors suspected of causing CCD, such as exposure of these insects to pesticides (especially neonicotinoid insecticides), antibiotics, environmental pollution, and pathogens (parasites, bacteria, fungi, viruses, and other insects). All of the above factors negatively affect the condition and health of honeybees by weakening their immunity, while making them susceptible to infections by pathogens such as *Paenibacillus larvae*. A growing number of studies report that the above factors, especially pesticides, adversely affect the microbiota of the honeybees' digestive tract, contributing to a disruption of its homeostasis and dysbiosis. For example, they may decrease levels of beneficial bacteria such as *Lactobacillales* and *Bifidobacterium* spp., and increase levels of opportunistic pathogens such as *Serratia* spp. A very important part of the honeybees' microbiota are lactic acid bacteria (LAB), which perform health-promoting functions in their bodies, such as protection against pathogens, detoxification of xenobiotics, and increase in immunity. In our project research, we isolated and characterized 51 LAB strains from the honeybee environment and tested them for antagonistic properties against honeybee pathogens such as *Paenibacillus* spp. or *Melissococcus plutonius*. Additionally, we tested the ability of selected LAB strains to detoxify insecticides (binding, metabolism, cyto- and genotoxicity in cell lines). For comparative purposes, we additionally included 51 collection LAB strains isolated from various environments (e.g., plant silages, human faeces), as well as a strain of *Apilactobacillus kunkeei* from the gut of a honeybee. We observed that LAB strains isolated from the honeybee environment displayed stronger antimicrobial activity against *Paenibacillus* species, while collection strains better inhibited the growth of opportunistic pathogens. LAB bound best to the cell wall chlorpyrifos and coumaphos, to the weakest extent imidacloprid. No metabolism of these compounds was observed. Lowering the concentration of pesticides was correlated with a decrease in their toxicity. The ability depended on the cell line, insecticide, and was rather strain-specific. As a result of the research, 10 strains with potentially the best probiotic properties were selected and then characterized for survival in the honeybee's digestive tract and sugar syrup, etc. These are *in vitro* preliminary studies, which require confirmation in field tests on honeybees.

Acknowledgements
Research supported by the project from the Provincial Fund for Environmental Protection and Water Management in Lodz (no. 729/BN/D/2019) entitled ‘Selection of microorganisms for the construction of ecological protective preparation for honeybee (*Apis mellifera* L.)’.

SESSION 8
BENEFICIAL MICROBES AND HUMAN HEALTH – PART 2

PROBIOTIC *BIFIDOBACTERIUM* IN THE PREVENTION OF COGNITIVE IMPAIRMENT – CLINICAL EVIDENCE AND MECHANISM STUDIES

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Dementia, such as Alzheimer's disease (AD), is increasing worldwide, and especially in Japan, it has become a major social problem with the arrival of a super-aging society. AD is one of the most prevalent neurodegenerative diseases. Studies have implied the association of gut microbiota with the pathogenesis of AD, via a bidirectional communication system such as neural, immune, endocrine, and metabolic pathways.

We explored the possibility of probiotics in preventing the risk of AD. In a pre-clinical study. Oral administration of *Bifidobacterium breve* strain MCC1274 (synonym *B. breve* A1) to AD model showed therapeutic potential in maintaining memory functions as well as suppressing inflammation in brain. In addition, in randomized double-blind trials, supplementation of this strain to middle-aged and elderly with mild cognitive impairment (MCI) led to significantly improved cognitive functions and suppressed brain atrophy. In the analysis of its mechanism of action in AD models, it has been confirmed that administration of this strain inhibited amyloid-β accumulation and inflammatory reactions in the brain, and some of the bacteria-mediated components involved in these effects have also been identified. In this presentation, I will introduce the latest research results and discuss the possibility of this probiotic strain as a game-changer in the prevention and rehabilitation of dementia.

ADVANCED ANALYSIS FOR IDENTIFICATION OF KEY MICROBIAL BIOMARKERS IN MICROBIOME DATA FROM CLINICAL TRIALS

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In recent years there is increased awareness of the importance of the microbiome in health and disease. The number of large microbiome-related clinical trials have significantly increased. Trials that link their outcome to the microbiome of the subjects require advanced biostatistical analysis combining the metadata and the microbiome taxonomic abundance data. Machine learning (ML) plays a fundamental role within the analysis of microbiome data by predicting and/or classifying outputs, inferring host phenotypes (metadata), and testing for associations.

Here, we present the Key Microbial Biomarker Bioinformatics pipeline that links microbial abundance tables (from amplicon and shotgun sequencing) with metadata. This biostatistical tool utilizes ML and statistical methods to compute the most relevant microbial biomarkers and signatures that explain the variation in the microbial abundance counts and metadata classes based on pre-defined metrics. The methods used in this pipeline include differential abundance analysis using SIAMCAT, LASSO regression method, Boruta and Random forest (RF) feature selection, Linear Discriminant effect size analysis (LEfSe). The use of an ensemble of dimension reduction, Differential Abundance Analysis and ML methods allows to bypass the shortcomings of using these methods individually, whilst retaining their predictive power. The pipeline was tested on three publicly available datasets, of which the outcomes were compared to previous published results. This biostatistical pipeline successfully identifies associations between host phenotypes and microbial taxonomic abundance by detecting Key Microbial Biomarker (KMB) species and signatures in microbiome samples. It was also able to determine accurately the biomarkers from the three literature datasets used as validation. Our results indicate that the biomarker detection is not obvious using only traditional beta-diversity analysis methods such as the redundancy analysis (RDA). By using an ensemble of methods, this pipeline allows to cover a wide range of assumptions for the datasets, which may not be met if either of the methods are used stand-alone. The KMBs detected by this biostatistical pipeline give leads to development of diagnostic tools for early disease detection, personalized medicine design and a better understanding of the mechanisms behind observed results in pre- and clinical trials. The Key Microbial Biomarker analysis pipeline (using the described ML models) is now available.

BACTERIAL PROBIOTIC AND POSTBIOTICS FROM *LACTOBACILLUS HELVETICUS* HA122 AND *L. PLANTARUM* HA-119 ALLOW REINFORCING GUT BARRIER FUNCTION IN A ZEBRAFISH MODEL

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Due to its high fecundity, genetic tractability, small size, rapid development, gut function and immune gene conservation with mammals, and availability of biomarkers, the zebrafish (*Danio rerio*) has become a widely used vertebrate model to study immune responses and gut barrier function under numerous conditions that matter for human and animal health. Because of those reasons, it also represents a good model to test the strain-specific modes of action of pro- and post-biotics. These products are supposed to exert a crosstalk with the host cells and to have positive impact on gut barrier and gut-organs axis. However, only few *in vivo* evidence is available.

Therefore, this study aimed at evaluating the dietary supplementation with either live or inactivated bacteria on the mucosal barrier function of healthy zebrafish. We carried out a 5-week study using wild-type adult zebrafish (25 fish/tank; 3 triplicates/treatment). Treatments consisted of (i) non-supplemented basal diet (control), and basal diet supplemented with 6×10^6 CFU/g feed (pro) and (post), i.e., (ii) *L. helveticus* HA122 probiotic (LHPro) and (iii) *Lactobacillus helveticus* HA122 postbiotic (LHPost) or (iv) *L. plantarum* HA-119 postbiotic (LPPost). The inactivation of the bacteria was obtained by tyndallisation and each tyndallized bacteria was characterized by various microscopy technics. At the end of the trial, intestinal and skin histomorphometry (n=9 fish/ treatment) as well as the intestinal immune response using ELISA, flow cytometry and gene expression analysis (n=6 fish/ treatment) were assessed. Differences between control and experimental groups were estimated by non-parametric permutation tests with significance accepted at $p<0.05$. The gut lysozyme concentration was higher in the LHPost and LPPost groups compared to the control; in line with a significant up-regulation in the gene expression of *lyz*. The expression of *catL* (coding for cathepsin L) was also up regulated in all groups compared to the control. Interestingly, compared to the control, goblet cell coverage was significantly higher in the LPPost group in both intestine and skin, while intestinal goblet cell density increased in the LHPost group. In the gut, the abundance of intraepithelial leukocytes (IELs) was also significantly higher in all supplemented diets. Healthy (> 0.9) and consistent ratios of CD4+ to CD8+ cells across all treatments, alongside with significant elevations of CD4+ and CD8+ cells in the LPPost group were observed.

This study allowed identifying the potential of postbiotics to strengthen barrier functions at two levels. Firstly, by promoting the goblet cell population that could lead to better antigen sensing via formation of goblet cell-associated antigen passages. Indeed, actively secreting goblet cells can take up antigenic material for further processing by dendritic cells and tissue specific macrophages for presentation to mobilise adaptive T-cell and B cell responses. Secondly, by stimulating innate humoral defences and increasing the presence of innate immune cells in the submucosa of the intestine as shown by elevated levels of lysozyme and IELs, CD4+, CD8+ cells respectively. Together these findings demonstrate the potential of bacterial postbiotics to support intestinal immunity and warrant further research on their application in target species subjected to different challenges.

LACTICASEIBACILLUS CASEI ISOLATE FROM THE HUMAN RESPIRATORY TRACT AS POTENTIAL PROBIOTIC AGAINST CYSTIC FIBROSIS

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Cystic fibrosis (CF) is the most common hereditary life-threatening condition in caucasian populations with an incidence of 1 in 2,500 newborns. Recurrent infections of the lungs of CF patients in combination with exaggerated immune responses lead to chronic lung disease, the major cause of morbidity and mortality in these patients. Administration of antibiotics is a standard treatment but has several disadvantages such as disturbance of the normal microbiota and multidrug resistant bacteria. The potential of probiotics, i.e., live microorganisms which when administered in adequate amounts confer a health benefit to the host, to reduce airway infections in CF patients is promising. However, current efforts focus on oral supplementation of probiotics, with the limitation that there is no direct interaction with the airway microbiome and epithelium. Topical administration of well-characterized probiotics to the upper respiratory tract (URT) can overcome this limitation and might hold potential as a novel treatment strategy for CF patients.

During a previous study, *Lacticaseibacillus casei* AMBR2 was isolated from a healthy human URT and showed beneficial properties including attachment to airway epithelium, antimicrobial and anti-inflammatory effects, and epithelial barrier enhancement. It was also successfully tested live in healthy volunteers via a nasal spray. This was found to be safe and overall well-tolerated. Here, we further evaluated the potential of *L. casei* AMBR2 against key CF-related pathogens *Pseudomonas aeruginosa, Achromobacter xylosoxidans* and *Stenotrophomonas maltophilia*. We observed anti-pathogenic and anti-inflammatory effects of *L. casei* AMBR2 against all tested pathogens via antimicrobial assays and immunomodulation experiments in human airway cells. Our data provides evidence that *L. casei* AMBR2 holds promise as microbiome-therapy in CF patients. To better understand the potential of this therapy for CF microbiome modulation, we are currently analysing the detailed composition and dynamics of microbial communities in the URT and LRT of CF patients via whole-genome Shotgun sequencing in order to link them with patients' health status, pulmonary exacerbation incidence, viral infection and antibiotic treatment. Ultimately, the future goal is to test topical application of *L. casei* AMBR2 in an appropriately selected cohort of CF patients.

TARGETING THE GUT-BRAIN AXIS IN AUTISM: MACHINE LEARNING ANALYSIS OF GUT MICROBIOME SHOWS ASSOCIATION WITH AUTISM SPECTRUM DISORDER

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Autism spectrum disorder (ASD) is a set of neurodevelopmental disorders characterized by reduced social functioning and repetitive behaviour. The gut microbiome has been considered as a potential target for treatments, as ASD individuals suffer from intestinal problems and show a differentiated gut microbiome. However, studies from different cohorts rarely agree on the specific bacterial taxa that are involved, and a recent study concluded that the gut microbiome has no relevance at all [1]. Despite this, faecal transplantation has been tentatively shown to alleviate both behavioural and intestinal symptoms [2].

Our aim is to find bacterial taxa, that are relevant to the ASD phenotype, and validate these results across 2 different cohorts. We processed 16S rRNA gene sequencing data of the gut microbiome of children with ASD and neurotypical children using the DADA2 pipeline. This was done for data from a sibling-controlled study [3], for feature selection, and two age-matched studies [4,5] to validate our findings. The data from the sibling-controlled study was analysed using recursive ensemble feature selection (REFS) [6] with 8 classifiers. As the gut microbiome is sensitive to environmental factors and siblings share most of these factors, the resulting features are more likely to be associated to ASD. REFS, applied to the sibling-controlled data, resulted in 20 bacterial features that predict ASD. Then, the reduced feature-set was evaluated with 5 classifiers not part of the ensemble, to avoid over fitting, in a nested 10-cross-validation scheme. The 20 bacterial features were applied to the validation data of 2 different cohort studies, after which the same evaluation method was applied to these reduced datasets.

Analysis of the gut microbiome has resulted in a set of bacterial taxa that can be used to predict the ASD phenotype of children in 3 distinct cohorts with over 80% accuracy. Bacteria from the class of *Clostridia* were enriched in ASD subjects, indicating that this class of bacteria might play a role in this disorder. Our results indicate that the gut microbiome has a robust association with ASD and should not be disregarded as a potential target for therapeutic interventions.

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PLENARY SESSION
STRUCTURE AND FUNCTION OF NON-DIGESTIBLE CARBOHYDRATES IN THE GUT MICROBIOME

STRUCTURE-FUNCTION RELATIONSHIPS IN DIETARY CARBOHYDRATES: WHAT DO WE KNOW AND WHAT DO WE NEED TO KNOW?

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In 2016, ILSI Europe convened an expert group to assess what was known about the structure function relationships in dietary carbohydrates and to come to conclusions about the state of our knowledge and the knowledge gaps that need to be filled. This group presented their findings in a review article published in Beneficial Microbes in March 2022 [1]. The scope of the expert group encompassed fermentation of plant derived polysaccharides such as arabinoxylans, glucans and pectins, of oligosaccharides such as galacto-oligosaccharides with a specific view to identify the structure-function relationships. We also covered methods of modelling the impact of non-digestible carbohydrates on the gut microbiome and emerging techniques for synthesis of novel candidate next-generation prebiotics. Direct interactions between carbohydrates and human physiological and immunological systems were also covered.

Recent research into the gut microbiome is uncovering the key roles of certain species in the degradation of complex carbohydrates and the extent of cross feeding in the gut ecosystem. The impacts on health of this changing pattern of metabolism in the gut are also being elucidated. This presentation will form part of a session outlining the main conclusions and future thoughts of the ILSI Expert Group. It will evaluate what is currently known about the relationships between carbohydrate structure and bacterial metabolism in the gut microbiome and suggest future priorities for investigation.

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SYSTEMIC EFFECTS OF PREBIOTICS

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Prebiotics are defined as substrates that are “selectively utilized by host microorganisms conferring a health benefit” (ISAPP definition). ‘Utilization’ in the gut may involve cross-feeding, which means products produced by primary prebiotic-degrading bugs (such as mono- or small oligosaccharides, or SCFAs) can then be further used by different members of the gut microbiota. Thus, it may take a series of complex steps to get to a final health outcome. However, selective utilization by microbial taxa and health benefits are always required for a substance to meet the definition of a prebiotic. The health benefit of a prebiotic can be local (in the gut) or systemic. Locally, prebiotics can act via fecal bulking, as they are typically types of fiber. In addition, they can lead to the production of short-chain fatty acids (SCFAs), which reduce gut pH and thereby can discourage pathogenic and toxigenic activity of gut microbes, increase calcium ion absorption and provide energy for gut epithelial cells.

This presentation will focus on systemic functions of prebiotic metabolism, which include them being used as substrates for microbes that produce or interact with host cells to produce molecules with neurochemical, metabolic or immune activity. Further, SCFAs can end up in the bloodstream and can reach organs like the liver, muscles, and the brain. The SCFAs interact with SCFA-receptors (e.g. GPCR41 and/or 43) and can lead to changes in gene-expression, the release of satiety hormones, or interaction with receptors in the liver, adipose tissue and muscle tissue, leading to reduced inflammation. Prebiotics can also interact directly with immune cells, although strictly speaking that activity, which does not involve the gut microbiota, is not considered to be ‘prebiotic’, despite that it might have a health benefit for the host by, e.g., priming the immune system. The overview of these systemic activities comes from the extensive review of ‘Structure and function of non-digestible carbohydrates in the gut microbiome’ commissioned by ILSI Europe, and published as a 73-page paper in Beneficial Microbes [1].

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MODELLING PREBIOTIC ACTIVITY

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This presentation will cover information on the range of *in vitro* gut model systems used for studying the prebiotic potential of carbohydrates. The following aspects of *in vitro* modelling will be addressed: (i) microbial inoculum (target groups, individual versus pooled faecal samples); (ii) a range of *in vitro* models used for the investigations of gut microbial fermentation processes; and (iii) the variety of sampling and downstream analysis options that can be implemented for the understanding of microbial community and metabolite dynamics. Furthermore, examples of recent advances in the uncovering of molecular structure-related prebiotic effects, as well as recommendations and hypotheses for future research will be shared.

FUTURE PREBIOTICS: ENABLING TECHNOLOGIES

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This presentation will cover the available technologies currently in use to develop non-digestible carbohydrates with prebiotic potential. Manufacture methods, including (i) extraction from natural sources, (ii) hydrolysis using physical, chemical or enzymatic methods, and (iii) synthesis using chemical or enzymatic engineering and microbial fermentation will be briefly discussed. A special attention will be given to recent findings and advances based on the use of unexplored agri-food sources and/or on enzyme engineering approaches aiming at the optimization and development of tailor-made non-digestible and fermentable carbohydrates that could promote the selective growth of specific beneficial intestinal bacteria not targeted by existing established prebiotic carbohydrates.

MICROBIOME AND NEUROLOGICAL DISORDERS: ALZHEIMER'S AND AGING.

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The human microbiome signifies the full range of microorganisms (the microbiota) that live on and in humans. It represents a diverse collection of microorganisms. Bacterial population alone is estimated at as high as 200 trillion individual organisms. We have assessed the role of a specific bacterial cell probiotic formulation on microbiota-derived impacts on energy homeostasis and role in Alzheimer's and longevity. Result shows that probiotic treatments accelerated the developmental phase-dependent 20-hydroxyecdysone and insulin receptor gene expression surges and altered the phasic expression of downstream insulin signalling factors including dAkt, dTOR and dFOXO indicating a deep level of nutritionally dependent regulatory control. TOR conservation indicates that probiotic has a high therapeutic potential towards Alzheimer's and aging. Details of these studies, potential and limitations of microbiome probiotic formulations as next generation of Alzheimer's and aging therapeutics will be discussed.

NUTRITIONAL STRATEGIES FOR HEALTH PROMOTION IN ELDERLY; PRESENT AND FUTURE OF THE 'BIOTICS' FAMILY

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Minimizing the physiological decline and comorbidities associated with ageing is perhaps the biggest societal challenge we may have to meet in the next decades. The importance of diet for maintaining health along aging is well recognized. Recently it has also been demonstrated the microbial community inhabiting our intestine, the intestinal microbiota (IM), regulate important functions, resulting essential for health maintenance. During the last decades the development of the next generation sequencing has facilitated the study of the microbiota and allowed evidencing the strong influence exerted by age and diet upon this IM. The ageing process is known to affect this intestinal microbiota, frequently accompanied by changes in the digestive system, modification of dietary patterns, and impairment of the immune system, all of them being part of the senescence process. Thus, given the role that alterations of the IM may have in impairing homeostasis and resilience, understanding the IM transition from adulthood to senescence constitutes a key step for promoting the microbiota at old age. Such knowledge shall allow the development of nutritional strategies aiming to counterbalance the specific alterations taking place on the microbiota during aging.

Moreover, the specific alterations on the microbiota composition present at old age may also suggest potentially anti-ageing microorganisms. Traditionally, probiotics belonging to the genera *Lactobacillus* or *Bifidobacterium* had been used for microbiota modulation in elderly, however, in the last years the evidence accumulated on the study of the senescence-associated microbiota has pointed our other potentially important microorganisms. Among these microorganisms such as *Akkermansia muciniphila* have been suggested. Preclinical data in different animal-models of ageing indicate the ability of this microbe to increase longevity. Moreover, administration of *Akkermansia* has been shown to promote the homeostasis of the immune system and to increase oxidative-stress resistance, resulting in an improved functional status. These preclinical data underline the interest of this microorganisms as a potential 'gerobiotic'.

To summarize; the data accumulated so far underline the importance of understanding the ageing process also from the microbiota perspective and point out at new 'biotic' agents with potential application by their anti-ageing effects.

TARGETING THE GUT TO PREVENT OR COUNTERACT AGE-RELATED METABOLIC DISTURBANCES AND PATHOLOGIES: COMBINING OLD AND NEW CONCEPTS IN NUTRITION

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In a context of aging population in Europe, an important challenge in the coming year will be to allow the people above 60-65 years old to age in a 'healthy' status, without (or delayed) development of age-related pathologies that are known to accelerate dependence. Indeed, and aside from the old individuals that present diagnosed pathologies, a part of the elderly population can be clinically defined as presenting a frailty syndrome, meaning that it is more prone to falls, incident disability, hospitalization and mortality [1]. This latter part of the old population is not necessarily dependent, or do not necessarily present diagnosed pathologies, but can be interesting responders to nutritional strategies capable to limit or reverse this state of frailty before disability or severe illnesses are installed. For this frail or pathologic old population, a loss of muscle mass and strength (sarcopenia) and impaired response to anabolic effect of the meal (and insulin resistance (IR)) are commonly observed, contribute to impair health status and accelerate dependence. If physical activity can limit sarcopenia and IR, it is not always applicable or less efficient in people with limited muscle function or with associated pathologies that can limit movement (e.g., osteoarthrosis – osteoarthritis). Nutritional supplements have also been developed and proven efficient both to favour muscle mass anabolism [2] (protein supplements, specific proteins, specific amino acids – leucine…) and increase muscle or whole-body insulin sensitivity (antioxidants, vitamins…). However, these nutritional strategies have shown their limits particularly in frail old individuals with low appetite where a combination of all strategies capable to favour anabolism require be combined to compensate for the low quantity of food ingested.

This is why we and others started to work on new targets and novel nutritional approaches capable, in combination with already developed nutritional solutions, to improve health status and limit sarcopenia in frail and pathologic old population [3]. In particular, it now well described that old populations' microbiota presents a decreased abundance in "beneficial species" whereas abundance in opportunistic pathogens is increased [4,5]. More than age, health status is the main driver of microbiota composition in these populations and recent data demonstrated in parallel that microbiota could also be a driver of age-related health impairment, including sarcopenia via the existence of a gut-muscle cross talk [6,7]. Such observations pave the way for the development of new research strategies targeting the gut and its microbiota to limit muscle impairment with aging and its associated IR. The aim of my presentation will be to develop how, by acting on gut physiology, metabolism and its associated microbiota, it is possible to develop some new nutritional strategies to improve health status and limit sarcopenia in the elderly population.

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BENEFICIAL MICROBES, SHOULD IT GET PERSONAL?

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Probiotics and prebiotics have gained tremendous popularity among the general public and scientific communities. However, their proofs of efficacy remain heterogenous and conflicted among the industry, medical and scientific communities. A precision approach addressing the heterogeneity pertaining to probiotic strains, prebiotic variety, individuals and their microbiome, would have the potential to bridge this gap. Lessons learnt from current approaches can guide the development of precision probiotics and prebiotics, that will need to leverage phenotypic and target-based discovery strategies and person-centric trials.

POSTER ABSTRACTS

P1 *Dietary fibres: the missing gap to optimize personalized nutrition?*
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P2 *Impact of the addition of autochthonous strains on physicochemical and microbiological parameters of Brazilian semi-hard artisanal goat cheese evaluated during maturation*
J.M. Caju de Oliveira[1], C.N. Meireles Costa[1], Â.M. da Silva Alves[2], K.Y..Gervazio de Lima[3], K. Olbrich dos Santos[4], E..Leite de Souza[1], M.T. Bertoldo Pacheco[5], M. Gonçalves Correia[6], M.E..Gomes de Oliveira[1] and **Adriane Elisabete Antunes de Moraes**[7]
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P3 *Lipid profile and atherogenicity, thrombogenicity and health promotion indices of Brazilian semi-hard artisanal goat cheese evaluated during ripening*
Â.M. da Silva Alves[1], J.M. Caju de Oliveira[2], C.N. Meireles Costa[2], S.R. Bagio[3], H. Polli[4], M.E. Gomes de Oliveira[2], A.S. do Egito[5], M.T. Bertoldo Pacheco[3] and **Adriane Elisabete Antunes de Moraes**[6]
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P4 *The use of a new microbial-based concept to minimize odours and ammonia emission, and to maintain microbial balance in cat litter*
I. Pélissié[1], J.-F. Hupé[1], L. Dunière[1,2], J. Plateau-Gonthier[1] and **Emmanuelle Apper**[1]
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P5 *Investigation of anthelmintic administration on the equine faecal microbiota*
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P6 *FerFood.CH: fermented foods for health*
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P7 *Deciphering the metabolic interactions between mucus associated Ruminococcus gnavus and Limosilactobacillus reuteri strains*
Raven Reynolds[1], **Andrew Bell**[1], T. Seecharran[1], S. Saha[1], M. Warren[1,2] and N. Juge[1]
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P8 *Ex vivo experimental approaches to explore immunomodulatory and homeostatic potential of pre- and probiotics*
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P9 *The role of galacto-oligosaccharides in the recovery from dysbiosis in patients on long-term atypical antipsychotic treatment*
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P10 *The effect of dietary supplements on gut microbiome composition among people with intellectual disabilities*
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P11 *Development of an in vitro model simulating the infant colon, to study composition and activity of the infant gut microbiome*
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P12 *Food by-products as substrates for growth and folate production by commercial probiotic strains*
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P13 *Selective fermentation of modified pectin from orange albedo and folate production by probiotic and starter cultures*
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P14 *Piloting an 'all-in-one' data collection platform, trialflare, for probiotic trials*
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P15 *Gut-brain axis mediated neurodegeneration in Parkinson's disease: TLR4 as a therapeutic target*
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P16 *Efficacy of using probiotics for the prevention of acute respiratory tract infection in older people*
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P17 *Developing an omics based rapid detection methods to ensure food safety of fermented or long shelf-life food products: TITAN innovations*
Isabelle Guelinckx[1], F. Bourdichon[2], A. Del Casale[3], A. Vontzalidou[4], A. Colafranceschi[1] and E.L. Sliwinski[5]
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P18 *Omics technologies for evaluation of starter and probiotic strains in the Brazilian-style beer Catharina sour*
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P19 *Survival of the probiotic strain Lacticaseibacillus paracasei subsp. paracasei F19 in beer formulations with high hop content (Humulus lupulus L.)*
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P20 *Viability of the Lacticaseibacillus (L.) paracasei subsp. paracasei F19 probiotic strain in a sour beer with fruit juice and by-product*
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P21 *Dietary probiotics, prebiotics and the gut microbiota in human health – updated **ILSI Europe** concise monograph*
O. Chen[1], M. Heyndrickx[2], A. Meynier[3], A. Ouwehand[4], B. Pot[5], B. Stahl[6,7], S. Theis[8], E. Vaughan[9] and M Miani[10]
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P22 *Study protocol: effects of butyrate on affective patterns, microbiome composition and depressive symptoms in young adults – a randomized clinical trial*
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P23 *Lacticaseibacillus rhamnosus HA-114: an innovative probiotic strain to support weight management efforts through the gut-brain axis*
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P24 *Dietary fibre combinations to counteract plant-protein colonic fermentation*
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P25 *Screening of candidate probiotics and microbial metabolites for the prevention of non-alcoholic fatty liver disease*
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P26 *Why multi-strain probiotics are becoming the standard*
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P27 *Potential of different dextrans as prebiotics to modulate human gut microbiota: an in vitro approach using human batch fermentation*
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P28 *Role of lipoteichoic acid from the genus apilactobacillus in inducing a strong iga response*
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P29 *Modulation of gut microbiota of healthy and prediabetic subjects by Asparagus officinalis dietary fibres in an in vitro model of the colon*
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P30 *Longitudinal gut mycobiota changes in japanese infants during first three years of life*
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P31 *Effect of a mix of Lacticaseibacillus casei LA205 and L. paracasei LA903 on behavioural, biochemical and gut microbial outcomes of male mice following chronic restraint stress*
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P32 *The effect of carbohydrates on the caecal microbial composition and metabolites in an in vitro model for broilers with impaired gut health*
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P33 *Short chain fatty acid inhibition of bacterial plasmid conjugation in broth and chicken caeca explants*
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P34 *Antimicrobial compounds produced by aquatic lactic acid bacteria inhibit the growth of food and fish pathogens*
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P35 *Feeding germ-free dams with a yoghurt activating the aryl hydrocarbon receptor increases intestinal group 3 innate lymphoid cells in the offspring*
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P36 *The life of the skin microbiome under stress*
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P37 *The gateway to our body: the oral microbiome*
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P38 *Faecal microbiota transplantation from autistic children modulates behaviour, intestinal parameters and inflammation in BALB/c mice*
Naika Prince[1], L. Peralta Marzal[1], L. Roussin[2], L. Naudon[3], J. Troisi[4], M. Lombardi[4], G. Corrivetti[5], S. Rabot[2], J. Garssen[1], A.D Kraneveld[1] and P. Perez-Pardo[1]
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P39 *Degradation of deoxynivalenol (DON) and zearalenone (ZEN) toxins using microbes for potential applications in animal feed to reduce the carryover of mycotoxins from animals to humans*
Z. Jakovčević, **Jog Raj**, H. Farkaš, S. Ćujić, J. Bošnjak-Neumüller and M. Vasiljević
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P40 *Towards a reduced feature selection pipeline in 16s rRNA microbiome data using machine learning*
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P41 *Identification of food safe microbial strains with postbiotic potential for application in fermented food products*
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P42 *Survival of Lacticaseibacillus paracasei subsp. paracasei F-19® and Bifidobacterium animalis subsp. lactis bb-12® in fermented red pitaya pulp under in vitro simulated gastrointestinal conditions*
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P43 *A predictive model for microbiome-dependent response to dietary fibres based on in vitro biological data predicts microbiota response to dietary intervention*
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P44 *Faecal microbiota diversity and composition are associated with ectopic fat in adults of African ancestry in the Tobago health study*
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P45 *The gut microbiota: master of puppets connecting the epidemiology of infectious, autoimmune, and metabolic disease*
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P46 *Probiotics for the management of infectious diseases: reviewing the state of the art*
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P47 *Modulation of gut microbiota composition and metabolites by different dietary fibres after in vitro fermentation in TIM-2*
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P48 *Reporting on the in vitro batch fermentation of (un)saturated homogalacturonan oligo-saccharides*
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P49 *The human milk oligosaccharide 2'-fucosyllactose alleviates liver steatosis, er stress and insulin resistance by reducing hepatic diacylglycerols and improved gut permeability in obese Ldlr-/-.Leiden mice*
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P50 *Current postbiotics in the cosmetic market – an update and development opportunities*
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P1
DIETARY FIBRES: THE MISSING GAP TO OPTIMIZE PERSONALIZED NUTRITION?

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Nutrition is crucial for the balance between health and disease. Dietary fibres (DF) are known for their health benefits, such as reducing risk to develop cardiovascular disease (CVD) and type II diabetes. DF are chemically diverse and structurally complex carbohydrates that resist digestion, reaching the large intestine almost intact and serving as an energy source for the gut microbiota (GM). Thus, DF are considered the main components capable of modulating and sustaining healthy gut microbial communities. Just as the concept ‘one diet fits all’ is not applicable, saying that DF are all the same and have the same effect on GM and health is also not correct. A food product may contain different types of DF with different chemical characteristics, monosaccharide compositions, glycosidic bonds and degrees of polymerization, thus presenting different biological functions. The lack of food-specific structural knowledge undermines our understanding and affects the interpretation of clinical data investigating the role of carbohydrates in the diet. For example, DF food labelling does not distinguish the characteristics of DF, such as soluble and insoluble, also denoting that DF would be a single and invariable molecule. Based on the average DF intake and its effect on CVD, as well as the beneficial role of functional fibres, an adequate intake (AI) for total fibre was defined for each age and gender group by multiplying 14 g/1000 kcal × median energy intake (kcal/1,000 kcal/d). The AI for total fibre in foods is set at 38 and 25 g/d for men and women, respectively. Although DF are present in a wide range of plant-based foods, inhabitants of countries with a Western-style diet do not consume sufficient amounts of fibre: the average DF intake ranges from 16.5 to 17 .9 g/d for men and 12.1 to 13.8 g/d for women. That is why achieving AI for DF has been a matter of debate among the scientific community. New DF concepts are emerging, but the physicochemical characteristics and their effects on the GM are not fully contemplated. Furthermore, none of these recommendations considers inter-individual features such as weight and height, and the most emerging factor linked to the health benefits of DF, the human GM. A novel approach for the DF intake recommendation, more specific and personalized, is needed. The best way to start is to consider that not all fibres are equal, and this should be taking into consideration by nutrition-authorities when developing food guidelines.

P2

IMPACT OF THE ADDITION OF AUTOCHTHONOUS STRAINS ON PHYSICOCHEMICAL AND MICROBIOLOGICAL PARAMETERS OF BRAZILIAN SEMI-HARD ARTISANAL GOAT CHEESE EVALUATED DURING MATURATION

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Coalho cheese is a typical dairy product from north-eastern Brazil, highly produced and consumed for over 150 years. Goat coalho cheeses have been investigated for their composition, with emphasis on artisanal cheeses, characterized by its unique flavour due to raw milk spontaneous fermentation. Aiming to protect its original characteristics and guarantee safety for consumption, the addition of potentially probiotic strains can be a good alternative to improve product quality. Thus, this study aimed to develop artisanal goat milk coalho cheeses added with *Lactiplantibacillus plantarum* CNPC003 and *Limosilactobacillus mucosae* CNPC007 strains and to evaluate its physicochemical and microbiological characteristics. Coalho cheeses were developed from raw or pasteurized goat milk with or without the strains and matured at 10 ±1 ºC, and had its physicochemical and microbiological characteristics evaluated after 1, 20, 40 and 60 days of maturation. Mesophilic aerobic bacteria (5.80 ±0.12 log CFU/ml) and high total coliform count were found in raw milk used in cheese production, while no pathogenic microorganism analysed was detected in pasteurized milk. Coalho cheeses presented similar yields between treatments, that varied from 156.67 to 165.83 g/l of milk. Elaborated cheeses presented medium to high moisture content (45.36 to 52.81%), as expected for coalho cheeses. Raw milk cheeses in general had lower moisture content, without significant differences during maturation. A significant change in syneresis was observed, where cheeses with *L. mucosae* showed a higher whey retention, while other treatments had a significant increase in syneresis. Lactose content decreased significantly throughout maturation, especially in raw milk cheeses, which presented values below the detection limit from the twentieth day of maturation. Formulations did not differ significantly in water activity and NaCl contents. The addition of potentially probiotic strains to cheeses significantly inhibited total and thermotolerant coliforms counts in raw milk cheeses. On the first day of maturation, staphylococci were detected in all raw milk cheeses, however by the twentieth day, it was only detected in raw milk cheese without added of potentially probiotic strains. *Salmonella* spp. and *L. monocytogenes* were not detected in any sample. As for LAB, all cheeses had counts greater than 8.0 log CFU/ml by the end of maturation. Therefore, cheeses added of both strains showed high LAB count and good microbiological quality after 60 days of maturation, being suitable for consumption and confirming the effectiveness of autochthonous strains addition in the microbiological control of cheeses produced with both raw and pasteurized milk.

P3

LIPID PROFILE AND ATHEROGENICITY, THROMBOGENICITY AND HEALTH PROMOTION INDICES OF BRAZILIAN SEMI-HARD ARTISANAL GOAT CHEESE EVALUATED DURING RIPENING

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Artisanal cheese is a consumer trend that has been increasing due to being produced on a smaller scale, with healthy and natural ingredients, reaching a differentiated market value. However, it must be produced with the highest quality and commercialized within the current regulations. In the present study, the lipid fraction of goat Coalho cheese produced with raw and pasteurized milk was analysed in the absence and addition of potentially probiotic *Limosilactobacillus mucosae* CNPC007 during 60 days of ripening. For the experimental protocol cheese production was done with pasteurized and raw milk. After that, the 2 batches of milk (raw and pasteurized) underwent a new division for the addition (or not) of the potentially probiotic culture. The treatment generated 4 different types of cheese that were matured for 60 days and analysed at 2 moments (0 and 60 days). Total lipids and fatty acid profile were determined to calculate indices of atherogenicity (AI), thrombogenicity (TI) and health promotion (HPI). Although the total lipid content was similar for all treatments at time zero, those made with raw milk (14.56-15.77) presented less variation at the end of the ripening, compared to cheese made with pasteurized milk (14.56-19.56). Consequently, cheese made with raw and pasteurized milk (with or without *L. mucosae*) had an average increase of 2.5 and 4.8% in saturated fat after 60 days of maturation, respectively. Mono- and poly-unsaturated lipids in cheese made with pasteurized milk (with or without *L. mucosae*) demonstrated an increase of 2.2 and 0.40%, respectively, at the end of maturation. On the other hand, after sixty days, cheese made with raw milk without the addition of *L. mucosae*, had a reduction in poly and mono-saturated fats, in relation to time 0. Considering raw and pasteurized milk, the range of atherogenicity (AI) and thrombogenicity (TI) indices calculated from the fatty acid in Coalho cheese were 3.46-3.54 and 4.44-4.62, respectively. However, despite the AI and TI indices being high, the health promotion index (HPI) had an average of 0.24. Therefore, cheese made with pasteurized milk with or without *L. mucosae*, presented a better lipid profile and quality index in relation to cheese made with raw milk, probably due to the competitive flora of raw milk resulting in greater metabolic activity.

P4

THE USE OF A NEW MICROBIAL-BASED CONCEPT TO MINIMIZE ODOURS AND AMMONIA EMISSION, AND TO MAINTAIN MICROBIAL BALANCE IN CAT LITTER

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With urbanization, cats become preferred companions as they can easily live inside home and defecate into litter box. However, malodours stemming from litter are major frustrations for cat owners. In addition, cat owners are sensitive to litters providing a 'safer microbial environment'. Nowadays, there is a large offer of litters with claims of odour control, but technologies are often chemically based and have not deeply been substantiated. We thus aimed at developing a new, GRAS and QPS microbial-based technology, and to scientifically proved its efficacy on odours management and litter microbial balance. To achieve our goals, we: (i) performed traditional microbiology tests to select the appropriate strains; and (ii) developed an *in vitro* model mimicking the evolution of a litter in a box for 7 days to test the efficacy of the solution (Cat Litter Pro). Selected bacteria were evaluated through an API gallery 20/NE to look for different metabolic activities in relation with malodorous compounds production. The selected bacteria did not produce malodorous compounds and were depleted in urease activity. Afterwards, prevention tests against *E. coli* CM454 by co-culture were performed. The presence of *E. coli* did not impact the number of bacteria in our positive biofilm, while the biofilm formed by *E. coli* CM454 was not as important when the selected strain was applied in a preventive manner. An *in vitro* model to mimic the live conditions of a litter box was developed. Briefly, 600 g of clean cat litter per replicate (n=3/treatment) supplemented with or without the solution Cat Litter Pro was put in a box, and 15% fresh mineral cat faeces was added at day 0 (D0). To maintain humidity all by providing an ammonia source, daily addition of synthetic urea diluted in water to reach typical concentration of cat urine was performed. Ammonia emissions were measured using Gastec® tube after a clothe recovered each box for 30 min to standardize the air volume at D0 and each day till D7. Faecal contamination was evaluated by enumeration of *Enterococcus spp*. at D7. Ammonia emissions were significantly lower with Cat Litter Pro (t test, $p<0.05$), with a continuous increase of ammonia in the control while remaining constant with the treatment. In addition, the solution significantly prevents the proliferation of *Enterococcus* spp., (-0.5 log, t-test, $p<0.001$). Cat Litter Pro is an efficient microbial-based solution to decrease malodorous compounds and to maintain a better microbial balance in the soiled cat litter. Other analyses are ongoing to measure further the impacts on other compounds, and on microbiota.

P5
INVESTIGATION OF ANTHELMINTIC ADMINISTRATION ON THE EQUINE FAECAL MICROBIOTA

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Horses are herbivorous; their hindgut harbours a complex microbiota to digest plant cell walls. The gastrointestinal microbiota also participates in maintaining gut homeostasis. Parasite infection can result in dramatic damage on gut health. In modern breeding practices, to counteract the parasitic load, routine worming is completed several times per year. However, anthelmintic drugs can impact microbiota balance which in turn can create softer faeces. The direct impact of worming on gastrointestinal microbiota is still not well described and may depend on the type of molecule used. The objective of this study was to assess the effect of worming on the gut microbiota of horses. To achieve that, 8 healthy horses (5 mares and 3 geldings, from 5 to 30 years old) were dewormed with a single dose of Praziquantel and Ivermectin administrated orally. Fresh faecal samples were collected at day 0 (D0) before deworming, then 24 (D1) and 72 hours (D3) after deworming and frozen at -80°C. DNA extractions were done with ZymoResearch Minikit and high-throughput sequencing of the V3-V4 regions of the 16S rRNA gene were performed using Illumina MiSeq and analysed with QIIME2. Alpha and beta diversity change with the age, with the gender, with the phylogenetic, but was not directly affected after the anthelminthic treatments. Instead, relative abundance of *Firmicutes* decreased while the one of *Bacteroidetes* increased from D0 to D1, leading to a significant reduction in the ratio *Firmicutes*/*Bacteroidetes* (F/B) ($p<0.05$). Moreover, *Firmicutes* relative abundance was still significantly reduced at D3 when compared to D0. Interestingly, a significant negative correlation between Shannon diversity index and the *Proteobacteria*/*Fibrobacteres* ratio was found and the faeces of 2 ponies exhibited an increase in the *Proteobacteria*; while a decrease in *Fibrobacteres* relative abundance just after deworming, suggesting that on top of the global trend in modulating the F/B ratio, there is individual pattern in response of a deworming drug. In conclusion, deworming horses with Praziquantel and Ivermectin affects the relative abundance of important phyla in animals and their ratio F/B. Individual patterns suggest that the microbiota of some horses can be more sensitive than other to the application of the product, leading to a composition already described in case of dysbiosis. Further studies using different types of deworming molecules, more horses infected with worms or not, are required to confirm this effect, to define the severity of the microbial imbalance (collectively and individually) and to elucidate if diversity is also constrained by the anthelminthic treatments. This will be helpful to develop efficient nutritional strategies, like probiotics, to prevent the modulation of the equine microbiota following a deworming treatment.

P6
FerFood.CH: FERMENTED FOODS FOR HEALTH

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In recent years, interest in the human microbiome and its ability to communicate with the human organism has increased remarkably. This communication takes place in different ways. One way is through the production of metabolites such as short-chain carboxylic acids and indoles, which can affect our metabolism in a variety of ways. Some of these effects are relevant to human health. In the past few years, a growing number of research studies have demonstrated the functional importance of these metabolites, even when ingested through the diet. Fermented products are of great importance in this context as the presence of bacteria in these products results in substances similar to those produced by intestinal bacteria and therefore gain more and more interest in current research. The objective of FerFood.CH is to develop fermented foods that translate the functional diversity contained in the genome of bacterial collections into metabolic profiles relevant to human health. As the strains used in this context are mainly from the Swiss dairy environment, the development of fermented dairy products is targeted. To achieve this goal, the genomes of bacteria is screened to explore the individual potential of each bacterial strain to produce specific bioactive compounds. Subsequently, fermented dairy products are produced by adding the selected strains in combination with starter strains. By measuring the bioactive compound of interest in a targeted approach and by an additional untargeted metabolomics approach, the individual fermented dairy products are characterized. Preclinical studies are conducted to verify an effect of the consumption of this fermented dairy product. Fermented products with encouraging preclinical results will subsequently be tested in human intervention studies.

P7

DECIPHERING THE METABOLIC INTERACTIONS BETWEEN MUCUS ASSOCIATED *RUMINOCOCCUS GNAVUS* AND *LIMOSILACTOBACILLUS REUTERI* STRAINS

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The gut microbiota is comprised of a complex community of microbes having a profound effect on human health. Cross-feeding activities between members of the gut microbiota help to shape and maintain homeostasis within this community. Vitamin B12 (cobalamin) is an essential metabolic cofactor synthesised by a limited number of bacteria [1]. Around 25% of gut microbes produce vitamin B12 derivatives while 80% encode cobalamin-dependent enzymes. Multiple variants of vitamin B12 can be produced by bacteria including cyano-, methyl-, and adenosyl cobalamin [2]. *Ruminoccocus gnavus* and *Limosilactobacillus reuteri* are gut symbionts with distinct strategies for adaptation to the intestinal mucosal surface. Under *in vitro* growth conditions *R. gnavus* is auxotrophic for vitamin B12 while *L. reuteri* is a known vitamin B12 producer. Our preliminary data suggest that co-culture of *R. gnavus* with *L. reuteri* in vitamin B12 deficient media increases growth of *R. gnavus* to similar levels to growth in media supplemented with vitamin B12. Similarly, we showed that *L. reuteri* was unable to grow in the defined media alone but could in co-culture with *R. gnavus*. Taken together these data suggest that cross feeding capabilities exist between the two organisms which may help them to colonise the mucosal niche. To further investigate these interactions at a molecular and biochemical level, we first performed bioinformatic analyses across 164 genome-sequenced *L. reuteri* strains to determine vitamin B12 producing strains and non-producing strains. LC-MS/MS analyses of supernatant and cell extracts of *L. reuteri* strains grown in LDMII showed cytosolic production of pseudocobalamin for strains bioinformatically predicted to synthesise vitamin B12 through the *pdu-cbi-cob-hem* cluster. *In silico* analyses of *R. gnavus* strains revealed 80 genome-sequenced *R. gnavus* strains were predicted to possess vitamin B12 transporters and/or vitamin B12-dependent enzymes. We then showed that the growth of *R. gnavus* strains in defined media was increased when supplemented with vitamin B12 or with the vitamin B12 precursor porphobilinogen, suggesting that part of the cobalamin synthesis pathway is functional in *R. gnavus* strains. Future work will make use of our ability to genetically manipulate *R. gnavus* to better understand its dependency on vitamin B12. In parallel metabolomic and transcriptomic approaches will be used to identify possible metabolites produced by *R. gnavus* that facilitate the growth of *L. reuteri* in co-culture.

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P8
EX VIVO EXPERIMENTAL APPROACHES TO EXPLORE IMMUNOMODULATORY AND HOMEOSTATIC POTENTIAL OF PRE- AND PROBIOTICS

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Intestinal microbiota plays a key role in human health, while the epithelium maintains the necessary barrier between the microbes and the internal organs. Disruption of this barrier provokes activation of local and systemic immune system, leading to a variety of inflammatory, metabolic, and neurodegenerative pathologies. Probiotics and prebiotics are extensively developed to aid maintaining and repairing the impaired balance between intestinal microbiota and host immune response. Their selection and validation is a complex process involving *in vitro* and *in vivo* models, while the former lacking the complexity of the physiological system and the latter often fails reflecting the effect in humans. Through collaborative projects, we explored *ex-vivo* experimental approaches involving human faecal microbiota and/or primary immune cells separated by intestinal epithelial barrier for pre- and probiotics evaluation. We demonstrate here examples of (i) the impact of synthetic polysaccharides digestion by faecal microbiota on epithelial homeostasis; (ii) the contribution of oral probiotics to immune response against SARS-CoV-2 (gut-lung axis); and (iii) the effect of probiotics on intestinal inflammation (using mid-throughput gut-on-a-chip model).

P9

THE ROLE OF GALACTO-OLIGOSACCHARIDES IN THE RECOVERY FROM DYSBIOSIS IN PATIENTS ON LONG-TERM ATYPICAL ANTIPSYCHOTIC TREATMENT

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Atypical antipsychotic (AAP) drugs are the gold-standard treatment for psychotic patients but are nowadays also widely prescribed among people with other mental disorders. Notwithstanding the benefits of AAP in terms of symptom improvement, there are severe adverse effects including the metabolic syndrome. A novel hypothesis is that part of these undesirable effects of antipsychotics could be mediated by their deleterious effects on the microbiome. This may result in dysbiosis, the disruption of bacterial species of the gut microbiota. Recently, dysbiosis has been linked to poor quality of life, depression, and anxiety through the gut-brain axis. Mounting evidence proposes that prebiotic consumption may be helpful in the recovery of dysbiosis, although this effect is unclear among long-term antipsychotic users. The main objective of this study is to assess the potential beneficial effects of the prebiotic galacto-oligosaccharides (GOS) in combination with 2′-fucosyllactose (2'-FL) on the gut microbiota, by showing a relative increase in Bifidobacteria in faecal samples following intervention. The secondary objective is to assess the effects of GOS on mental wellbeing and sleep. We hypothesize that GOS+2'FL supplementation will improve gut health and mental wellbeing. The tertiary objective is to assess the effect of GOS+2'FL on metabolic parameters. Data will be collected 4 weeks prior to the start of the intervention during an observation only phase (t0), at baseline (t1), and after 2 (t2) and 6 (t3) weeks of GOS+2'FL intake. A follow-up will take place at week 10, 4 weeks after the intervention (t4). The study is a single-arm pilot study (non-randomized and non-blinded). We aim to include 20 psychiatric patients on long-term atypical antipsychotic use, irrespective of their specific psychiatric disorder, with a BMI >25 kg/m^2. Following a run-in period of 4 weeks (no intervention but all other aspects of the study), the participants will consume GOS+2'FL daily during the first consumption moment of the day for 42 days. The primary endpoint is the change in Bifidobacteria in faecal samples from week 0 to week 6 (versus the change from week -4 tot week 0 as the reference).

P10
THE EFFECT OF DIETARY SUPPLEMENTS ON GUT MICROBIOME COMPOSITION AMONG PEOPLE WITH INTELLECTUAL DISABILITIES

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The gut microbiome has a major impact on human physiology and is affected by the diet. Yet, it is unknown whether dietary supplements may also influence the composition of the microbiome. To study the effect of supplementation of vitamins, minerals, and omega-3 fatty acids on the gut microbiome in people with intellectual disability (ID). We conducted a double blind randomized controlled crossover trial. The intervention lasted 16 weeks and consisted of a daily supplement of vitamins, minerals and omega-3 fatty acids, or placebo. The participants were between 12 and 39 years old (M=24.5, SD=7.6) and had an ID or borderline intellectual functioning. We sampled faeces at baseline and endpoint from 38 individuals (23.7% female), of whom 13 participated in a crossover arm. The faecal samples were analysed using the 16s RNA technique, in which abundance and alpha diversity were determined. Also, the between groups difference in change of the 25 most common families and genera were explored. The differences were not significant on any of the main outcome measures. The Bray-Curtis analysis of the pre-post treatment gut microbiome did not show a systematic effect. Contrary to our expectations, we did find a statistical trend towards a decrease in alpha diversity in the active versus placebo group. At the level of genera, there was a decrease in Eubacterium coprostanoligenes and a trend of a decrease in Coprococcus in the active versus placebo group. Both those genera are associated with health-promoting effects. Our research does not support the hypothesis that supplementation with vitamins, minerals and omega-3 fatty acids contributes to an improvement of the gut microbiome in people with ID.

P11

DEVELOPMENT OF AN *IN VITRO* MODEL SIMULATING THE INFANT COLON, TO STUDY COMPOSITION AND ACTIVITY OF THE INFANT GUT MICROBIOME

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The early life stages are critical for the development of the gut microbiome. Variables, such as antibiotics exposure, birth-mode, and feeding mode have been associated with developmental disruptions of this ecosystem, which has been related to adverse health effects in later life. However, studying the effects of microbiome modulating strategies (such as pre- and/or probiotics) in infants, is challenging due to limited accessibility and ethical constraints. We, therefore, further developed and optimized an *in vitro* system simulating the infant colon using TIM-2, a validated, computer-controlled, dynamic gastrointestinal model. The model, consisting of four separate compartments, fed with adapted infant simulated ileal efflux medium, was inoculated with faeces from healthy, exclusively breastfed, 2-4 months old infants. Experiments with faeces from four infants, displaying distinctive microbiota profiles, were performed. Each lasted five days with two compartments additionally fed with a mix of 5 human milk oligosaccharides (HMOs), 2'FL, 3'FL, LNT, 3'SL and 6'SL. Microbial load and composition were determined by shotgun metagenomics and qPCR. Metabolic output was determined by LC-MS. Dominant bacterial genera of the inocula were replicated in the model. Additionally, bacterial load, metabolite ratios and concentrations were consistent with data published for infant faeces. HMOs strongly modulated microbiome composition by stimulating bifidobacteria, which consequently decreased diversity. This affected the metabolic output and resulted in an increased production of mainly acetic and formic acid. Furthermore, species level compositional dynamics and associated HMO consumption patterns were donor dependent. Our *in vitro* model reproduced the metabolic activity and maintained the dominant bacterial populations of the infant inocula. It represents a valid model to study the infant gut microbiome and the effects of various microbiome modulating strategies.

P12
FOOD BY-PRODUCTS AS SUBSTRATES FOR GROWTH AND FOLATE PRODUCTION BY COMMERCIAL PROBIOTIC STRAINS

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The biotechnological potential of probiotics and LAB to produce folate and bio-enriched fermented food has been extensively demonstrated. Food by-products, such as whey and fruit by-products, are natural sources of bioactive compounds, carbohydrates, and phenolic compounds. They can be applied to ferment foods as substrates for the growth and folate production by LAB. The present study aims to evaluate the ability of three commercial probiotic cultures to ferment whey and fruit by-products and to produce folate. *Streptococcus thermophilus* TH-4, B*ifidobacterium longum* subsp. *infantis* BB-02, and *Lacticaseibacillus rhamnosus* LGG were selected after screening in a folate-free medium (FACM). An *in vitro* fermentability test was carried out in modified MRS broth, with phenol red and without carbohydrate source (MRS-m), supplemented with 1% grape, pitaya or passion fruit by-product. Each strain was inoculated (5-6 log CFU/ml) in 5 ml of MRS-m and incubated at 37°C for 48 h. Samples were withdrawn at times 0, 24, and 48h for folate determination and viable cell counts by drop plate in specific agar for each strain. Afterwards, they were tested for folate production (microbiological assay) in previously pasteurized whey and milk at different concentrations (30, 50 and 70% whey), with or without water extract of the three fruits by-product, for 24 h at 37°C. The by-product that best-favoured vitamin production in the MRS-m medium was grape (101.2 ± 11.18, 96.4 ± 6.04, and 73.3 ± 9.14 ng/ml, respectively, for LGG, TH4, and BB02). The highest folate concentration was observed for fermentation by *B. infantis* BB-02 at 70% whey (193.8 ± 11.5 ng/ml) and at 30% whey (188.6 ± 10.4 ng/ml) and by *S. thermophilus* TH-4 at 30% (186 ± 16.1 ng/ml) and 50% (185.7 ± 18.9 ng/ml) of whey. Fermentation with LGG had the lowest folate content at all whey concentrations compared to the other strains. The presence of the fruit water extract increased the strains growth during fermentation at different whey concentrations. The highest folate levels were found in the fermentation with TH-4, with grape extract (249.8 ± 21.1 ng/ml) in 70% of whey, followed by BB-02 at the same condition (234.6 ± 22.1 ng/ml) and LGG was the strain that produced the lowest folate concentrations. Cultivation conditions had a remarkable influence on folate production, and agro-industrial food by-products, such as fruit by-products and whey, in specific concentrations, can be used as substrates for the growth of beneficial microorganisms and folate production.

Acknowledgements
This study was supported by FAPESP #2018/12190-2, #2013/07914-8, CAPES and BMBF #031B0875.

P13

SELECTIVE FERMENTATION OF MODIFIED PECTIN FROM ORANGE ALBEDO AND FOLATE PRODUCTION BY PROBIOTIC AND STARTER CULTURES

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Orange albedo, a by-product of orange industry, is rich in water-soluble dietary fibres, such as pectins. This by-product generated by the industries is either used as animal feed or discarded in the environment, causing contamination problems. However, evidence suggest that pectins can be considered emerging prebiotics with ability to modulate the gut microbiota. Additionally, studies have reported that citrus pectin modified by heat treatment may have advantages regarding the prevention and treatment of different types of cancer. Therefore, the ability of heat-modified orange albedo pectin (MP) to support the growth of the probiotic (*Lacticaseibacillus rhamnosus* LGG and *Bifidobacterium longum* subsp. *infantis* BB-02) and starter (*Streptococcus thermophilus* TH4) strains and to stimulate the folate production by these microorganisms were assessed *in vitro*. The evaluation of growth promotion of the strains in the presence of MP was determined on selective agar before (0 h) and after 24h and 48h of aerobic incubation at 37°C. The basic medium was modified MRS broth plus phenol red supplemented with MP (1%, w/v). Samples were taken at 0 and 24 h for quantifying folate by microbiological assay using *L. rhamnosus* NCIMB 10463 (as folate consumer indicator strain). There was a significant increase (> 2.0 log CFU/ml) in the population of probiotic and starter strains in phenol red broth with MP after 24 h of incubation ($p<0.05$). The greatest increase was observed in the population of BB-02 (3.0 log CFU/ml), followed by TH-4 (2.6 log CFU/ml) and LGG (2.2 log CFU/ml). In general, bacterial populations kept stable after 48 h. However, there was an increase ($p<0.05$) in the population of probiotic and starter strains in phenol red broth without MP (negative control) after 24 h of incubation. The populations of BB-02, TH-4, and LGG increased, respectively, by 2.1 log CFU/ml, 2.6 log CFU/ml, and 1.9 log CFU/ml after 24 h of incubation in the negative controls. Regarding folate production, there was an increase in folate content after 24 h for all strains ($p<0.05$). The greatest increase was observed for LGG (61.3 ± 8.3 ng/ml), followed by BB-02 (44.0 ± 2.6 ng/ml), and TH-4 (41.7 ± 6.0 ng/ml). Therefore, the results showed that MP can significantly stimulate folate production by the three strains tested and promote an increase in the BB-02 growth after 24 h. Although pure culture models do not reflect bacterial interactions in the host, this study reinforces that the ability to metabolize different substrates like MP and folate production are strain-dependent.

Ackonowledgements
Financial suppor by the: University of São Paulo, USP (Project PIPAE # 2021.1.10424.1.9) and FAPESP (Projects #2013/07914-8 and #2018/12190-2).

P14
PILOTING AN 'ALL-IN-ONE' DATA COLLECTION PLATFORM, TRIALFLARE, FOR PROBIOTIC TRIALS

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Human trials are the tried-and-tested gold standard of product efficacy testing, whether it's pharmaceutical or nutritionally focused. Such trials are associated with a substantial administrative burden and extensive associated costs. New platforms operating by so-called 'decentralised clinical trial' (DCT) methodologies allow trials to be conducted digitally, capturing data securely on the web and on smartphone devices. Such technologies can improve cost, efficiency and data security, provide non-redundancy and full visibility for audit purposes and can offer new ways of capturing additional metadata not supported by more traditional 'pen and paper' methods. We provide evidence of the usability, functionality and effectiveness of the Trialflare DCT platform in human nutritional studies with a probiotic consortium. In three trials, we piloted the use of the Trialflare platform. We used the system to support product compliance by use of smartphone push notifications. Participants used the Trialflare Apple and Google Play Store app to remind participants to take their product daily and to submit their data in a timely manner. Trialflare was also used to gather data relating to wellbeing and cognitive performance in an independently-managed, double-blind, randomised placebo controlled probiotic trial hosted at a dedicated trial centre. The app is also currently in use for a probiotic study "in the field" gathering participant-reported outcomes from volunteers across South Wales without the input of multiple trial administrators or healthcare professionals (HCPs). We report the outcomes of these studies showing that the Trialflare platform is fit for purpose in encouraging participant product compliance during human nutritional studies with a probiotic supplement. Trialflare was also highly effective at capturing accurate data throughout the probiotic trials. The dedicated trials centre was based outside the UK and the Trialflare app was fully functional across different languages/countries. These pilot studies aimed at challenging the flexibility of the Trialflare DCT platform indicated the user friendliness of this particular system.

P15
GUT-BRAIN AXIS MEDIATED NEURODEGENERATION IN PARKINSON'S DISEASE: TLR4 AS A THERAPEUTIC TARGET

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Parkinson's disease (PD) is the second most prevalent neurodegenerative disease and, so far, no treatment exists that can halt the neurodegenerative process [1]. Inflammation, both locally in the brain and in the periphery, has been shown to be a key driver of neurodegeneration in PD [2]. The microbiome as a potential source of this inflammation is an area of study that has garnered increased attention in recent years (3). Studies have found that patients with PD have a more proinflammatory microbiota profile, with increases in Lipopolysaccharide (LPS) producing bacteria and therefore increased intestinal levels of LPS being a key feature [3–6]. The exact mechanisms by which the microbiota might influence PD progression remains to be elucidated. However, one proposal involves the sensing of LPS by the ubiquitous innate immune receptor, toll-like receptor 4 (TLR4). Recent studies have demonstrated that inflammatory processes triggered by the activation of TLR4 and its accessory molecules are important in the onset and exacerbation of PD symptoms [7–10]. Thus, the blockade of these TLR4 mediated inflammatory signals could present a new therapeutic strategy, with the aim to prevent further neurodegeneration and finally present a disease modifying therapy. Therefore, to evaluate TLR4 as a druggable target we tested two different antagonists in a well-established PD mouse model. One treatment consisted of a small molecule inhibitor of TLR4 that can freely pass the blood-brain barrier, the other was an antibody raised against TLR4, therefore not able to pass through to the brain. These treatments were chosen to investigate if the inhibition of TLR4 in the brain in addition to the periphery was necessary to prevent neurodegeneration, compared to inhibition in the periphery alone. Furthermore, by implementing these two different areas of inhibition, insight can be gained into the mechanisms underlying TLR4 mediated neurodegeneration in PD. It was seen that treatment with either antagonist could decrease the degree of muscle strength loss seen in the PD model and resolve gastrointestinal symptoms. These preliminary data suggest that the targeted antagonism of TLR4 could provide a promising intervention targeting microbiome mediated inflammation in the early stages of PD. Furthermore, due to both antagonists having efficacy in reducing PD severity, it can be inferred that TLR4 mediated inflammation in the periphery and not the brain is most important in initiating neurodegeneration.

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P16

EFFICACY OF USING PROBIOTICS FOR THE PREVENTION OF ACUTE RESPIRATORY TRACT INFECTION IN OLDER PEOPLE

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Acute upper respiratory tract infections (URTIs) are illnesses caused by infection of mucosal surfaces in the nose, sinuses, pharynx and/or larynx and large airways with viruses or bacteria. Older people are at higher risk for worse outcome due to epidemiological elements, immunosenescence and malnutrition, as well as many age-associated physiological and anatomical alterations, including physiological changes in the diversity and loss of resilience of the intestinal microbiota. A large pool of evidence in well-designed reviews has suggested that probiotic supplementation reduces episodes of common infectious diseases, including respiratory tract infections, through improvement of immune function. We performed a systematic review to investigate the indirect influence of probiotics on the incidence and duration of acute upper respiratory-tract infections in older people, by regulating the immune system. Eight randomized, placebo-controlled clinical trials met the inclusion criteria, considering the threshold of older people being 60 years and over. Single strain probiotics were used in all studies, including three probiotic strains used in fermented foods: *Lactobacillus delbrueckii* subsp. *bulgaricus* OLL1073R-1, *Lacticaseibacillus paracasei* subsp. *paracasei* CNCM I-1518 and *Lacticaseibacillus paracasei* Shirota, and three probiotic strains used as food supplements: *Loigolactobacillus coryniformis* K8 CECT5711, *Bacillus subtilis* CU1 and *Lacticaseibacillus rhamnosus* GG. Adverse events were minor and not related to probiotic consumption. The mode of action of probiotics was most probably systemic immunomodulation via interaction of the microorganisms with the mucosal immune system by various methods, including colonization resistance, trans-epithelial resistance, increased number and activity of natural killer cells, release of certain cytokines and bacteriocins, enhanced antibody response, stimulation of non-specific immunity, enhancing humoral and cellular immunity as well as co-mediation of metabolic and immune homeostasis, leading to better communication between the gut-lung axis. Current evidence showed that certain probiotic strains were better than placebo in lowering the incidence or number of older people experiencing acute upper respiratory tract infections; however, not all probiotic strains were efficient, and not all studies reported statistically significant outcomes. More high quality large-scale properly controlled clinical studies focusing on older people are warranted.

P17

DEVELOPING AN OMICS BASED RAPID DETECTION METHODS TO ENSURE FOOD SAFETY OF FERMENTED OR LONG SHELF-LIFE FOOD PRODUCTS: TITAN INNOVATIONS

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Transparency solutions for transforming the food system, abbreviated as TITAN, is a 4-year project funded by the European Union through the Horizon Europe Framework Programme to enhance transparency and food safety in the food value chain (https://titanproject.eu/). Supported by 28 partners from 14 countries, the overall objective of TITAN is to provide an extensive platform for the development of a wide range of innovations that aid transparency and address key challenges identified in the European Green Deal, such as climate change and reduction of food waste. This ambitious goal is based on an interactive co-creative approach involving technology providers, research centres, and business actors. The TITAN innovations, all transparency related, address the following themes: (i) enhancing transparency in agri-food businesses with a focus on SMEs; (ii) improving food choices by providing more transparent information to the consumer; (iii) using improved transparency to enhance food safety and authenticity of products; (iv) and providing improved information on the health and sustainability of food products. Overall, TITAN will showcase 21 innovations covering these themes, with TRLs moving on average from TRL 5 to 7 within the lifetime of the project. For the 9th Beneficial Microbes Conference, we would like to mention three specific pilots which are concerned with the development and/or application of rapid detection methods: (i) microbiology of fermented food products, safety demonstration of food cultures; (ii) omics and molecular approaches for microbial and chemical quality of long shelf-life food products; and (iii) real-time and intelligent data sharing for verification of honey and herbs suppliers. By bringing together business strategy, the latest technology innovations, policy and the consumer, TITAN will provide the blueprint of a demand driven economy that provides healthy, sustainable, and accessible food for its citizens.

P18

OMICS TECHNOLOGIES FOR EVALUATION OF STARTER AND PROBIOTIC STRAINS IN THE BRAZILIAN-STYLE BEER CATHARINA SOUR

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The craft beer market is growing worldwide, especially regarding sour beers, which are produced by lactic acid bacteria (LAB) and may have probiotic potential. Catharina sour is the first internationally recognized Brazilian beer. It is characterized by fermentation by LAB and the addition of fruit juice. This study aimed to evaluate the use of the starter culture *Streptococcus thermophilus* TH-4 (TH-4), and the probiotic cultures *Lacticaseibacillus paracasei* F19 and *L. paracasei* 431 (431), in association with *Saccharomyces cerevisiae* US-05, in the absence (control) or presence of passion fruit or peach juices. Evaluation proceeded during fermentation and storage by enumeration, using pour-plate and qPCR; gene expression of genes associated with hops resistance, including *horA*, *horB*, *horC*, *hitA*, *bsrA*, and *recA*; proteome by liquid chromatography tandem mass spectrometry (LC-MS/MS); and odour, flavour, and metabolome by headspace solid-phase microextraction (HS-SPME), coupled with the gas chromatography-mass spectrometry (GC-MS) analysis. We concluded that probiotics strains tested in this study may be the good candidates to be used in sour beers brewery, due the several defence mechanisms, such as membrane adhesion proteins and H+ pump. All formulations showed a probiotic viability between 5 and 8 log CFU/ml throughout the steps. In addition, the expression of the *horC* gene seemed to be involved with microbial resistance in control and passion fruit juice formulations while *hitA* in peach juice, however, these genes may not be determinant for survival in beer. Proteome analysis indicated that strain 431 may be best adapted to beer conditions, and flavouring analysis indicated that the strain used may interfere with flavour and odour, due to the variation of compounds produced during fermentation with the different strains of LAB.

Acknowledgements
This study was supported by FAPESP (#2018/21584-4, #2018/12190-2, #2013/07914-8, #2019/ 02583-0, and #2021/08621-0), CAPES (#88881.187323/2018-01, #88882.376972-2019-01, #88887. 473545/ 2020-00), and CNPq (#305380/2019-2).

P19

SURVIVAL OF THE PROBIOTIC STRAIN *LACTICASEIBACILLUS PARACASEI* SUBSP. *PARACASEI* F19 IN BEER FORMULATIONS WITH HIGH HOP CONTENT (*HUMULUS LUPULUS* L.)

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Consumption of craft beers is increasing, along with the number of small independent breweries. Among the craft beers, today the sour beers stand out. The sour beer brewing process has an additional lactic fermentation step prior to alcoholic fermentation by yeast, which can be performed by a lactic acid probiotic bacteria strain. However, beer is a beverage that has several factors that prevent microbial proliferation, among them, hops (*Humulus lupulus* L.) stand out. The typical hop aroma in beers is attributed to a large group of volatile aromatic compounds from the lupulin glands of female hop cones, collectively known as essential oils. Thus, this study aimed to evaluate the survival of the probiotic *Lacticaseibacillus paracasei* subsp. *paracasei* F19 (F19) with, or without, the yeasts *Saccharomyces cerevisiae*, strains US-05 and Kveik, and *Saccharomycodes ludwigii* (non-alcohol), in formulations with IBU (International Bitterness Unit) 0 and 20, which corresponds to the amount of hop. The pH and specific gravity (SG) were measured during fermentation, as well as the yeast count and their viability was performed by the Oculyze microscope (Wildau, Germany) and the F19 count by qPCR. The yeast count by the Oculyze microscope showed that there was no difference in the yeast count between the strains, ranging from 6.96 to 7.64 log UFC/ml in 24 h. The F19 count ranging from 8.39 to 8.89 log CFU/ml in 24 h in IBU 0 and from 6.61 to 7.89 log CFU/ml in IBU 20. There were no differences between with F19 pure formulations with IBU 0 (7.93 log CFU/ml) and with IBU 20 (7.71 log CFU/ml). We can conclude that the F19 strain survive in beers with high amounts of hops after 24 h of fermentation, and there is no relationship with the presence of yeast, contrary to what was shown in previous studies.

Acknowledgements
This study was supported by FAPESP (#2018/21584-4, #2018/12190-2, #2013/07914-8, and #2019/02583-0), and CNPq (#305380/2019-2).

P20

VIABILITY OF THE *LACTICASEIBACILLUS* (L.) *PARACASEI* SUBSP. *PARACASEI* F19 PROBIOTIC STRAIN IN A SOUR BEER WITH FRUIT JUICE AND BY-PRODUCT

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In general, the beer fermentation process is carried out by a monoculture of *Saccharomyces cerevisiae* for ales beer, or *Saccharomyces pastorianus*, for lager beers. Nevertheless, beverage variations can be obtained by co-fermentation from yeasts and lactic acid bacteria (LAB), featuring sour or acidic beers. Fruits by-products are substrates rich in fibres, sugars, nutritional and bioactive compounds, and may be used in fermentation to produce other types of food. Thus, this study aimed to evaluate the feasibility of the application of *Lacticaseibacillus* (L.) *paracasei* subsp. *paracasei* F19 in the production of a sour-type beer with the addition of *Spondias mombim* L. juice and by-product. The sour beer was produced in a laboratory-scale through four types of formulations, according to the addition of by-product and/or fruit juice and received two stages of fermentation: alcoholic (*S. cerevisiae*) and lactic (*L. paracasei* F19). For the microbial growth analysis, samples were collected during the beer fermentation and during storage and were also evaluated regarding pH and acidity. The fermentation assay revealed the ability performance of *L. paracasei* F19 to ferment the *S. mombim* by-product in MRS medium, reaching 9.28 log CFU/ml in 24 h. Regarding the hopped wort, the probiotic microorganism had a lower viability. The bacterial strain was able to survive in the formulations and promote acidification of the medium. However, the addition of S. mombim juice and/or by-product did not support the growth/survival of *L. paracasei* F19. Only the control formulation composed only of wort and hops, without the addition of extra ingredients, reached high bacterial population values, around 7.28 log CFU/ml after 28 days of production. Thus, the application of *Spondias mombim* juice and/or by-product is not appropriate to produce sour beer with *L. paracasei* F19 in co-culture with *Saccharomyces cerevisiae* and other combinations between fruit juices and by-products and strains ought to be tests.

Acknowledgements
This study was supported by FAPESP (#2018/21584-4, #2018/12190-2, #2013/07914-8, #2019/02583-0, and #2021/08621-0), CAPES (#88887.473545/2020-00), and CNPq (#305380/2019-2).

P21
DIETARY PROBIOTICS, PREBIOTICS AND THE GUT MICROBIOTA IN HUMAN HEALTH – UPDATED ILSI EUROPE CONCISE MONOGRAPH

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While the participants of the 9th Beneficial Microbes conference are very well aware of what probiotics and prebiotics are, this is less so for the lay audience that make up the consumers of these products. There is therefore a need for reliable and objective information. In 2013, the International Life Sciences Institute (ILSI) Europe published a concise monograph that addressed this topic. The monograph was the fruit of a collaboration between the Probiotic and Prebiotic Task Forces and external academic experts. The monograph can be downloaded for free and has been one of ILSI's most frequently downloaded. In the past eight years, much has happened and therefore there was a need to update the monograph. The expanded understanding of the role the intestinal microbiota plays in health and wellbeing has been expanded; most notably with the gut-brain axis. The probiotic section discusses many of the proposed health benefits of probiotics and has seen an update in the definition and taxonomy of microbes, in particular the lactobacilli. Also, the prebiotic section describes the putative health benefits and has seen an update of the definition; expanding beyond the traditional fibre-like carbohydrates and has been expanded with human milk oligosaccharides. Although there are still gaps, there is an improved understanding of the mechanisms of action for both probiotics and prebiotics. The glossary has been updated as well as the list with further reading. The new edition provides an up-to-date explanation of the intestinal microbiota, probiotics, and prebiotics. It is a freely usable tool for everybody with an interest in these topics. In addition to the interested lay person, the monograph may also be useful for undergraduate students who need an introduction in the topics.

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P22

STUDY PROTOCOL: EFFECTS OF BUTYRATE ON AFFECTIVE PATTERNS, MICROBIOME COMPOSITION AND DEPRESSIVE SYMPTOMS IN YOUNG ADULTS – A RANDOMIZED CLINICAL TRIAL

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Major depressive disorder (MDD) in young adults is a serious mental health issue associated with poor mental and physical health outcomes. Because adolescence is a developmentally sensitive period, besides being a risk factor, it also offers a window of opportunity to mitigate (the worsening of) depression. The aetiology and pathophysiology of depression may be influenced by intestinal dysbiosis and targeting the microbiota-gut-brain axis with probiotic and synbiotics have shown promise in reducing depressive symptoms. Such beneficial effects have been proposed to be (partly) contributed by an increase of microbial butyrate-production. However, studies have not yet assessed therapeutic effects of directly administering butyrate in depressed youth. Here, we describe a study protocol for a randomized clinical trial with the aim to assess the effects of oral butyrate supplementation on affective patterns and depressive symptoms in young adults with MDD. To gain insights into butyrate's potential mechanism of action on affective patterns and depressive symptoms, secondary outcomes will include faecal microbiome composition, faecal and plasma metabolites, plasma immune markers and stress hormones, and intestinal permeability. Treatment-naive young adults with MDD (18-25 years) will receive oral capsules containing 4 grams tributyrin (n=10) or placebo (n=10) daily for eight weeks, besides receiving treatment as usual. To assess affective patterns, participants will report their positive and negative affect five times per day through a mobile phone application during and prior to the intervention. Secondary outcomes will be measured before (week 0), during (week 4) and directly after (week 8) intervention, and at follow-up (week 24). Depressive symptoms will be measured with the Hamilton Depression Rating Scale (HDRS) and the Quick Inventory of Depressive Symptomatology (QIDS). In addition, fasting blood and faecal samples will be obtained. Microbiome composition will be determined by 16S rRNA gene sequencing. Faecal and plasma metabolites will be measured with liquid chromatography-mass spectrometry. Proxies of intestinal permeability will include levels of faecal zonulin and plasma lipopolysaccharide binding protein. In this proof of principle study, we aim to see whether targeting the intestinal microbiota with a microbial metabolite – butyrate – shows therapeutic promise in treatment of MDD. Findings from this pilot will help unravel the influence of butyrate on the microbiota-gut- brain axis, and how this relates to affective patterns and depressive symptoms.

P23
LACTICASEIBACILLUS RHAMNOSUS HA-114: AN INNOVATIVE PROBIOTIC STRAIN TO SUPPORT WEIGHT MANAGEMENT EFFORTS THROUGH THE GUT-BRAIN AXIS

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Bidirectional correlations between the prevalence of overweight/obesity and depression have been identified worldwide. Recent weight management guidelines now recognize the importance of the microbiota-gut-brain axis on the mutual influence between mental health/wellbeing/mood and food intake/behaviours. Hence, probiotics are considered as potential tools to support weight loss and help counteract the rising incidence of obesity-associated metabolic syndrome and cardiometabolic disorders, although clinical evidence remains limited. The study described herein was aimed at assessing the effect of *Lacticaseibacillus rhamnosus* HA-114, in combination with a weight loss intervention, on body weight and composition, metabolic markers, eating behaviours and psychological wellness in healthy participants with overweight or obesity (ClinicalTrials.gov identifier: NCT02962583). The selected probiotic strain was previously shown to reduce fat accumulation in *Caenorhabditis elegans* and to improve cognitive phenotypes in mice, suggesting potential metabolic and psychobiotic effects. In this randomized, double-blind, placebo-controlled trial, 152 participants with overweight/obesity (BMI: 27-39.9kg/m^2) were randomly assigned to receive either *L. rhamnosus* HA-114 (10 billion colony-forming units (CFU)/day) or placebo during a 12-week calorie-restricted nutritional intervention (-500 kcal/day). Baseline characteristics were similar between groups, and metabolic marker values were in the higher limit of the normal range, suggesting a potentially increased risk of MetS or CMDs. Although both groups experienced a similar extent of weight loss over the 12 weeks (on average, body weight -3.96kg, BMI -1.41kg/m^2, waist circumference -3.27cm), probiotic supplementation significantly decreased LDL-cholesterol and triglycerides, plasma insulin, and the HOMA-IR (insulin resistance index) (all $p<0.05$). Importantly, psychological well-being and eating behaviours, assessed using several validated questionnaires with overlapping parameters (TFEQ, BSQ, FCQ-S, FCQ-T, STAI-T, PSS and BDI), were significantly improved in the probiotic-supplemented group. *L. rhamnosus* HA-114 significantly increased cognitive restraint of eating (+42%) while decreasing disinhibition (-18%), hunger (-39.9%), binge-eating behaviour (-38.1%), intense desire to eat (-19.6%) and lack of control (-22.4%). Additionally, mental health questionnaires demonstrated that the 12-week probiotic supplementation also reduced anxiety, perceived stress, and depression scores ($p<0.05$). This first clinical trial demonstrated that *L. rhamnosus* HA-114 supplementation enhances metabolic health and supports weight management efforts by improving mood and eating-behaviours, which could be important for nutritional habit changes and weight loss maintenance over time. Additional preclinical and clinical studies are ongoing to better understand the mechanisms underlying the beneficial psychobiotic effects of HA-114 via the microbiota-gut-brain axis.

P24
DIETARY FIBRE COMBINATIONS TO COUNTERACT PLANT-PROTEIN COLONIC FERMENTATION

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Western diet is characterized by a high intake of protein and fat but low in dietary fibre. The deficiency of dietary fibre in the colon leads the gut microbiota to ferment protein at a higher rate. Proteolytic fermentation yields undesired products such as branch-short chain fatty acids (BSCFA), ammonia and hydrogen sulphide. Moreover, the western diet is transiting from animal-origin proteins to be rich in plant-origin proteins. These kinds of proteins are expected to be less digestible and to affect the metabolic profile in the colon. On the other hand, fermentation of dietary fibres produces short-chain fatty acids (SCFA) which have diverse benefits for immune, metabolic and brain health. Our research aims to investigate the impact of selected dietary fibres on the metabolic profile of saccharolytic and proteolytic fermentation, as well as on the gut microbiota composition. For this purpose, an *in vitro* batch fermentation model will be implemented using human faecal inoculum and SIEM medium to emulate colonic conditions. Three different dietary fibres (inulin, pectin, and potato fibre) and three different plant-origin proteins (fava bean, pea and potato protein) will be tested in this *in vitro* model. The SCFA and BSCFA production will be measured by high-performance liquid chromatography. Protein controls will be included to compare the effect of dietary fibres on proteolytic fermentation.

P25
SCREENING OF CANDIDATE PROBIOTICS AND MICROBIAL METABOLITES FOR THE PREVENTION OF NON-ALCOHOLIC FATTY LIVER DISEASE

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Metabolic health and related diseases pose world-wide challenges and are expected to rise dramatically by 2030. Non-alcoholic fatty liver disease (NAFLD) is strongly associated with metabolic syndrome, type 2 diabetes mellitus and deprived gut health. Increased gut permeability, by disturbance of tight junction proteins, allows passage of damaging microbial components that upon reaching the liver may cause release of inflammatory cytokines and generation of cellular stress. Recently, frontier research has suggested utilization of targeted probiotic supplements as preventive therapy improving gut barrier function and tight junctions. Furthermore, specific microbial metabolites induce secretion of NAFLD-preventive hormones such as GLP-1 and might alleviate hepatic lipid impairment. In this screening study, 43 bacterial strains (*Lactobacillus* and *Bifidobacterium* spp.) were selected based on literature searches and novelty factor. Transepithelial electrical resistance (TEER) response by human colonic cells (CACO-2) co-incubated with selected strains was explored and metabolomics were performed on assay supernatants to correlate outcome with specific metabolites. Moreover, GLP-1 secretion from STC-1 cells was investigated by MSD platform and promising candidate strains were further tested on human small intestinal organoid 2D monolayer for transcriptomics. Likewise, outstanding bacterial metabolites were assessed in primary murine hepatocytes for the evaluation *de novo* lipogenesis inhibition and AMPK signalling. All strains displayed positive impact on TEER, however to various degrees. High TEER showed strong correlation with microbial metabolites such as: choline and Indole-3 lactic acid (ILA). GLP-1 release was in general induced by the Bifidobacteria, with only few high stimulating candidates. Best performing strains were investigated in an *in vivo* study exploring the preventive effect of probiotics in a diet induced obesity and NASH mouse model.

P26
WHY MULTI-STRAIN PROBIOTICS ARE BECOMING THE STANDARD

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For the long-term sustainability of human health, animal health and meat production, it is imperative we reduce antibiotics use to an absolute minimum. Probiotics have already shown their use in antibiotic reduction and improvement of animal health. Spore-forming *Bacillus spp.* are often used since they have a measurable positive effect as a probiotic as well as other beneficial properties such as long shelf life. Single strain probiotics are commonly added to feed to improve flock health, but the vast majority of new probiotics are multi-strain. Here, we will present a trial with a triple-strain probiotic (*Bacillus* spp. ATCC PTA-6737, PTA-127113 and PTA-127114) and its effect on a range of markers, including the microbiome. A total of 1040 female day-old Ross 308 broilers were used for the study. Broilers were distributed into 4 treatments (20 broilers/pen, 13 pens/treatment, pen=experimental unit): (i) T1, negative control; (ii) T2, $3.0x10^8$ CFU/kg of the multi-strain probiotic (recommended dose); (iii) T3, $3.0x10^9$ CFU/kg; and (iv) T4, $3.0x10^{11}$ CFU/kg (tolerance test). Diets were formulated in three phases to meet or exceed breed recommendation. Chickens and feed were weighed weekly until 35 days of age. Average body weight, average daily gain, average daily feed intake and feed conversion ratio (FCR) were calculated from these measurements. Two birds per pen were slaughtered on both days 14 and 36 for carcass and internal organ parameters. Ileal and caecal content were sampled on days 14 and 35 (one bird/pen). Total microbiome DNA was extracted and subsequently, 16S rRNA amplicon sequencing was performed using Illumina next generation sequencing. On day 35, the probiotic-treated birds showed higher body weights ($p<0.05$) for all dosages (1,897 g in T2, 1,904 g in T3 and 1,894 g in T4 versus 1,774 g in control). Final FCR was also significantly ($p<0.05$) improved for all probiotic-treated broilers. For example, the FCR of 1.81 in control birds reduced to 1.57 when fed with the recommended dose of probiotic (T2). The breast meat yield showed a significant increase from 23% in the control to 25% in T2. Furthermore, the trial results showed that the probiotic improved gut health by reducing the formation of gases in the ileum and reducing the intestinal lesion score. At 14 days of age, microbial diversity in the cecum was significantly increased and the relative abundances of *Firmicutes* and *Lachnospiraceae* were increased. In conclusion, the multi-strain probiotic can significantly improve performance and intestinal health parameters when fed to broiler chickens.

P27

POTENTIAL OF DIFFERENT DEXTRANS AS PREBIOTICS TO MODULATE HUMAN GUT MICROBIOTA: AN *IN VITRO* APPROACH USING HUMAN BATCH FERMENTATION

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Dextrans are exo-homopolysaccharides (EPS) of D-glucose synthesized by lactic acid bacteria (LAB). EPS have several technological applications of interest as well as gut health-beneficial effects, including prebiotic properties to improve functionalities of food and the host health. EPS properties will strongly depend on their strain-dependent structures. Therefore, it is necessary to characterize each EPS with relevant tools and *in vitro* models to assess human colonic fermentation. The aim of this study was to assess the microbiota modulation properties of 3 types of dextran *in vitro* with a SHIME® (Simulator of Human Intestinal Microbial Ecosystem) batch fermentation using two healthy subjects. Each dextran (D1-3) differentiated by their molecular weight (D1, 1.5-2.8 MDa; D2, 5-40 MDa; and D3, <40MDa) has been evaluated at 3 doses (2, 3.5 and 5g/l) and up to 48 h colonic fermentation. Inulin has been included as a positive control. Microbiota modulation was investigated by combining 2 analytical approaches to study both taxa composition (16S rRNA metabarcoding) and microbial activity (SCFAs, BCFAs and lactate). Following colonic fermentation, all dextrans increased total SCFA levels in a dose -dependent manner and irrespective of the size of dextran tested. However, among the main SCFA evaluated, different levels of acetate and propionate were detected according to the size of dextran used. Notably, D2 showed a significative increase of acetate and propionate compared to D1 and D3 and a profile comparable than inulin. It suggested a dextran specific modulation of microbial activity. A shift of microbiota composition was observed after fermentation with specific signatures linked to the treatment. Indeed, D2 treatment had the highest ratio of *Bacteroidetes/Firmicutes*, irrespective of the dose applied. In addition, among *Bacteroidetes* phylum, *Bacteroidaceae* and *Tannerellaceae* family (up to 60 and 10% assigned OTUs, respectively) were enriched in this treatment. Therefore, the high abundance of *Bacteroidaceae* taxa following D2 treatment can explain the predominance of acetate levels associated to their known saccharolytic metabolism. In conclusion, by using short-term colonic fermentation, our study allowed to characterize and compare different dextrans with a focus on their microbiota modulation properties. Additional *in vivo* work is warranted to validate this predictive approach to assess dextran prebiotic functions.

P28

ROLE OF LIPOTEICHOIC ACID FROM THE GENUS *APILACTOBACILLUS* IN INDUCING A STRONG IgA RESPONSE

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The components of lactic acid bacteria that exert immunostimulatory effects are of increasing interest for therapeutic and prophylactic options, such as alternatives to antibiotics, cognitive enhancements, and vaccine adjuvants. Lipoteichoic acids (LTAs), which are a cell wall component of gram-positive bacteria such as lactic acid bacteria, are well-known to act as immunostimulatory molecules in the host's innate immune system by interacting with pattern recognition receptors. However, LTA structures differ among species, therefore, detailed knowledge of the structure-function relationship for immunostimulatory effects is required. Immunoglobulin A (IgA) is a critical component of mucosal immunity and is secreted onto mucosal surfaces to neutralize dietary antigens, toxins, and pathogenic microorganisms. Using murine Peyer's patch cells, we compared the IgA-inducing activity of 30 species of lactic acid bacteria and found that species belonging to the genus *Apilactobacillus* (*A. kosoi* 10H^T, *A. apinorum* JCM30765^T, and *A. kunkeei* JCM16173^T) possessed significantly higher activities than the others. Furthermore, administration of *A. kosoi* 10H^T cells to mice stimulated intestinal IgA production. We purified LTAs from the three *Apilactobacillus* species, and their IgA-inducing activity was compared to those from *Lactiplantibacillus plantarum* JCM1149^T and *Lacticaseibacillus rhamnosus* GG, because LTA structures and their ability to activate immune cells have been reported in both strains. The results demonstrated that LTAs from the genus *Apilactobacillus* possess distinctive activities to stimulate IgA production from Peyer's patch cells. Furthermore, murine bone marrow-derived dendritic cells stimulated by *A. kosoi* 10H^T LTA showed upregulation in the expression of IL-6, retinal dehydrogenase 2, IL-10, and inducible nitric oxide synthase genes, revealing that IgA-production was stimulated through stronger induction of IL-6, retinoic acid, IL-10, and nitric oxide. We also compared the LTA structures of *A. kosoi* 10H^T, *L. plantarum* JCM1149^T and *L. rhamnosus* GG. Although D-alanine or both D-alanine and carbohydrate residues were substituents of free hydroxyl groups in the poly-glycerol phosphate structure in LTAs from *L. plantarum* JCM1149^T and *L. rhamnosus* GG, D-alanine residue was not found in LTA from *A. kosoi* 10H^T by ^{1}H NMR analysis. MALDI-TOF MS analysis of the glycolipid structure of LTA revealed that LTA from *A. kosoi* 10H^T contained dihexosyl glycerol, whereas trihexosyl glycerol was detected in the LTAs of other strains. These structural differences of LTA from *Apilactobacillus* species may be related to strong IgA-inducing activity. Our findings should help in developing the use of lactic acid bacteria for functional foods and pharmaceutical applications of immunostimulatory molecules.

P29

MODULATION OF GUT MICROBIOTA OF HEALTHY AND PREDIABETIC SUBJECTS BY *ASPARAGUS OFFICINALIS* DIETARY FIBRES IN AN *IN VITRO* MODEL OF THE COLON

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Growing evidence indicates that the gut microbiota (GM) plays a role in the aetiology of type 2 diabetes (T2D) through the fermentation of indigestible dietary fibres (DF), which are associated with the production of short-chain fatty acids (SCFA). *Asparagus officinalis* possesses several functional properties, including hypolipidemic, hypoglycaemic, and antioxidant activity, as well as prebiotic potential due to its DF content. Given that the relationship between DF and GM in patients with T2D is not fully understood, we aimed to evaluate *A. officinalis* effect on GM composition and activity using a validated, dynamic, *in vitro model* of the colon (TIM-2) and associate these findings with the chemical characterization of its DF. Fermented asparagus (FA) or asparagus peels (AP) were provided to a standardized microbiota from healthy and pre-diabetic adults in TIM-2 over a 3-day experimental period. Lumen samples (0, 24, 48, and 72 h) were used to analyse GM composition (sequencing of the V3-V4 region of the 16S rRNA gene), and both lumen and dialysis samples were analysed for SCFA concentrations (GC-MS). Kruskal-Wallis test (with FDR correction) was applied to compare α-diversities (Shannon index) among different treatments and time points, and the Wilcoxon test was used for pairwise comparison. Spearman correlations (FDR-corrected) between SCFA production and taxa were evaluated. Besides, the DF content of FA and AP was determined by the enzymatic-gravimetric method (AOAC 991.43). The isolated DF were analysed for their monosaccharide composition, by derivatization to alditol-acetates, and molecular weight by HPSEC. *A. officinalis* modulated the GM composition in TIM-2. At the genus level, the analysis showed that ~20% of all sequenced taxa were *Bacteroides*, followed by around 18% *Faecalibacterium* and 8% *Alistipes*. Butyrate production was higher on AP, while FA showed the highest acetate production compared to control. The production of propionate was correlated to *Roseburia* (q=0.02, rho=0.660) and *Lachnospira* (q=0.119, rho=0.692) abundance, while butyrate production was correlated to *Catenisphaera* (q=0.133, rho=0.686). *Bacteroides* and *Faecalibacterium* are two of the bacterial genera most often related to preventing the development of T2D, with butyrate studied the most. Studies have shown a tight association of butyrate with glucose homeostasis, insulin resistance, and appetite. DF analysis revealed different monosaccharide composition, molecular weight, and solubility that might be associated with the different responses on GM analyses and SCFA production. In conclusion, the modulation of SCFA production through DF fermentation by the GM can be a useful strategy to prevent the development of T2D.

P30

LONGITUDINAL GUT MYCOBIOTA CHANGES IN JAPANESE INFANTS DURING FIRST THREE YEARS OF LIFE

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Although fungi can have a great impact on host health through the stimulation of the immune system, not many studies have addressed the potential contributions of gut mycobiota during infancy, a period sensitive to intestinal stimuli. This study aims to capture the succession of gut mycobiota in infancy. Faecal samples were collected from Japanese infants at 1, 3, and 6 months and 1, 2, and 3 years. Internal transcribed spacer 1 region was sequenced using MiSeq. Infants had two major phyla, Ascomycota and Basidiomycota, and the most abundant genus was *Saccharomyces*, followed by *Malassezia*, *Candida*, *Meyerozyma*, and *Penicillium*. Alpha-diversity significantly decreased with age, suggesting adaptive selection in the gut environment of the colonizing species. Beta-diversity analysis divided infant mycobiota into age-related clusters, and showed discrete separation before and after weaning, suggesting microenvironmental shift through weaning. At 1 month, the ratio between moulds and yeasts was almost equal whereas yeasts significantly increased after 3 months, indicating that compared to aerobic moulds, yeasts, which are facultative anaerobes, can adapt to the anaerobic environment in the intestinal tract. In the initial stage, *Penicillium* and *Meyerozyma* highly colonized and, in lactation period, they were overtaken by *Malassezia*, which is a skin commensal yeast. After weaning, *Saccharomyces* became dominant genus, which is frequently detected from adult mycobiota. Additionally, our study revealed that *Malassezia* were abundant in breast-fed infants compared to mixed-fed ones. In conclusion, this study suggests that gut mycobiota develops in infancy, in which *Malassezia* colonizes in association with breast milk feeding and is thereafter replaced by *Saccharomyces* in association with solid food intake. Further studies are needed to shed light on how the passage of the series of fungal colonisations in infancy affects the development of the host immune system and the other homeostasis involved in health later in life.

P31

EFFECT OF A MIX OF *LACTICASEIBACILLUS CASEI* LA205 AND *L. PARACASEI* LA903 ON BEHAVIOURAL, BIOCHEMICAL AND GUT MICROBIAL OUTCOMES OF MALE MICE FOLLOWING CHRONIC RESTRAINT STRESS

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This study evaluated the potential anxiolytic properties of a probiotic mix of *Lacticaseibacillus casei* LA205 and *L. paracasei* LA903 in mice undergoing chronic restraint stress (CRS). The 4 groups of male BALB/cByJrj mice were non-stressed/solvent (NS-PBS), non-stressed/probiotics (NS-Probio), CRS/solvent (S-PBS), CRS/probiotics (S-Probio). Probiotic mix ($2x10^9$ CFU) and solvent (PBS 1x) were administered daily *per os* from two weeks prior to the CRS until the end of protocol (including 21 days of CRS (4 h per day) + 2 days of behavioural testing). Elevated-plus maze test (EPM) was performed on the first day and open-field test (OF) on the second day. Faeces were collected at the beginning of the experiment and the day before the behavioural tests. During necropsies, performed the day after the behavioural tests, blood, brain and gut samples were collected. CRS resulted in a significant decrease in body weight gain, which was attenuated by the probiotic mix. Although CRS was not associated with differences in performance in the EPM and OF tests between NS-PBS and S-PBS mice, S-Probio mice spent more time in the open arms in the EPM and in the centre area in the OF than their NS counterpart. The 16S rRNA gene sequencing analysis of the gut microbiota revealed that alpha and beta diversities were not different between the four groups. However, differences in bacterial genera proportions were observed. Analysis of the relative proportions of short-chain fatty acids in the caecal content, an index of fermentation activity, showed that CRS was accompanied by a decrease in the proportion of acetate in S-PBS mice vs. their NS counterpart, but not in the probiotic groups. Regarding gut inflammation and permeability, treatment with the probiotic mix induced a decrease in faecal lipocalin in S-Probio mice vs. their NS counterpart, and an increase in colonic claudin-2 mRNA expression in S-Probio vs. S-PBS mice. Finally, in serum, CRS induced an increase in the kynurenine/tryptophan ratio and a decrease in serotonin in S-PBS mice vs.their NS counterpart, but not in the probiotic groups. In the hippocampus, the expression of dopamine and serotonin transporters mRNAs was decreased in S-Probio vs.S-PBS mice. These data show that the probiotic mix could mitigate some of the consequences of CRS. Potential correlations between these data and the composition of the gut microbiota under the influence of the probiotic mix remain to be determined.

P32

THE EFFECT OF CARBOHYDRATES ON THE CAECAL MICROBIAL COMPOSITION AND METABOLITES IN AN *IN VITRO* MODEL FOR BROILERS WITH IMPAIRED GUT HEALTH

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Broiler chickens are prone to develop the intestinal disease coccidiosis, caused by *Eimeria* spp. infections, which has a major impact on health and production performance of broiler flocks worldwide. Coccidiosis is a predisposing factor for overgrowth with pathogenic bacteria, such as *Clostridium perfringens*, resulting in severe gut inflammation. Carbohydrates, such as mannan-oligosaccharide (MOS) have been shown to reduce the impact of these gut infections and can promote mucosal immune fitness, but the exact mode of action remains unclear. This study aimed to investigate the prebiotic effects of different novel carbohydrates on the microbial composition and its metabolites in the ceca, using an *in vitro* model mimicking necrotic enteritis in broilers. To study the effect of the potential prebiotic compounds, the bacterial composition of the caecum of broiler chickens was examined with the *in vitro* fermentation system Chicken ALImEntary tRact mOdel-2 (CALIMERO-2). This system was inoculated with caecal content collected at slaughter from healthy 35-day-old broilers and was spiked with *C. perfringens* to mimic the infection associated with necrotic enteritis. To study the effects on microbial composition and activity, product X and three different pectic polysaccharides were fed to CALIMERO-2. The bacterial composition was analysed by sequencing amplicons of the V3-V4 region of the 16S rRNA gene and was compared to the composition of the basic medium, i.e., standard ileal effluent media (SIEM), and the positive control MOS. Fermentative metabolites, i.e., branched- and short-chain fatty acids from CALIMERO-2, were quantified with ion exclusion chromatography. The sugar content of the pectins was determined by analysing the alditol acetate derivatives of the constituent monosaccharides, and the molecular weight by high-pressure size exclusion chromatography. There is a significant decrease in observed OTUs for the three pectins compared to SIEM. Product X shows a similar α-diversity pattern compared to MOS and SIEM. For the β-diversity, there is a significant difference between Product X and the other substrates, and also one of the pectins is significantly different compared to the other substrates. The monosaccharide composition of the pectins showed only small differences and does not fully explain the differences observed between the three pectins. At the genus levels, several genera have been identified that were specifically (and significantly) modulated by one or more of the substrates using non-parametric Kruskal-Wallis analysis with Benjamini-Hochberg FDR correction. Further deep characterization of the substrates, e.g., degree of blockiness and linkage pattern of the pectins, may explain some of the differences observed and may lead to structure-function relationships.

P33

SHORT CHAIN FATTY ACID INHIBITION OF BACTERIAL PLASMID CONJUGATION IN BROTH AND CHICKEN CAECA EXPLANTS

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The emergence and rapid spread of multidrug resistance (MDR) around the globe is an urgent concern for human and animal health. MDR emergence is driven by the direct transfer of plasmids from bacterium to bacterium. This process occurs rapidly and ubiquitously in the guts of animals and humans. As such, the inhibition of this process is of dire importance to curtail the spread of MDR, and interventions that target the process of plasmid transfer directly are desired. The probiotic derived short chain fatty acid (SCFA) metabolite class confer a wealth of host and microbial benefits, from increasing tight junction function and epithelial health to modifying the distribution of beneficial and deleterious microbes in the gut. However, their role in the process of bacterial plasmid conjugation is currently not understood. Here we aimed to test the effect of SCFA on the *in vitro* and *ex vivo* transfer of MDR and virulence plasmids of both broad (IncP-1) and narrow (IncFII, IncI1) host range incompatibility groups. Conjugations between donor *E. coli* (MG1655, APEC-O2-211) and the plasmidless *E. coli* (HS-4) strains were conducted in the presence (20 mM) or absence (0 mM) of SCFA at chicken ceca physiological levels for a period of six hours. Ceca explant conjugations utilized fresh ceca tissue from 14-day old commercial DeKalb white leghorn layer chickens. Following conjugation, donor, recipient, and transconjugant populations were enumerated on selective agar plates. In broth conjugation conditions, the addition of SCFA at physiological levels found in the chicken ceca resulted in a significant decrease ($p<0.005$) in enumerated transconjugant populations regardless of plasmid incompatibility type. Furthermore, the supplementation of SCFAs significantly ($p<0.005$) reduced the transfer of the avian associated narrow (IncFII) host range plasmid pAPEC-O2-211A-ColV, which confers resistance to multiple antibiotics, such as macrolides, nitroimidazoles, aminocoumarins, beta-lactams, aminoglycosides, tetracyclines, nitroimidazoles, aminocoumarins, and fluoroquinolones. Overall, this study demonstrates the potential role of the SCFA metabolite class in the reduction of horizontal gene transfer with minimal effect on commensal bacteria in the gut environment. Inhibition of the transfer of plasmids and their associated resistance to last resort antibiotics is of dire importance to combat the emergence and spread of MDR bacteria. Application of this approach has potential to lead to the reduction of AMR and virulence transfer and thus emergence of new bacteria in both agricultural animals as well as in the gut of humans.

P34

ANTIMICROBIAL COMPOUNDS PRODUCED BY AQUATIC LACTIC ACID BACTERIA INHIBIT THE GROWTH OF FOOD AND FISH PATHOGENS

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The aquatic environment has a significant impact on the microbiota of several animals, many of which are potential sources of biotechnologically important microorganisms. Alternative antibiotic-resistant pathogen control methods, such as bacteriocinogenic probiotic bacteria, have recently been considered a promising agent in this scenario. Bacterial strains isolated from aquatic environments were bioprospected for probiotic profiles and antimicrobial effects against fish and food pathogens in this study. Two isolates, identified via 16S rRNA sequencing as *Lactococcus lactis* (L1 and L2) and one as *Enterococcus faecium* 135 (EF), produced a bacteriocin-like antimicrobial substance (BLIS), active against *Listeria monocytogenes, Salmonella* Choleraesuis and *Salmonella* Typhimurium. The protein nature of the BLIS was confirmed by sensitivity to high temperatures and exposure to proteolytic enzymes. Moreover, the probiotic potential of both isolates was assessed by antibiotic-, virulence factor-, pH- and bile salts-resistance assays. Finally, bacteriocin genes were detected in total DNA extracted from L1, L2 and EF isolates. The molecular and physiological evidence indicates that all tested bacterial isolates could be used as natural antimicrobial agents and may be considered safe for probiotic application. All of the isolates investigated in this study had bacteriocin, demonstrated antimicrobial activity against important fish and food pathogens, were sensitive to all antibiotics tested, had a high rate of adherence to Caco-2 cells and did not express haemolysin as well as gelatinase virulence factors. It was shown that isolates L1 and L2 from rainbow trout were not able to resist low pH. However, isolates L2 and EF (from starfish) demonstrated good resistance to the action of bile salts, and EF was also resistant to pH 2.5 and 3. For this reason, future tests to evaluate the protective effect of microencapsulation on the viability of the isolates and their effect on an animal model will be carried out. There is no doubt that the new discoveries in the field of probiotics will bring countless changes in this area of study, which will result in ever higher quality foods and consumer health whilst having lower impacts on nature.

Acknowledgements
FAPESP (n° 2018/25511-1 and 2021/01570-1), CNPq (n° 312923/2020-1 and 408783/2021-4), FONDECYT/Postdoctoral (N° 3180765) and FONDECYT / Regular (Nº 1211246).

P35

FEEDING GERM-FREE DAMS WITH A YOGHURT ACTIVATING THE ARYL HYDROCARBON RECEPTOR INCREASES INTESTINAL GROUP 3 INNATE LYMPHOID CELLS IN THE OFFSPRING

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Among bioactive microbial compounds, metabolites from the tryptophan pathway, and in particular indole derivatives, are considered promising given their role in the gut immune homeostasis. Such property has been attributed to their ability to bind to the intestinal transcription factor aryl hydrocarbon receptor (AhR), modulating IL22 production by intestinal group 3 innate lymphoid cells (ILC3). Interestingly, it has been shown in mice that indole derivatives from the gut microbiota of a pregnant mother could reach the intestinal epithelium of the foetus and neonate and drive the early postnatal ILC3 development through activation of AhR. Apart from the gut microbiota, the production of such indole compounds has also been reported in milk fermented by lactic acid bacteria (LAB). Indole derivatives were found in fermented dairy foods (yoghurt or cheese), as well as in blood of individuals having consumed these products. The aim of our study was therefore to evaluate if feeding germ-free pregnant mice with a specific fermented product could activate the pups' intestinal innate immune system. To this end, a selection of 135 strains from Agroscope's collection were used to produce 135 yoghurts; their ability to activate AhR was evaluated *in vitro* and compared to that of a conventional yoghurt using HepG2-AhR-Luc cell line. The most efficient yoghurt, as well as a conventional yoghurt, were given as sterile pellets to germ-free pregnant mice (n=4) for 14 days and 10 days postnatal. The development of the innate immune system was evaluated in the pups (n=15) 14 days postnatal in the small intestine by flow cytometry. The experiment was replicated (n=4 mice, n=12 pups). After consumption of the test yogurt by the mothers, a significant increase in the frequency of NKp46+ILC3+ ILC3s was observed in the pups compared to the conventional yogurt. The result was confirmed in the replicated experiment. The selection of a specific LAB based on its ability to produce a fermented dairy product able to activate AhR was an effective approach to obtain a yoghurt with immunomodulatory properties. In pregnant germ-free mice, the intake of fermented dairy products can be considered as a source of bioactive microbial compounds leading to a modulation of the innate immune development in the pups.

P36
THE LIFE OF THE SKIN MICROBIOME UNDER STRESS

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Abrupt changes, such as UV exposure and the use of chemicals disturb the skin microbiome. In this discovery presentation, it is described how the impact of alcohol, preservatives, surfactants, and chemical peelings on the skin microbiota has been *in vivo* tested and quantified. The evolution of the skin microbiota after such a disturbance has also been monitored. A particle test was set up to evaluate the influence of UV light on the skin microbiota and how it evolves after this exposure. Besides evaluating a healthy skin microbiome, the surprising behaviour of the microbiota of acne-prone skin is demonstrated after washing. The supporting effect of 2 different prebiotics was quantified in all these distress situations. The results are translated into a concrete strategy to formulate skin microbiome-derived cosmetics.

P37
THE GATEWAY TO OUR BODY: THE ORAL MICROBIOME

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Our teeth and gums are protected by the synergy of saliva and a balanced oral microbiome. This presentation explores why we need to brush twice a day. How sugar and alcohol affect oral hygiene is demonstrated. The origin of carries and bad breath is explained through the oral microbiome. The oral microbiome goes beyond the protection of teeth and gums. It is also responsible for our total health. An out-of-balance oral microbiome is associated with many known chronic diseases such as diabetes, obesity, and many others. Many other unexpected influences of the oral microbiome are discussed. The use of a specially refined fructo-oligosaccharide in toothpaste, mouth wash, and chewing gum is tested under different destress circumstances. These different circumstances were sugar, soft drinks, and alcohol. The restoring and protective effect on the oral microbiome of this prebiotic is shown. These patented findings are a game-changer for future oral care strategies.

P38

FAECAL MICROBIOTA TRANSPLANTATION FROM AUTISTIC CHILDREN MODULATES BEHAVIOUR, INTESTINAL PARAMETERS AND INFLAMMATION IN BALB/c MICE

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Autism spectrum disorder (ASD) is a cluster of neurodevelopmental disorders characterized by impairments in communication and behaviour. Increasing evidence highlights the involvement of the microbiota-gut-brain axis in ASD. Gastrointestinal (GI) deficits and gut microbiota dysbiosis have been suggested to contribute to the development or severity of autistic behaviour. To validate the role of the gut microbiota in ASD and investigate mechanisms though which they interact with the gut and brain, we depleted and transplanted conventional BALB/cAnNCrl mice with stools of children diagnosed with ASD with GI symptoms or their siblings as control. After depletion of the murine gut microbiota with a cocktail of antibiotics (ampicillin (200 mg/kg), neomycin (200 mg/kg), metronidazole (200 mg/kg) and vancomycin (100 mg/kg)), bowel cleansing solution or the combination of both, 3-weeks old male BALB/cAnNCrl mice were transplanted either a pool of ASD+GI stools or sibling control stools for three consecutive days by gavage (200µl) (hFMT). Behavioural tests were performed to assess ASD-like phenotype. Here, we report that hFMT with ASD+GI stools decreased exploratory behaviour of the mice. Additionally, ASD+GI hFMT induced intestinal and systemic inflammation in recipient mice when compared to control transplanted mice. Moreover, hFMT with ASD+GI, but not sibling control stools resulted in an increased intestinal barrier permeability. Overall, our findings enlighten the alterations in the intestinal physiology and behaviour of mice transplanted with ASD+GI stools and provide evidence for hFMT as a promising tool to study ASD physiopathology or potential treatments.

Acknowledgements
The research was funded by European Commission by means of the Horizon 2020 program (call H2020-SC1-BHC-03-2018) with the project ID 825033.

P39

DEGRADATION OF DEOXYNIVALENOL (DON) AND ZEARALENONE (ZEN) TOXINS USING MICROBES FOR POTENTIAL APPLICATIONS IN ANIMAL FEED TO REDUCE THE CARRYOVER OF MYCOTOXINS FROM ANIMALS TO HUMANS

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Aflatoxins (AF), zearalenone (ZEN), fumonisins (FB1 and FB2), T-2/HT-2, ochratoxin A (OTA) and deoxynivalenol (DON) are considered the most economically important mycotoxins in terms of their high prevalence and significant negative effects on animal performance. DON is a type B trichothecene mycotoxin and ZEN is a potent estrogenic metabolite produced by several *Fusarium* species that co-infest maize, wheat, barley, and oats. Food and animal feed co-contaminated with DON and ZEN present a significant health risk for livestock and can enter humans via livestock-based foods. Among many remediation methods in animals, biological degradation of mycotoxins has shown promise because it works under mild and environmentally friendly conditions. In this research work, 5 soil samples were collected from different fields of alfalfa crop and pooled into one sample. Microbe, identified as *Devosia lucknowensis* by NCIMB (UK), was isolated by enrichment culture procedure using mineral salts medium and 1 ppm of DON as a sole carbon source. *D. lucknowensis* degraded 100% of DON in a 24-hour period at 30°C, under aerobic conditions. The microbe, was able to reduce DON at pH levels ranging from 6-9 and temperatures ranging from 30-40°C. DON reduction level was quantified using LC-MS/MS. For the isolation of ZEN degrading microbe, *Aerobium tamlense*, a soil sample was collected from a field of wheat and contaminated with ZEN rich material. Samples were taken every six days. After it was determined that the level of ZEN has been decreasing in a span of 2 months, an enrichment culture procedure has been started, by using mineral salts medium and 1 ppm of ZEN as a sole carbon source. *A. tamlense* degraded 100% of ZEN in a 2-hour period at 30°C, under aerobic conditions. The microbe was able to reduce ZEN at pH levels ranging from 4-9 and temperatures ranging from 30-40°C. ZEN reduction level was quantified using LC-MS/MS. Based on this data *D. lucknowensis* NCIMB 30593 and *A. tamlense* NCIMB 30596 have the potential for use in animal feed to reduce the absorption of DON and ZEN in animals and their carryover from animals to humans

P40

TOWARDS A REDUCED FEATURE SELECTION PIPELINE IN 16s rRNA MICROBIOME DATA USING MACHINE LEARNING

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The use of machine learning (ML) in the analysis of microbiome data is becoming more common due to the ability to process high dimensional data with small number of samples [1]. There are several pipelines for microbiome data analysis that allow researchers to perform statistical analysis to identify representative sequences related to a disease. Once these sequences are identified, researchers apply ML techniques to differentiate between groups, e.g., between healthy controls and patients [2]. Nevertheless, it has been shown that working with a small number of samples can cause overfitting. A proven solution to avoid overfitting is to use nested cross-validation [1] to produce robust and unbiased results regarding the number of samples. In this work, we propose a novel pipeline that combines DADA2 [3], which is an open-source package for modelling and correcting errors in Illumina-sequenced amplicon inferring sample sequences, and a Recursive Ensemble Feature Selection (REFS) [4,5], method to discover biomarkers, applied to 16s rRNA sequencing gut microbiome dataset PRJEB33711 [6]. The aim of the selected study [6] is to show that the gut microbiome of inflammatory bowel disease (IBD) patients is less diverse compared to healthy individuals. In [6], authors use DADA2 pipeline and phyloseq [7] to process and perform statistical analysis on 16s sequences identifying a substantial imbalance in four major bacterial phyla (*Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Actinobacteria*). As study [6] reported gut dysbiosis in IBD patients, we postulated that a novel ML approach could be used to analyse gut microbiome data for predictive IBD diagnostics. To test the effectivity of our proposed pipeline, we compared the classification accuracy of control group and IBD group using all sequences (features) in the four bacterial phyla identified in [6] (2,505 features) and the features selected in REFS (5 features – bacterial phyla *Bacteroidetes* and *Firmicutes*). The accuracy obtained by using the 5 features selected with REFS in a nested cross-validation (10-fold cross-validation) gives an area under the curve (AUC) of 0.818 in comparison to 0.5 obtained by using the 2,505 features reported in the original IBD study. The AUC helps us estimate the diagnostic accuracy of a given methodology [8]. Thus, an AUC in a 10-fold cross validation close to 1.0 is a successful discriminative test [8]. Our results point that REFS can be a suitable option in the analysis of microbiome data using DADA2 to build a pipeline to process the 16s rRNA sequencing data.

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P41

IDENTIFICATION OF FOOD SAFE MICROBIAL STRAINS WITH POSTBIOTIC POTENTIAL FOR APPLICATION IN FERMENTED FOOD PRODUCTS

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Postbiotics are a new category of biotics that have the potential to confer health benefits but, unlike probiotics, do not require living cells to induce health effects and thus are not subject to the food safety requirements that apply to live microorganisms. Postbiotics are defined as a “preparation of inanimate microorganisms and/or their components that confers a health benefit on the host” [1]. While research into postbiotics is in its infancy, there is increasing evidence that postbiotics have the potential to modulate human health. Chronic gut diseases, such as inflammatory bowel disease and irritable bowel syndrome, are characterised by inflammation and depleted barrier function in the gut and have been associated with oxidative stress resulting from the excessive production of reactive oxygen species (ROS) [2-4]. Due to their highly reactive nature, ROS can modify other oxygen species, DNA, proteins or lipids, and excessive amounts of ROS can cause genomic instability [5]. Antioxidants are compounds that scavenge oxygen free radicals or inhibit the oxidation process in a cell [6,7]. This investigation is apart of a wider project identifying food safe microbial strains with postbiotic potential that can be added to food products and when fermented provide a health benefit to the consumer. Specifically, the aim of this investigation was to assess the total antioxidant capacity of 128 probiotic lactic acid bacteria strains in order to identify the specific strain with the highest antioxidant capacity. The 128 strains were made up of 30 different species; therefore, the antioxidant capacity of different strains of the same species was assessed to determine the extent to which different strains of the same species have an antioxidant capacity. The total antioxidant capacity was determined using a colorimetric analysis assay where in the presence of an antioxidant Cu^{2+} ion is reduced by antioxidants to Cu^{+}. Specific strains of *Limosilactobacillus fermentum* and *Pediococcus pentosaceus* displayed very high antioxidant capacities compared with the other strains, including other strains of the same species. One of the most interesting findings of this investigation is that antioxidant capacity displayed strain-dependent variation, i.e., strains of the same species do not have similar antioxidant capacities. Different strains of the same species displayed strain dependent variation, which explains inconsistencies with literature and highlights the importance of analysing multiple strains of the same species in order to identify the strain with the optimal desired characteristics.

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P42

SURVIVAL OF *LACTICASEIBACILLUS PARACASEI* SUBSP*. PARACASEI* F-19® AND *BIFIDOBACTERIUM ANIMALIS* SUBSP*. LACTIS* BB-12® IN FERMENTED RED PITAYA PULP UNDER *IN VITRO* SIMULATED GASTROINTESTINAL CONDITIONS

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Pitaya has been increasingly cultivated and marketed in Brazil and has aroused great interest for its nutritional and functional properties, including antioxidant and prebiotic effects. The development of non-dairy foods with probiotics has become a demand mainly to meet the public with dietary restrictions related to dairy products. Moreover, selecting a suitable food matrix to be used as probiotic carrier is very important in the development of probiotic foods. This study aimed to evaluate the probiotic survival in fermented red pitaya pulp (RPP) under *in vitro* simulated gastrointestinal tract (GIT) stress. Two formulations were evaluated: RPP1 (RPP+ *Lacticaseibacillus paracasei* subsp*. paracasei* F-19®) and RPP2 (RPP + *Bifidobacterium animalis* subsp*. lactis* BB-12®). The fermented pulps were stored at 4±2°C for 28 days and the probiotic *in vitro* survival was evaluated on days 1, 14, and 28 of storage. Enumeration of F-19® and BB-12® was carried out in aliquots collected from triplicate samples of each digestion phase. Aliquots of 1 ml were pour-plated in suitable growth medium for each strain, and populations were counted after 72 h of anaerobic incubation at 37°C. Initial populations were 8.5 (day 1), 8.6 (day 14), and 8.8 (day 28) log CFU/ mL for RPP1, and 8.1 (day 1), 7.1 (day 14), and 6.9 (day 28) log CFU/ ml for RPP2. Comparing the survival of strains in freshly prepared probiotic cultures and fermented pulps (day 1), RPP promoted the F-19® resistance during passage through the GIT, since the counts after gastric and intestinal phases did not differ from the initial ($p>0.05$). This result was similar for all periods evaluated. However, in general, BB-12® populations showed a considerable reduction at the gastric and intestinal phases for all periods evaluated ($p<0.05$). The highest decrease was observed in the gastric phase on days 1 and 28 of storage (on average, 3.24 and 3.50 log CFU/ ml, respectively). Therefore, RPP may be considered good vehicle for F-19® and could play an important role in its protection against gastrointestinal juices.

Acknowledgements
Financial support: FAPESP (#2018/12190-2) and CNPq (#140487/2020-4).

P43

A PREDICTIVE MODEL FOR MICROBIOME-DEPENDENT RESPONSE TO DIETARY FIBRES BASED ON *IN VITRO* BIOLOGICAL DATA PREDICTS MICROBIOTA RESPONSE TO DIETARY INTERVENTION

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The intestinal microbiome is a dense and diverse microbial community and a key contributor to human health. One of the central roles of the microbiome is the breakdown of complex carbohydrates that are otherwise not digestible by the host. This breakdown is achieved through a metabolic cascade that is distributed across different microbial taxa. While microbiomes are frequently characterized using genomic techniques to enumerate the taxa, the mapping of the taxa onto the different functional steps in the metabolic cascade remains unclear, and this lack of clarity is one of the main obstacles impeding our ability to design microbiome interventions and therapies. We have developed a predictive platform, called NicheMap™, to map taxa onto the relevant functional niches of the intestinal carbohydrate fermentation. Our predictive model is based on *in vitro* data of whole human faecal microbiomes cultured in a panel of for gut microbes. This panel comprises complex dietary fibres, simpler carbohydrates, and typical intermediate fermentation products. By performing a comparative analysis across the different conditions, we identify the realized functional potential of intestinal 12 different representative carbon sources bacteria within their natural microbiome community. Specifically, we performed enrichment experiments in strict anaerobic batch fermentations with diluted faecal samples from human donors as inocula. By measuring the changes in microbial composition during enrichment along with the produced metabolites and performing comparative analysis across conditions, we were able to predict specialist taxa that were likely responsible for the specific metabolic conversions observed in the data. We challenged these predictions with data from a nutritional intervention study using one of the tested carbon sources—resistant dextrin—and found that our prediction of those bacteria which are specialist degraders of resistant dextrin matched with those taxa that increased most in abundance during the nutritional intervention study. The NicheMap™ is thus able to deliver predictions of how different human microbiomes will metabolize dietary fibres based on their taxonomic composition. Our platform is thus a powerful tool to predict the *in vivo* effect of candidate prebiotics.

P44

FAECAL MICROBIOTA DIVERSITY AND COMPOSITION ARE ASSOCIATED WITH ECTOPIC FAT IN ADULTS OF AFRICAN ANCESTRY IN THE TOBAGO HEALTH STUDY

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Storage of fat within and around muscle and internal organs is considered ectopic and metabolically pernicious. Gut microbiota is associated with overall obesity and with ectopic visceral fat; however, few microbiota studies include measures of ectopic fat in other anatomical locations. Differences in non-visceral ectopic fat depots explain some of the race/ethnic disparities in the burden of cardiometabolic disorders, which are particularly high in persons of African ancestry. The objective of this study is to examine associations of gut microbiota diversity and composition with pericardial, abdominal and thigh ectopic fats in adults of African ancestry. The Tobago health study is a longitudinal cohort of African Ancestry middle-aged and older men from the Caribbean Island of Tobago, Trinidad and Tobago. From 2014 to 2018, a subset of n=198 participants with complete chest, abdominal, and thigh computed tomography scans provided faecal samples for 16S V4 rRNA gene sequencing. We measured ectopic fat tissue (visceral, pericardial, and intermuscular (abdominal psoas, abdominal paraspinous, thigh muscles)) and intra-organ fat accumulation (psoas, paraspinous, and thigh muscle attenuation; liver attenuation). We examined associations of faecal microbiota diversity with BMI or ectopic fat measures using linear regression for microbiome alpha diversity (observed operational taxonomic units (OTUs), Pielou's evenness, Shannon diversity) and permutational analysis of variance (PERMANOVA) for Bray-Curtis dissimilarity. We then tested for differential abundance of bacterial OTUs using analysis of compositions of microbiomes with bias correction (ANCOM-BC). All models were adjusted for age, education, and hours walked per week for physical activity. Our analytic sample had a mean age of 62.2 years and BMI of 28.3 kg/m^2. Visceral fat was inversely associated with Shannon diversity and community evenness, and it explained ~1.5% of Bray-Curtis dissimilarity. Several OTUs associated with short chain fatty acid producing bacteria (e.g., *Lachnoclostridium*, *Acidaminococcus*, and *Roseburia*) were positively associated with multiple fat tissue and intra-organ fat accumulation measures; the greatest number of OTU associations were with paraspinous intermuscular fat, followed by visceral fat. Compared to visceral fat, BMI was similarly associated with diversity metrics, but BMI associations with OTUs were more attenuated. In conclusion, in a sample of adult African Caribbean men, faecal microbiota diversity was associated with visceral fat and BMI, while microbiota compositions were more associated with visceral and intermuscular fats. Longitudinal studies are needed to examine the contribution of microbiota to ectopic fat accumulation.

P45
THE GUT MICROBIOTA: MASTER OF PUPPETS CONNECTING THE EPIDEMIOLOGY OF INFECTIOUS, AUTOIMMUNE, AND METABOLIC DISEASE

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Infectious, autoimmune, and metabolic diseases put an enormous pressure on both quality of life and the economy. For all three disease types, it is known that the quality of the gut microbiota composition is correlated to both onset and progression of disease. As such, the epidemiological trends of these disease types may serve as proxies for the integrity of the human gut microbiota. Incidence data were retrieved from different public sources regarding prototypical infectious diseases (tuberculosis and measles), autoimmune disorders (type-1 diabetes and multiple sclerosis), and the prevalence of metabolic syndrome. The presented data covered incidences and vaccination coverage in 7 Western countries (Finland, France, Germany, Italy, the Netherlands, UK, and USA) from 1980 until now. It was revealed that prototypical autoimmune disease incidence and metabolic disorder prevalence are still steadily increasing. This is accompanied by a plateauing decline in prototypical infectious disease incidence. Moreover, the data show a strong association between the level of eradication of infectious disease and the population coverage of vaccination programs. The findings of this study suggest that the status of the gut microbiota is deteriorating, as reflected by the proxies. The epidemiological trends that were studied may serve as a starting point for a mechanistic understanding of the interplay between these different disease types that can be used for future prevention and mitigation strategies such as targeted stimulation and suppletion of microorganisms by means of, e.g., fermented foods, prebiotics, and probiotics.

Funding/conflict of interest
OL is also Senior Manager Science at Yakult Nederland B.V.

P46

PROBIOTICS FOR THE MANAGEMENT OF INFECTIOUS DISEASES: REVIEWING THE STATE OF THE ART

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Maintaining and potentially restoring the microbiota of humans is considered a vital aspect of health, protecting against many infectious and inflammatory diseases. Considering the rise of emerging infectious diseases like COVID-19, microbiota targeting interventions such as probiotics could be of great value to combat infectious diseases. The potential of probiotics as a clinical modality was investigated by creating an overview of the state of the art of research and development efforts as shown by patents and clinical trials since 1999. Data were retrieved from patent and clinical trial databases to reflect the long- and short-term developments of probiotics research. The data were analysed to extract information on the total number of patents and clinical trials for each indication, application date and location, and applicant/sponsor type. A total of 80 infectious diseases were investigated, precipitating in 789 patents and 602 clinical trials for 67 indications studied as targets of probiotic based interventions. An increasing trend was seen for the number of patents and clinical trials per year, with the highest number of patents and clinical trials targeted to digestive tract, respiratory, and urogenital indications. Overall, research demonstrated a substantial interest in probiotics targeting infectious diseases, which was in line with reported unmet needs and global probiotics market estimates. However, a declining rate of translation from patents to clinical trials was observed. This could indicate that there are some barriers obstructing the research process, which may be detrimental to probiotic innovation.

Funding/conflict of interest
The contribution of LB is financed by the project Preparedness for Emerging Infectious Diseases with project number VI.Veni.201S.044 of the research program Veni SGW, which is financed by the Dutch Research Council (NWO). The contribution of CW is financed by Yakult Nederland B.V. OL is also Senior Manager Science at Yakult Nederland B.V.

P47

MODULATION OF GUT MICROBIOTA COMPOSITION AND METABOLITES BY DIFFERENT DIETARY FIBRES AFTER *IN VITRO* FERMENTATION IN TIM-2

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The gut microbiota has a great impact on host health and metabolism. This community is greatly affected by host diet, with dietary fibres (DF) as key substrates. DF are carbohydrates with ≥10 monomeric units, which cannot be hydrolyzed by the endogenous enzymes of humans. However, DF can affect the gut microbiota and human health through different microbial metabolites produced from fermented substrates. In order to defining a structure-function relationship for DF, which would allow a rational choice of DF for health-promotion and disease-prevention, the aim of this study was to investigate the mechanistic connections between the gut microbiota composition with its ability to use DF as substrates. Therefore, inulin, pectin, and soluble maize fibre (SCF) were tested using a standardized pool of human faeces in a validated, dynamic, *in vitro* model of the colon (TIM-2). We investigated changes in gut microbiota composition and production of short-chain fatty acids (SCFA) and correlated these with the findings of the chemical characterization of DF. The chemical analysis of DF revealed contrasting structures, demonstrated by the different molecular weights, relative solubility, and monosaccharide composition. TIM-2 experiments showed that the different DF caused different α- and β-diversity changes in microbiota composition. For example, inulin increased the relative frequency (RF) of *Blautia*, *Ruminococcaceae UCG-014*, and *Faecalibacterium*, while pectin decreased the RF of *Blautia*, *Prevotella 9*, and *Bacteroides*. SCF decreased the RF of *Prevotella 9* and *Bacteroides*, but also increased the RF of *Blautia* and *Faecalibacterium*. This might be related to the genetic ability of bacteria to degrade these fibres. SCFA production was also altered by DF. Compared to the standard medium, cumulative acetate production was increased by SCF and pectin, propionate production was increased only by pectin and butyrate production was only increased by inulin. DF can influence RFs of specific bacteria, and their ability to produce SCFA are changed under the influence of different DF. The different DF modulate the gut microbiota differently, both in composition and activity, which may be strongly related to the structural differences of DF. Thus, our results illustrate that the chemical characteristics of DF can differently affect the gut microbiota, although, the lack of knowledge on very basic structures of DF, still preclude the detailed interpretation of results on gut microbiota research. The deep mechanisms underlying a structure-function relationship require further research in the future, which we intend to do by studying many more fibres, which is currently ongoing.

P48

REPORTING ON THE *IN VITRO* BATCH FERMENTATION OF (UN)SATURATED HOMOGALACTURONAN OLIGOSACCHARIDES

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Novel prebiotic compounds, designed for steering the gut microbiota towards a healthy composition, are in high demand as the relationship between gut microbiota and human health is becoming increasingly clear [1]. This study reports on the poorly understood structure-dependent *in vitro* fermentation of galacturonic acid oligosaccharides (GalA-OS), enzymatically derived from pectin and their possible use as a novel prebiotic. Treatment of low methyl esterified and high methyl esterified pectin with the enzymes polygalacturonase and pectin lyase respectively resulted in two series of GalA-OS up to a DP (degree of polymerization) of 10. Subsequent saponification of a portion of both materials using NaOH, which removes the native methyl-esters from the GalA-OS backbone, resulted in four series of OS differing in the presence of both methyl-esterification at C6 and a double bond between C4&C5. The double, unsaturated, bond present in two of the four series is caused by the β-elimination mechanism of pectin lyase, the enzyme used to produce GalA-OS from the high methyl esterified pectin substrate [2]. The four series of GalA-OS were fermented *in vitro* using three distinct human adult faecal inocula. Short chain fatty acid (SCFA) production, the metabolization of oligosaccharides by the gut microbiota and the level of methyl esterification were monitored over a 48h time period by HPLC, HPAEC and MALDI-TOF-MS respectively. Methyl-esterification was determined to be a major determinant of the efficient fermentation of GalA-OS, with methyl esterified GalA-OS significantly suppressing fermentation compared to non-methyl-esterified galacturonic acids of similar chain length and (un)saturation. Acetate, propionate, and butyrate were produced after fermentation of all oligomeric substrates by all donors, regardless of saturation or methyl-esterification. Preliminary results of 16S rRNA DNA sequencing suggests that *in vitro* fermentation of unsaturated GalA-OS significantly alters the microbiota composition compared to saturated oligosaccharides of similar degrees of methyl-esterification, highlighting the importance of the unsaturated GalA residue on GalA OS fermentation.

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P49

THE HUMAN MILK OLIGOSACCHARIDE 2'-FUCOSYLLACTOSE ALLEVIATES LIVER STEATOSIS, ER STRESS AND INSULIN RESISTANCE BY REDUCING HEPATIC DIACYLGLYCEROLS AND IMPROVED GUT PERMEABILITY IN OBESE LdIr-/-. LEIDEN MICE

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Non-alcoholic fatty liver disease (NAFLD) is a complex multifactorial disorder that is associated with gut dysbiosis, enhanced gut permeability, adiposity, and insulin resistance. Prebiotics such as human milk oligosaccharide 2'-fucosyllactose are thought to primarily improve gut health and it is uncertain whether they would affect more distant organs. This study investigates whether 2'-fucosyllactose can alleviate NAFLD development in manifest obesity. Obese hyperinsulinemic Ldlr-/-.Leiden mice, after an 8-week run-in on a high-fat diet (HFD), were treated with 2'-fucosyllactose by oral gavage until week 28 and compared to HFD-vehicle controls. 2'-fucosyllactose did not affect food intake, body weight, total fat mass or plasma lipids, but significantly decreased circulating pro-inflammatory factor MCP-1/CCL2. 2'-fucosyllactose altered the faecal microbiota composition, which was paralleled by a suppression of HFD-induced gut permeability at t=12 weeks. 2'-Fucosyllactose significantly attenuated the development of NAFLD by reducing microvesicular steatosis. These hepatoprotective effects were supported by upstream regulator analyses showing that 2'-fucosyllactose activated ACOX1 (involved in lipid catabolism), while deactivating SREBF1 (involved in lipogenesis). Furthermore, 2'-fucosyllactose suppressed ATF4, ATF6, ERN1, and NUPR1 all of which participate in endoplasmic reticulum stress. 2'-fucosyllactose reduced fasting insulin concentrations and HOMA-IR, which was corroborated by decreased intrahepatic diacylglycerols. In conclusion, long-term supplementation with 2'-fucosyllactose can counteract the detrimental effects of HFD on gut dysbiosis and gut permeability and attenuates the development of liver steatosis. The observed reduction in intrahepatic diacylglycerols provides a mechanistic rationale for the improvement of hyperinsulinemia and supports the use of 2'-fucosyllactose to correct dysmetabolism and insulin resistance.

P50
CURRENT POSTBIOTICS IN THE COSMETIC MARKET – AN UPDATE AND DEVELOPMENT OPPORTUNITIES*

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Postbiotics (or fermented cosmetics), a new class of health-promoting molecules, are defined by the International Scientific Association for Probiotics and Prebiotics (ISAPP) as a “preparation of inanimate microorganisms and/or their components that confers a health benefit on the host”. These molecules can be enzymes, polysaccharides, teichoic acid, as other metabolites and exert relevant biological effects for cosmetic and skincare applications. The most relevant benefits are immunomodulatory, anti-inflammatory, antioxidant, antimicrobial, anti-proliferative, and anti-aging activities, promotion skin microbiota equilibrium, skin enzymes activity inhibition, as well antimicrobial effect over opportunistic pathogen and skin infectious bacteria. Recently, other benefits, such as the treatment of dermatological diseases (e.g., *Alopecia areata*) have been scientifically proven for some postbiotics-based products. The main advantages identified for the use of postbiotics are related to their higher specificity of action on resident microbiota and interaction with cells of the host compared to probiotics. Besides that, postbiotics have longer shelf life than probiotics, greater safety and do not require viability in the topical formulation, which turns them into an innovative approach within the cosmetic ingredients market. Moreover, they also can be safely administered to immune-deficient or compromised patients for which live probiotics are not allowed. Postbiotics can be produced/obtained especially through fermentative processes, and major part of the commercial products available are derived from lactic acid bacteria, *Lactobacillus* genera and/or yeasts, especially *Saccharomyces cerevisiae*. Also, most of the postbiotics-based products present in the cosmetic market claims are based on their anti-aging effect, skin defence/barrier/immunity boost, skin regeneration, skin elasticity improvement, anti-wrinkles effect, positive skin microbiota modulation and antioxidant defences improvement, among others. The main players are companies that operate in several areas, such as food innovation, and chemical, pharmaceutical, and cosmetic industries, and the critical trends for production of these compounds include energy efficiency, emission-free mobility, conservation of finite resources and renewable raw material utilization. This communication intents to give an update on the major postbiotic products available at the market and identification of the major opportunities on this field.

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