THE 10TH BENEFICIAL MICROBES CONFERENCE

PRE- AND PROBIOTICS FOR LIFELONG Human AND Animal HEALTH

ABSTRACTS OF LECTURES & POSTERS

27-29 NOVEMBER 2023

AMSTERDAM

THE NETHERLANDS

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Secretariat
Bastiaanse Communication
P.O. Box 179
3720 AD Bilthoven
the Netherlands

T +31 30 2294247
BMC@bastiaanse-communication.com
www.BeneficialMicrobes2023.org

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Key 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

The Beneficial Microbes Conference series started in 2008. During the years, it has become an important meeting point for academia and industry providing a reference source for professionals who want to be updated on the advances in research on beneficial microbes (probiotics, prebiotics, postbiotics, synbiotics, fermented foods, …) for human and animal health across the lifespan. The conference promotes the creation of new initiatives for the customised application of beneficial microbes in food, feed, and healthcare in a networking environment.

The programme. The **10th Beneficial Microbes Conference** provides an overview of the latest scientific results and future developments related to beneficial microbes and their importance to human and animal health. Topics include the societal impact, the gut microbiome, the oral-gut microbiome axis, the gut-brain axis, the microbiome beyond the gut, the holomicrobiome (the interactions between the many microbiomes in humans, animals, plants, soil and water), and more.

The science battle. The science battle is a valued part of the conference. This year's science battle is about the (non-)sense of commercial microbiome testing. How to look at commercial microbiome testing from different perspectives? Two speakers will take a different side of the topic followed by a panel discussion and Q&A with the audience.

The power of networking. The **10th Beneficial Microbes Conference** is a networking event par excellence, the common theme being HOW CAN WE LEARN FROM EACH OTHER? The conference aims to bring together scientists with different expertise with the possibility of cross-fertilizing each other and to develop new views and applications. Take part and experience the power of networking.

CONFERENCE CHAIR

Prof. Koen Venema	Beneficial Microbes Consultancy, the Netherlands

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Dr Gabriel Vinderola	Instituto de Lactología Industrial, Universidad Nacional del Litoral, Argentina

PROGRAMME AT A GLANCE

MONDAY 27 NOVEMBER 2023

12:45 – 13:00	Opening of the **10th Beneficial Microbes Conference**
13:00 – 14:00	Plenary session *Beneficial microbes – What's up, Doc?*
14:00 – 15:45	Plenary session *Societal impact of beneficial microbes*
15:45 – 16:15	Networking break
16:15 – 17:30	Plenary session *The gut microbiome and beneficial microbes*
17:30 – 17:55	Speed presentations *Short presentations by selected poster presenters*
18:00 – 19:00	Happy hour

TUESDAY 28 NOVEMBER 2023

08:45 – 10:05	Session 1 *Why should human microbiome scientists be interested in animal studies?*	Session 2 *Oral-gut microbiome axis*
10:05 – 10:30	Poster tour	
10:30 – 11:00	Networking break	
11:00 – 12:35	Session 3 *Beneficial microbes in animal health and nutrition*	Session 4 *Beneficial microbes, human health & well-being*
12:35 – 14:00	Lunch break	
14:00 – 15:20	Session 5 *Fermented foods and health*	Session 6 *Beneficial microbes and the gut-brain axis*
15:20 – 15:45	Poster tour	
15:45 – 16:15	Networking break	
16:15 – 17:30	Plenary session *The battle: (Non-)sense of commercial microbiome testing*	

WEDNESDAY 29 NOVEMBER 2023

08:45 – 10:45	Plenary session *The focus on prebiotics and synbiotics*
10:45 – 11:15	Networking break
11:15 – 12:30	Plenary session *The holomicrobiome – beneficial microbes from farm to fork*
12:30 – 12:40	Poster award ceremony
12:40 – 13:00	Lessons learned
13:00	Closing of the **10th Beneficial Microbes Conference**

MONDAY 27 NOVEMBER 2023

12:45 Introduction to the **10th Beneficial Microbes Conference**
Prof. Koen Venema, conference chair

PLENARY SESSION: BENEFICIAL MICROBES – WHAT'S UP, DOC?

Chair: Prof. Koen Venema, Beneficial Microbes Consultancy, the Netherlands

13:00 *One Health, Old Friends, and the gut microbiota*
Prof. Olaf Larsen, Athena Institute, VU Amsterdam and Yakult Nederland, the Netherlands

13:20 *From the use of probiotics in gut health to new perspectives in humans: focus on mental and women's health*
Dr Mélanie Le Barz, Lallemand Health Solutions, France

13:40 *Beyond the brain: microbial-derived metabolites as an early indicator of age-related cognitive decline and dementia*
Prof. David Vauzour, Norwich Medical School, University of East Anglia, UK

PLENARY SESSION: SOCIETAL IMPACT OF BENEFICIAL MICROBES

Chair: Prof. Koen Venema, Beneficial Microbes Consultancy, the Netherlands

14:00 *Democratizing beneficial microbes – Its time to bring real world science to the consumers*
Dr Adam Baker, Chr. Hansen, Denmark

14:20 *Probiotics in perspective*
Dr Anne van der Geest, Athena Institute, VU Amsterdam, the Netherlands

14:40 *Fine-scale mapping of the vaginal microbiome with citizen science*
Dr Sarah Ahannach, Department of Bioscience Engineering, University of Antwerp, Belgium

14:55 *What impact will revisions to EU regulatory frameworks have on R&D programs for microbiome-based medicinal products?*
Dr Céline Druart, Pharmabiotic Research Institute, France

15:15 *The power of networking*
Michiel Hesseling, MBTM, the Netherlands

15:45 **Networking break**

MONDAY 27 NOVEMBER 2023

PLENARY SESSION: THE GUT MICROBIOME AND BENEFICIAL MICROBES

Chair: Prof. Sarah Lebeer, University of Antwerp, Belgium

16:15 *Diet-host-microbe interaction in preterm infants*
Dr Andrea Masi, Translational and Clinical Research Institute, Newcastle University, UK

16:35 *Human milk bacteria individually or as a synthetic community exhibited contrasted immunomodulatory profiles and impact on the gut epithelial barrier*
Charles Le Bras, Research Unit Science and Technology of Milk and Eggs (STLO), INRAE, France

16:50 *Revolutionizing microbiome research: Unleashing the power of machine learning*
Dr Laura Judith Marcos-Zambrano, Computational Biology Group, IMDEA Food Institute, Spain

17:10 *Space Odyssey 2023: exploring temporal and regional metabolomes in the human gut*
Prof. Oliver Fiehn, West Coast Metabolomics Center and Department of Food Science and Technology, University of California, Davis, USA

PLENARY SESSION: SPEED PRESENTATIONS
Short presentations (5-minutes) by selected poster presenters

Chair: Prof. Koen Venema, Beneficial Microbes Consultancy, the Netherlands

17:30 – 18:00

- *Development of a synbiotic mixture to decrease intestinal neuroinflammation and restore short-chain fatty acid production in Parkinson's disease patients*
  Dr Charlotte De Rudder, University of Luxembourg, Luxembourg
- *Synbiotics, a promising approach to reduce the exacerbated allergic airway immune responses in offspring maternally exposed to cigarette smoke*
  Dr Ali Dehghani, Utrecht University, the Netherlands
- *Christensenella minuta alleviates psychiatric and cardiac alterations induced by early life stress in mice*
  Dr María Tamayo, IATA-CSIC, Spain
- *Engineering lactic acid bacteria as delivery vehicles for Clostridioides difficile antitoxin proteins*
  Abida Zahirović, Jožef Stefan Institute, Slovenia

18:00 – 19:00
Happy hour – drinks & snacks

TUESDAY 28 NOVEMBER 2023

SESSION 1: WHY SHOULD HUMAN MICROBIOME SCIENTISTS BE INTERESTED IN ANIMAL STUDIES?

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

08:45 *Bovine animal model for studying the role of microbiome in the developmental origins of health and disease*
Dr Samat Amat, Department of Microbiological Sciences, North Dakota State University, USA

09:05 *NODding to growth: Lactiplantibacillus plantarum*[WJL] *supports mouse juvenile growth in chronic undernutrition*
Dr Martin Schwarzer, Laboratory of Gnotobiology, Institute of Microbiology of the Czech Academy of Sciences, Czech Republic

09:25 *From the unique properties and specific metabolism of Saccharomyces boulardii cncm i-1079 to probiotic effects beyond gut microbiota modulation: What impact on host metabolism and immunity?*
Dr Caroline Achard, Lallemand Animal Nutrition, France

09:45 *Why mammalian milk contains lactose*
Prof. Richard Ducatelle, Department of Pathology, Pharmacology and Zoological Medicine, Ghent University, Belgium

10:05 **Poster tour**
Presenting authors are requested to be available at their posters during the Poster Tour. A Best Poster Award is given to the best poster presented at the conference. It rewards a combination of excellent research, innovation, and presentation. The conference participants will vote for the best poster. The winner will be recognized publicly at the final plenary session.

10:30 **Networking break**

SESSION 3: BENEFICIAL MICROBES IN ANIMAL HEALTH AND NUTRITION

Chair: Dr Frédérique Chaucheyras-Durand, Lallemand SAS and Clermont Auvergne University, INRAE, France

11:00 *Visualising and quantifying rumen microbiome responses to live microbials and prebiotics*
Dr Greta Reintjes, Microbial-Carbohydrate Interactions Group, University of Bremen, Germany

11:20 *Response of the gut-liver axis to stimulation during embryonic development in chickens*
Dr Aleksandra Dunisławska, Department of Animal Biotechnology and Genetics, Bydgoszcz University of Science and Technology, Poland

11:40 *Prebiotic galacto-oligosaccharide feed enhances broiler chicken productivity and Salmonella clearance*
Prof. Ian Connerton, School of Biosciences, Nottingham University, UK

12:00 *Development of probiotic feed for salmonids with immunomodulatory potential*
Prof. Dagmar Mudroňová, Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy, Slovakia

12:20 *Probac-seq technology, a powerful tool to screen new solutions impacting necrotic enteritis toxin B-like toxin and C. perfringens cytotoxicity*
Dr Marion Bernardeau, IFF, France

12:35 **Lunch break**

TUESDAY 28 NOVEMBER 2023

SESSION 2: ORAL-GUT MICROBIOME AXIS

Chair: Prof. Michiel Kleerebezem, Wageningen University & Research, the Netherlands

08:45 *Oral microbiome: a friend or a foe?*
Prof. Egija Zaura, Department of Preventive Dentistry, Academic Centre for Dentistry Amsterdam, the Netherlands

09:05 *The oral-gut microbiome axis in health and disease*
Dr Benoît Kunath, Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Luxembourg

09:25 *Gut-mouth axis in periodontitis*
Dr Jun-ichi Nagao, Department of Functional Bioscience, Fukuoka Dental College, Japan

09:45 *Metagenomic analysis of the gut and oral microbiomes reveals cross-cohort signatures to predict pancreatic cancer*
Dr Suguru Nishijima, Structural and Computational Biology Unit, European Molecular Biology Laboratory, Germany

10:05 **Poster tour**
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10:30 **Networking break**

SESSION 4: BENEFICIAL MICROBES, HUMAN HEALTH & WELL-BEING

Chair: Prof. Koen Venema, Beneficial Microbes Consultancy, the Netherlands

11:00 *The potential of Dolosigranulum pigrum ambr11 as beneficial microbe for respiratory health*
Dr Ilke De Boeck, Department of Bioscience Engineering, University of Antwerp, Belgium

11:20 *Extracellular vesicles and surface layer proteins as the post-biotic active ingredient of the probiotic bacterium Propionibacterium freudenreichii against colitis and mucositis*
Dr Gwénaël Jan, Research Unit Science and Technology of Milk and Eggs, INRAE, France

11:40 *Interactions of microplastics with the human gut microbiota of adults and infants using in vitro gut models*
Dr Elora Fournier, Laboratoire des Adaptations Métaboliques à l'Exercice en conditions Physiologiques et Pathologiques, Université Clermont Auvergne, France

12:00 *Uncovering the mechanisms of Lactiplantibacillus plantarum mediated type I interferon induction: Role of immunomodulatory surface proteins*
Selvin Solis, School of Biosciences, University of Surrey, UK

12:20 *Daily lactose supplementation in lactase non-persistent individuals induces colonic adaptation and reduces intolerance symptoms*
Dr Lonneke Janssen Duijghuijsen, Wageningen Food and Biobased Research, Wageningen University & Research, the Netherlands

12:35 **Lunch break**

TUESDAY 28 NOVEMBER 2023

SESSION 5: FERMENTED FOODS AND HEALTH

Chair: Dr Jiro Nakayama, Kyushu University, Japan

14:00 *Fermented foods and health, an industry perspective*
Dr. Janneke Ouwerkerk, NIZO food research, the Netherlands

14:20 *Harnessing microbiome data to create the next generation of fermented foods*
Dr Paul Cotter, Department of Food Biosciences, Teagasc, Ireland

14:40 *Fermented dairy and legume-based food products: From smart design of lactic acid bacteria to innovative products*
Dr Valérie Gagnaire, Research Unit Science and Technology of Milk and Eggs (STLO), INRAE, France

15:00 *Raw milk and raw milk kefir for the dietary management of allergic diseases*
Prof. Johan Garssen, Utrecht University and Danone Nutricia Research, the Netherlands

15:20 **Poster tour**
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15:45 **Networking break**

PLENARY SESSION: THE BATTLE – (NON-)SENSE OF COMMERCIAL MICROBIOME TESTING
How to look at commercial microbiome testing from different perspectives? Two speakers will take a different side of the topic followed by a panel discussion and Q&A with the audience.

Chair: Prof. Koen Venema, Beneficial Microbes Consultancy, the Netherlands

16:15 Chair's introduction to the debate

16:20 The relevance of microbiome testing for the consumer
Dr Eline Klaassens, BaseClear, the Netherlands

16:35 Is microbiome testing genuinely helpful for the consumer?
Prof. Karen Scott, The Rowett Institute, University of Aberdeen, UK

16:50 Debate and Q&A with the audience

17:30 **End of day 2**

TUESDAY 28 NOVEMBER 2023

SESSION 6: BENEFICIAL MICROBES AND THE GUT-BRAIN-AXIS

Chair: Prof. Michiel Kleerebezem, Wageningen University & Research, the Netherlands

14:00 *Modulation of the maternal microbiota and behavioural effects in the offspring*
Dr Daniel Radford-Smith, Department of Pharmacology, University of Oxford, UK

14:20 *Gut microbiome composition and functionality impact the responsiveness to a dairy-based product containing galacto-oligosaccharides for improving sleep quality in adults*
Dr Arjen Nauta, FrieslandCampina, the Netherlands

14:40 *Early-life gut microbiome and neurodevelopmental outcomes*
Dr Rochellys Diaz Heijtz, Department of Neuroscience, Karolinska Institutet, Sweden

15:00 *Disorders of the gut-brain axis as a target for probiotics: Insight into developments of the Industry*
Cato Wiegers, Athena Institute, VU Amsterdam, the Netherlands

15:20 **Poster tour**
Presenting authors are requested to be available at their posters during the Poster Tour. A Best Poster Award is given to the best poster presented at the conference. It rewards a combination of excellent research, innovation, and presentation. The conference participants will vote for the best poster. The winner will be recognized publicly at the final plenary session.

15:45 **Networking break**

PLENARY SESSION: THE BATTLE – (NON-)SENSE OF COMMERCIAL MICROBIOME TESTING
How to look at commercial microbiome testing from different perspectives? Two speakers will take a different side of the topic followed by a panel discussion and Q&A with the audience.

For details, see page 8.

WEDNESDAY 29 NOVEMBER 2023

PLENARY SESSION: THE FOCUS ON PREBIOTICS AND SYNBIOTICS

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

08:45 *Vitamins and effects on the gut microbiome*
Dr Robert Steinert, dsm-firmenich, Switzerland

09:05 *Polyphenol-rich extracts from olive leaves modulate gut microbiota composition and metabolism in the in vitro MicroColon model, and show protective effects on the intestinal barrier function*
Dr Guus Kortman, NIZO food research, the Netherlands

09:25 *Prebiotics for the athlete and active consumer – the evidence and current state of play*
Dr Neil Williams, Sport Health and Performance Enhancement Research Centre, Nottingham Trent University, UK

09:45 *Human milk oligosaccharides, infant gut microbiome and health: Results from the HELMi birth cohort*
Dollwin Matharu, Human Microbiome Research Program, University of Helsinki, Finland

10:05 *Modifications of the raffinose family oligosaccharides profile in peas by plant breeding influence the human gut microbiota structure and function*
Aryana Zardkoohi, Quadram Institute, UK

10:25 *Effects of synbiotics in children and adolescents with obesity*
Prof. Ener Cagri Dinleyici, Department of Pediatrics, Eskisehir Osmangazi University, Türkiye

10:45 **Networking break**

PLENARY SESSION: THE HOLOMICROBIOME – BENEFICIAL MICROBES FROM FARM TO FORK
In our food system, the many separate microbiomes together form one large and tight network: a 'holomicrobiome'. Knowledge about the interaction of all the contributing parts will lead to new and better products for agriculture, food production and healthcare, and thus to a society with better health and more sustainability.

Chair: Prof. Koen Venema, Beneficial Microbes Consultancy, the Netherlands

11:15 *Beyond holobiont: Microbiome interconnectedness throughout environments*
Dr Tanja Kostić, Center for Health and Bioresources, AIT Austrian Institute of Technology GmbH, Austria

11:40 *Soil microbiome and soil health as basis of crop quality and gut health*
Dr Emilia Hannula, Institute of Environmental Sciences, Leiden University, the Netherlands

12:05 *Does the (agricultural) soil microbiome affect human health?*
Prof. Harro Bouwmeester, Swammerdam Institute for Life Sciences, University of Amsterdam, the Netherlands

12:30 **Poster Award Ceremony**

12:40 **Lessons learned**
Prof. Koen Venema, conference chair

13:00 Closing of the **10th Beneficial Microbes Conference**

TAKE YOUR PACKED LUNCH TO EAT ALONG THE WAY!

LECTURE ABSTRACTS

PLENARY SESSION
BENEFICIAL MICROBES – WHAT'S UP, DOC?

ONE HEALTH, OLD FRIENDS, AND THE GUT MICROBIOME

Olaf Larsen

Athena Institute, VU Amsterdam and Yakult Nederland, the Netherlands

olarsen@yakult.nl

Adequate gut microbiota management requires a One Health approach. In this presentation, epidemiological data on both communicable and non-communicable diseases will be presented. New data on macronutrient intake will also be presented, demonstrating that these data are not sufficient for an adequate explanation of the rise in metabolic disorders. Altogether, the data will be put in the perspective of a deterioration of the gut microbiota quality which calls for, among others, a targeted intervention using keystone microbial taxa and guilds. Nutrition can be one of the interventional modalities towards such restoration of the gut microbiota quality but suffers from an inherently low effect size. Therefore, results will be presented focusing on optimizing the statistical power for clinical trials using nutritional intervention. To facilitate a more rational approach towards gut microbiota intervention, a regiment will be proposed that carefully balances between a one-size-fits-all intervention and the supplementation of future personalized microbial consortia. As a first step towards such interventions, computer simulations estimating the ecological dimensions of these future personalized microbial consortia will be presented.

FROM THE USE OF PROBIOTICS IN GUT HEALTH TO NEW PERSPECTIVES IN HUMANS: FOCUS ON MENTAL AND WOMEN'S HEALTH

Mélanie Le Barz

Lallemand Health Solutions, France and Rosell® Institute for Microbiome and Probiotics, Canada

mlebarz@lallemand.com

Diet is recognized, since several decades, as a major influence on general physiological functions and maintenance of overall health. It is also well-known that the gastrointestinal tract and the microorganisms that populate these ecological niches – the microbiome - represent the first surface of interactions between the environment and our endogenous system. In individuals suffering from various types of pathologies, the composition and functions of the intestinal microbiome may be altered. Originally, in the 1930s, beneficial bacteria – today's probiotics – were used as food supplements to improve digestive disorders such as diarrhoea and even a mood disorder named melancholia. Through numerous scientific advances, including increasingly advanced technologies to analyse ever more complex model systems, it has been demonstrated that some variations in the intestinal microbiome could be associated with metabolic disorders, neurodegenerative diseases, sensation of well-being, infant development, decline in the elderly, and environmental changes, among others. These findings open up new fields of interest and numerous novel applications for probiotics.

This presentation will focus on two booming markets: (i) the beneficial effects of probiotics in mental health, with the specific history of our documented psychobiotic Cerebiome® and future research perspectives; and (ii) the use of probiotics in women's health, from the balance of vaginoma versus vaginosis to new innovative, personalized applications and solutions.

BEYOND THE BRAIN: MICROBIAL-DERIVED METABOLITES AS AN EARLY INDICATOR OF AGE-RELATED COGNITIVE DECLINE AND DEMENTIA

David Vauzour

Norwich Medical School, Faculty of Medicine and Health Sciences, University of East Anglia, UK

d.vauzour@uea.ac.uk

Due to our ageing population, incidences of cognitive decline and dementia are on the rise, with estimates suggesting up to 153 million cases worldwide by 2050. In the absence of effective therapeutic interventions, identifying novel risk factors assisting in the early detection and prevention of disease progression becomes increasingly vital. Nutrition has emerged as a key influencer of cognitive function. Dietary compounds can shape gut microbiota composition, as well as provide vital precursor molecules to form key host and microbial-derived metabolites affecting the central nervous system. Microbial-derived metabolites, as well as neural, endocrine and immune pathways, form the bidirectional communication systems of the microbiota-gut-brain axis. Accumulating evidence indicates alterations in gut composition (i.e., dysbiosis) occur during preceding stages of dementia, such as mild cognitive impairment, and Alzheimer's disease (AD). Dysbiosis can dysregulate the microbiota-gut-brain axis, altering the production of bioactive metabolites to toxic concentrations, promoting neuroinflammation, neural injury and ultimately cognitive decline.

This presentation will look at the metabolites currently linked to cognitive health and how they may represent a novel panel of risk factors for the early identification of AD. Together these gut-derived metabolites may highlight early metabolic mechanisms contributing to AD progression, having critical clinical implications in today's ageing society.

PLENARY SESSION
SOCIETAL IMPACT OF BENEFICIAL MICROBES

DEMOCRATIZING BENEFICIAL MICROBES – ITS TIME TO BRING REAL WORLD SCIENCE TO THE CONSUMERS

Adam Baker

Chr. Hansen, Denmark

dkadb@chr-hansen.com

During the last decade here has been an explosion in research and understanding of what the microbiome is and how important it is throughout our life. We know more now about how important and what different roles microbiomes can play at different stages in our lives. This expanding knowledge has led to an increase in the health areas that are now connected and linked with microbiome research. Central to this the gastrointestinal microbiome is now considered to have fundamental roles in our health.

We will discuss the science that is being developed and the technologies that are being used and will be used to help us learn more about the microbiome and how beneficial microbes can play an important role in our health. Moreover, we will discuss the use of real-world data and consumer focused science in terms of microbiome and One Health.

PROBIOTICS IN PERSPECTIVE

Anne van der Geest

Athena Institute, VU Amsterdam, the Netherlands

a.m.vander.geest@vu.nl

Probiotics, defined as 'live microorganisms, which when administered in adequate amounts, confer a health benefit on the host,' have shown associations with various health benefits, including reduced pain and symptom severity for individuals with irritable bowel syndrome, prevention of antibiotic-associated diarrhoea, lower risk of food allergies, and prevention of necrotizing enterocolitis in low-weight preterm infants. Despite numerous studies indicate the potential of probiotics in addressing unmet medical needs, there remains a gap in understanding their adoption within primary healthcare. To bridge this knowledge gap, we studied the use of probiotics in clinical setting, evaluated the prevalent safety and efficacy assessment tools, and delved into the considerations of healthcare professionals (HCPs) when advising probiotics to their patients.

More than half of general practitioners and over 90% of dietitians recommend probiotics. HCPs recommend probiotics for a broad spectrum of conditions, frequently for gastrointestinal issues but also for less apparent conditions such as allergies, vaginal complaints, and mental stress. It is noteworthy that significant uncertainty exists among these HCPs regarding whether or not to continue advising probiotics. This suggests that advisory percentages may fluctuate over time. Although the effectiveness of probiotics for specific indications partly relies on product choice, up to 43% of HCPs reported experiencing difficulty in selecting a probiotic product. Following the evaluation of probiotics' safety and efficacy, we observed significant heterogeneity in both probiotic formulations and irritable bowel syndrome (IBS) patient populations. This raises a valid concern about the feasibility of employing a generalization-based method, such as a meta-analysis, to assess the efficacy and safety of these interventions. Furthermore, we noted inconsistencies in safety reporting, adding complexity to the establishment of a comprehensive safety profile for probiotic interventions in the context of irritable bowel syndrome (IBS). This complicates the ability of HCPs to make informed decisions. Notably, HCPs express concerns about the RCTs outcomes' relevance to their diverse patient populations, as they may not reflect the homogeneity seen in RCTs. Subsequently, HCPs emphasize the importance of research conducted in real-life settings, suggesting user experience research could be valuable to HCPs for evaluating the perceived effectiveness and safety of probiotics. HCPs' practices are influenced by a combination of considerations, including the recognition of individual patients' needs and preferences, reliance on the best available research evidence, and their own clinical expertise. However, there appear to be challenges in reconciling these factors at times, suggesting that balancing these considerations can be complex.

To date, the lack of recognition by authorities, such as the European Food Safety Authority (EFSA), has posed challenges hindering the full realization of the potential of probiotic innovations. Nevertheless, our study demonstrated that HCPs are increasingly adopting probiotic interventions in their practice, even in the absence of formal regulations or guidelines. In summary, we argue that probiotics, once considered a niche innovation, are now gaining widespread acceptance in the primary healthcare setting, reflecting a shift in the way HCPs think, organize, and act.

FINE-SCALE MAPPING OF THE VAGINAL MICROBIOME WITH CITIZEN SCIENCE

Sarah Ahannach[1], T. Gehrmann[1], S. Wittouck[1], T. Eilers[1], S. Condori[1], J. Dillen[1], L. Vander Donck[1], G. Donders[2,3,4], V. Verhoeven[5] and S. Lebeer[1]

[1] Department of Bioscience Engineering, Research Group Environmental Ecology and Applied Microbiology, University of Antwerp, Belgium
[2] Department of Obstetrics and Gynaecology, University Hospital Antwerp, Belgium
[3] Regional Hospital Heilig Hart, Belgium
[4] Femicare, Clinical Research for Women, Belgium
[5] Department of Family medicine and Population Health (FAMPOP), University of Antwerp, Belgium

sarah.ahannach@uantwerpen.be

As the cornerstone of women's health and reproduction, the vaginal microbiome is steadily gaining more attention from both the general population and the global microbiome research field. In contrast to the gut, interindividual variations in the vaginal microbiome have remained largely under-investigated in large-scale population studies outside the clinic. This lack of a reference framework hampers much-needed innovations in diagnostics and therapeutics. We therefore launched the Isala project in Belgium (https://isala.be/en/). To engage sufficient women to donate vaginal samples and provide intimate information via large questionnaires, we organized a citizen science-based campaign that included an elaborate communication plan for optimal commitment and community creation. Next, we remotely mapped the vaginal microbiome of 3345 women with 16S rRNA amplicon sequencing and additional metagenomics.

Here, we found that 78% of the swabs were dominated by *Lactobacillus* taxa, most notably *Lactobacillus crispatus* and *Lactobacillus iners*. In 15% of the women, these species co-occurred in similar amounts demonstrating a continuum in the vaginal microbiome and arguing against previously described suggestions of discrete community state types. We further found that most vaginal taxa show small to moderate positive or negative abundance correlations with other taxa and that positively interacting vaginal taxa can be summarized by grouping them into four modules (i.e., *L. crispatus-, Gardnerella-, Prevotella-,* and *Bacteroides*-modules). Interestingly, we found that the *Limosilactobacillus* genus was prevalent in almost 50% of the vaginal samples and positively correlated with *L. crispatus* and *L. jensenii*. In addition, we found that a small but significant part of the vaginal microbiome is associated to lifestyle and life course events, suggesting a potential to improve vaginal health through lifestyle interventions. In particular, our results indicate that besides age, previous pregnancies displayed the strongest association with the vaginal microbiome as well as the phase in the menstrual cycle. But also BMI, sexual intercourse, menstrual hygiene products, sleep and dietary habits were associated with the vaginal microbiome diversity and particular modules.

Finally, we highlighted that given conscious communication tools and style, women are eager to participate in taboo-breaking conversations as well as scientific studies aimed at improving their health. We therefore endorse citizen science as a powerful approach to facilitate large-scale intimate microbiome research and challenge the scientific status quo by proposing novel insights in the vaginal microbiome constellation. In addition, it empowers citizens to impact their individual and community-level health by promoting open science-based communication on common taboo subjects.

WHAT IMPACT WILL REVISIONS TO EU REGULATORY FRAMEWORKS HAVE ON R&D PROGRAMS FOR MICROBIOME-BASED MEDICINAL PRODUCTS?

Céline Druart

Pharmabiotic Research Institute, France

celine@pharmabiotic.org

The microbiome's importance in human health has been established and the first proofs-of-concept of the possibility to treat pathologies by acting on the microbiome have recently manifested in the marketing approval of medicines based on or targeting faecal (US and Australia) and vaginal microbiomes (EU). These products open a path for a myriad of drug developments in other pathologies. With these recent approvals, microbiome samples are now recognized more than ever as a source of innovative treatments, and the current regulatory developments are helping to clarify the regulatory pathway to market authorization. Indeed, human microbiome samples will be included in the scope of the new European regulation on standards of quality and safety for substances of human origin (SoHO) intended for human application, which is expected to come into force in 2026. The inclusion of human microbiomes within the scope of this new SoHO regulation means that human microbiomes used as SoHO preparation and/or starting material for the production of microbiome-based medicinal products will have to follow the standards and requirements set by this new SoHO regulation. This proposal is currently under discussion by the Council and the European Parliament.

Concomitantly, the European General pharmaceutical legislation is also under revision. In the context of microbiome-based medicinal product development, this piece of legislation will be of the utmost importance as it will specify the interplay between the SoHO regulation and Pharma legislation, including the definition of 'SoHO-derived medicinal products other than ATMPs'. Indeed, it is likely that microbiome-based medicinal products will fall into this new category of medicinal products. In this presentation, we will summarize the impacts these revisions to the EU regulatory frameworks will have on R&D programs for microbiome-based medicinal products. We will also emphasize the importance for all the actors involved in microbiome-based medicinal product development to follow the regulatory developments and be prepared for the major anticipated changes induced by the revision of these 2 key European legislations.

THE POWER OF NETWORKING

Michiel Hesseling

MBTM, the Netherlands

michiel@mindpepper.nl

In today's world, networking is a necessity. A mountain of research shows that professional networks lead to more business opportunities, broader and deeper knowledge, improved capacity to innovate, and faster advancement. Are you open to build and nurture professional relationships?

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DIET-HOST-MICROBE INTERACTION IN PRETERM INFANTS

Andrea Masi

Translational and Clinical Research Institute, Newcastle University, UK

andrea.masi@newcastle.ac.uk

Preterm infants face an increased risk of abnormal microbial colonization in their gut, potentially leading to conditions like necrotizing enterocolitis (NEC). Probiotics are being increasingly employed in neonatal intensive care to encourage the colonization of beneficial bacteria. To gain a deeper understanding of the influence of probiotics on the preterm gut microbiome, we conducted metagenomic sequencing on a vast dataset of stool samples (comprising 1,431 samples) collected over time from 123 preterm infants born before 32 weeks of gestation, all of whom did not develop intestinal complications. During this sampling period, two distinct probiotic products were introduced: initially, Infloran (containing *Bifidobacterium bifidum* and *Lactobacillus acidophilus*), and later, Labinic (comprising *B. bifidum*, *Bifidobacterium longum* subsp. *infantis*, and *L. acidophilus*). Our analysis, exploring the taxonomic composition, highlighted the pivotal role of probiotics in shaping the gut microbial community. Each of the two probiotics led to establishment of distinct preterm gut community types enriched with specific Bifidobacterium species, which were also associated with increased maturity. Notably, differences between the two probiotics were observed, particularly in the prevalence and persistence of *B. bifidum* and *L. acidophilus*.

In a separate cohort, we investigated the impact of human milk oligosaccharides (HMOs) on NEC and the establishment of the gut microbiome, with a specific focus on Bifidobacterium species. In this cohort, we analysed HMOs in 33 infants with NEC and 37 matched healthy controls, and conducted longitudinal stool metagenomic sequencing in a subset of 48 infants (14 with NEC; stool n=644). We found a significant decrease in the concentration of a specific HMO, disialyllacto-N-tetraose (DSLNT), in the breast milk received by infants with NEC compared to controls. Moreover, infants receiving breast milk with low DSLNT were less likely to transition into preterm gut community types dominated by Bifidobacterium species.

These studies offer valuable insights into the impact of different probiotics on the taxonomic composition of the preterm gut microbiome and suggest that human milk composition might influence the colonisation of probiotic species.

HUMAN MILK BACTERIA INDIVIDUALLY OR AS A SYNTHETIC COMMUNITY EXHIBITED CONTRASTED IMMUNOMODULATORY PROFILES AND IMPACT ON THE GUT EPITHELIAL BARRIER

Charles Le Bras[1,2], L. Rault[1], N. Jacquet[1], N. Daniel[1], V. Chuat[1], F. Valence[1], A. Bellanger[3], L. Boursarghin[2], Y. Le Loir[1], I. Le Huërou-Luron[2] and S. Even[1]

[1] STLO, INRAE, Institut Agro, France
[2] Institut NUMECAN, INRAE, INSERM, Université Rennes, France
[3] Centre Hospitalier Universitaire de Rennes, Département de Pédiatrie, France

charles.le-bras@inrae.fr

Breastfeeding is recommended for the first 6 months of life. Many bioactive compounds of the human milk (HM) support the development of the intestinal immune system and barrier functions in infants. Our hypothesis was that HM microbiota contributes to these health benefits. Our objective was to characterise *in vitro* the role of HM bacteria, either individually or combined in synthetic communities (SynCom), on gut homeostasis. A collection of bacterial isolates, reflecting HM microbiota composition, was made from 28 healthy mothers exclusively breastfeeding. Firstly, the immunomodulatory profile of 84 HM bacterial isolates belonging to 38 species was characterized using blood mononuclear cells (PBMC). Secondly, the impact of a subset of 29 strains was deeply investigated on epithelial immune and barrier functions using a quadricellular (Caco2, HT29-MTX-E12, M cell, THP1 cells) model of the intestinal epithelium. Strains were characterized for their ability to modulate cellular IL-10 and TNF-α production and the expression of genes related to the barrier, immune and apoptosis/proliferation functions. Based on these results, 2 SynComs were designed and characterized on the quadricellular model.

HM bacteria displayed a large range of immunomodulatory properties. Using MultiDimensionate Scaling (MDS) on IL-10 and TNF-α production by PBMC, isolates were classified into 5 groups with specific signatures, highlighting the anti- and/or pro-inflammatory profiles of HM bacteria. Further, the MDS analysis of cytokine production and gene expressions of the quadricellular model stimulated by each of the 29 bacteria, classified strains into 3 groups named Quadri1, 2 and 3, according to their immunomodulatory activity and their impact on the epithelial barrier function. The composition of each group did not reveal major taxonomic biases between the 3 groups, but a diversity of the HM bacteria impact on gut epithelium within each genus or species. Quadri3 and, to a lesser extent, Quadri1 strains stimulated the immune function whereas Quadri2 hardly affected it. Besides, Quadri1 and 3 strains reinforced the epithelial barrier whereas an opposite effect was observed with Quadri2 strains. Finally, strains belonging to prevalent HM genera and with contrasted immunomodulatory profiles were assembled in two HM-like SynCom of 11 strains. The 2 Syncoms displayed different immunomodulatory properties, yet less contrasted than individual strains, whereas they both exhibited beneficial impact on barrier function.

This study showed the great diversity of immunomodulatory potential and impact on the barrier function of HM bacteria, individually or assembled in SynCom, highlighting the potential of the HM microbiota to modulate the intestinal development.

REVOLUTIONIZING MICROBIOME RESEARCH: UNLEASHING THE POWER OF MACHINE LEARNING

Laura Judith Marcos-Zambrano

Computational Biology Group. IMDEA Food Institute, Spain

judith.marcos@imdea.org

The surge in microbiome-related studies has significantly increased the amount of data available on the composition and function of the human microbiome. These studies provide valuable information for exploring the relationships between the microbiome and the development of complex diseases. To make the most of this data, we need better analytical tools that take into account the unique characteristics of microbiome data, which can be complex, varied, and sparse. The ability to predict a person's health status based on their microbiome, using specific features related to the types of microorganisms present, is a crucial step towards personalized medicine. Machine learning (ML) plays a key role in creating models that can classify and predict outcomes in microbiology and help us understand how the microbiome is linked to disease. To support research in this area and make it easier to find studies that combine machine learning and microbiome data, we have developed MoLTRES (Machine Learning meTagenomic REsearch Scraper), a web tool for locating ML studies that use human microbiome data. This tool covers various aspects, including feature selection, biomarker identification, disease prediction, and treatment strategies. The creation of MoLTRES followed a thorough review of the latest ML methods and software tools used in human microbiome research as part of the COST Action ML4Microbiome activities.

Furthermore, as we delve deeper into microbiome research, we face a pivotal challenge – translating our findings into valuable clinical applications, encompassing risk assessment, disease diagnosis and prognosis, and monitoring the efficacy of treatments. This presents an opportunity to refine existing bioinformatics methods, improve species identification from microbiome sequencing data, develop robust predictive models, and seamlessly integrate microbiome data with other omics information. The process of conducting such analyses brings about various challenges, underlining the importance of developing and optimizing statistical methods and workflows capable of handling the distinctive characteristics of microbiome data, thereby ensuring the accuracy and reproducibility of microbiome research.

Finally, in this context, we present a case study focused on classifying individuals with Celiac Disease (CD) based on their microbiome data. We used the random forest (RF) algorithm and data from the lower gastrointestinal tract, including stool and duodenum samples, to distinguish between healthy individuals and those with CD. We built six different models, considering whether individuals had consumed gluten and using binary-encoded information from the 16S rRNA hypervariable regions. The key genera contributing to the models' discriminatory abilities remained consistent across the various models. Furthermore, we created a separate RF classifier utilizing only stool data, which achieved an accuracy of 0.85 for CD prediction and 0.75 when applied to a population without CD. This underscores the potential of using stool-based markers to diagnose the disease. This progress hints at a future where microbiome research is at the forefront of healthcare innovation, offering personalized and effective medical treatments.

SPACE ODYSSEY 2023: EXPLORING TEMPORAL AND REGIONAL METABOLOMES IN THE HUMAN GUT

J. Folz[1], R. Neal Culver[2], J. Montes Morales[1], J. Grembi[3], G. Triadafilopoulos[4], D.A. Relman [3,5-7], K. Casey Huang[5,6,8], D. Shalon[9] and **Oliver Fiehn**[1]

[1] West Coast Metabolomics Center, University of California, Davis, USA
[2] Department of Genetics, Stanford University School of Medicine, USA
[3] Department of Medicine, Stanford University School of Medicine, USA
[4] Silicon Valley Neurogastroenterology and Motility Center, USA
[5] Department of Microbiology and Immunology, Stanford University School of Medicine, USA
[6] Chan Zuckerberg Biohub, USA.
[7] Infectious Diseases Section, Veterans Affairs Palo Alto Health Care System, USA
[8] Department of Bioengineering, Stanford University, USA
[9] Envivo Bio, USA

ofiehn@ucdavis.edu

Most utilization of human diets occurs in the small intestine, which remains largely unstudied. Stool is an inadequate surrogate for intestinal microbiome or metabolome studies for these diseases. Peroral or endoscopic gut aspirates and mucosal biopsies are highly invasive, and are conducted in the fasted state. Instead, we used a novel non-invasive, ingestible sampling device to probe the spatiotemporal variation of upper intestinal luminal contents during routine daily digestion in 15 healthy subjects. To demonstrate the biological and clinical utility of the capsule sampling device, we profiled the microbiome and metabolites present in these samples. We analyzed 274 intestinal samples and 60 corresponding stool homogenates by metabolomics and 16S rRNA sequencing. By combining data from three LC-MS/MS assays, we identified 1,909 metabolites, including sulfonolipids and novel bile acids.

275 samples were taken from 15 healthy humans using four capsules after lunch and again after dinner over two subsequent days. Samples were analyzed using untargeted lipidomics and biogenic amines by CSH- and BEH amide LC with +/- ESI Orbitrap MS/MS and primary metabolites by GC-TOF MS. Bile acids were targeted by C18-QTRAP 6500 MS/MS. Data analysis was performed by MS-DIAL 4.8 with compound annotations using MassBank.us and NIST20 libraries. We identified 1,909 metabolites, including novel bile acids, in addition to numerous unknown compounds. Stool metabolomes were dramatically different than intestinal tract samples. Trends in chemical abundance were observed based on intestinal location, subject-specific metabolite expression, and diet-linked factors. As expected, essential nutrients and dietary lipids decreased along the intestinal tract, while other diet related metabolites increased along the intestine. Interestingly, bile acids showed both expected and novel variations. Two subjects with antibiotic treatments up to 6 months prior to collection showed markedly different metabolome patterns. Exposome metabolism could be readily followed in different modifications along the intestinal tract. From inter-individual variation, we annotated *Blautia* species as a candidate to be involved in FAHFA metabolism.

For the first time, the metabolome of the human intestine was analyzed to reveal subject specific chemical phenotypes.

PLENARY SESSION
SPEED PRESENTATIONS
Short presentations (5-minutes) by selected poster presenters.

The abstracts can be found in the section 'Poster abstracts' (pages 61-113).

P9
Development of a synbiotic mixture to decrease intestinal neuroinflammation and restore short-chain fatty acid production in Parkinson's disease patients
Charlotte De Rudder
Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Luxembourg

P10
Synbiotics, a promising approach to reduce the exacerbated allergic airway immune responses in offspring maternally exposed to cigarette smoke
Ali Dehghani
Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, the Netherlands

P37
Christensenella minuta alleviates psychiatric and cardiac alterations induced by early life stress in mice
María Tamayo
Microbiome, Nutrition and Health Research Unit, Institute of Agrochemistry and Food Technology, Spanish National Research Council (IATA-CSIC), Spain

P45
Engineering lactic acid bacteria as delivery vehicles for *Clostridioides difficile* antitoxin proteins
Abida Zahirović
Department of Biotechnology, Jožef Stefan Institute, Slovenia

SESSION 1
WHY SHOULD HUMAN MICROBIOME SCIENTISTS BE INTERESTED IN ANIMAL STUDIES?

BOVINE ANIMAL MODEL FOR STUDYING THE ROLE OF MICROBIOME IN THE DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE

Samat Amat

Department of Microbiological Sciences, North Dakota State University, USA

samat.amat@ndsu.edu

Maternal gut microbiome has been shown to influence immune, metabolic and neurodevelopmental programming of offspring from the embryonic stage, suggesting a potential role in the Developmental Origins of Health and Disease (DOHaD). Whereas many still support the 'sterile-womb hypothesis' that neonatal microbiome acquisition occurs only during and after birth, very recent studies have provided evidence showing the existence of *in utero* microbial colonization. Thus, these recent developments call for further research to evaluate the maternal microbiome, *in utero* microbial colonization, and their role in offspring development and foetal programming. Although much progress has been made in early-life microbiome research based primarily on preterm infants, the ethical and legal constraints associated with human embryo and foetus-related research hinder research progress in terms of a developmental and mechanistic understanding of the role of the maternal microbiome in fatal programming. Rodent models have proven very good for studying the role of the maternal microbiome in foetal programming. However, some inherent limitations in these animal models make it challenging to study perinatal microbial colonization from a biomedical standpoint.

In this presentation, I will discuss the potential use of bovine animals as a biomedical model to study the maternal microbiome and developmental programming. In addition, I will briefly present the results of our recent work focused on the evaluation of alterations of maternal diet during early gestation on the maternal gut and reproductive microbiota, and perinatal microbial colonization in offspring beef calves.

NODding TO GROWTH: *LACTIPLANTIBACILLUS PLANTARUM*^{WJL} SUPPORTS MOUSE JUVENILE GROWTH IN CHRONIC UNDERNUTRITION

Martin Schwarzer

Laboratory of Gnotobiology, Institute of Microbiology of the Czech Academy of Sciences, Czech Republic

schwarzer@biomed.cas.cz

The intestinal microbiota is known to influence postnatal growth. We previously found that a strain of *Lactiplantibacillus plantarum* (strain LpWJL) buffers the adverse effects of chronic undernutrition on the growth of juvenile germ-free mice. Next, we were curious to explore if the growth promoting capability of administered LpWJL is retained also in conventional animals, i.e., animals with intestinal microbiome. We found that daily administration of LpWJL sustains the postnatal growth of malnourished conventional animals and supports both insulin-like growth factor-1 (IGF-1) and insulin production and activity. We have identified cell walls isolated from LpWJL as sufficient cues to stimulate animal growth despite undernutrition. Further, we found that NOD2 is necessary in intestinal epithelial cells for LpWJL-mediated improvement of intestinal crypt cell proliferation, type I interferon–regulated gene induction, IGF-1 production, and postnatal growth promotion in malnourished conventional animals. Our results demonstrate that bacterial cell walls or purified NOD2 ligands are sensed by the pattern recognition receptor NOD2 in the intestinal epithelial cells and sustain postnatal juvenile growth despite chronic undernutrition. We posit that one of the mechanisms by which LpWJL and its cell wall exert its postnatal growth-promoting properties is the buffering of the deleterious effect of undernutrition on small intestinal crypt cell proliferation through NOD2-dependent bacterial cell walls sensing.

Our results suggest that, coupled with renutrition strategies, supplementation of evidence-based probiotics, such as LpWJL, or defined bacteria-derived postbiotics, such as LpWJL cell walls, and/or NOD2 agonists, have the potential to alleviate persistent stunting, one of the long-term sequelae of undernutrition that still affects >149 million children under 5 years of age in low- and middle-income countries.

Acknowledgements
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FROM THE UNIQUE PROPERTIES AND SPECIFIC METABOLISM OF *SACCHAROMYCES BOULARDII* CNCM I-1079 TO PROBIOTIC EFFECTS BEYOND GUT MICROBIOTA MODULATION: WHAT IMPACT ON HOST METABOLISM AND IMMUNITY?

Caroline Achard[1], M. Schiavone[1], B. Bertaud[1], F. Bravo De Laguna[2], F. Chaucheyras-Durand[1,3], M. Castex[1], E. Chevaux[1], L. Dunière[1,3], M. Le Barz[1], P. Lebreton[1], D. Saornil[2], C. Villot[1] and E. Apper[1]

[1] Lallemand SAS, France
[2] Lallemand Espana SL, Spain
[3] Clermont Auvergne University, INRAE, France

cachard@lallemand.com

Originally classified as a separate species of Saccharomyces, *Saccharomyces cerevisiae* var. *boulardii* exhibits a genome similar to *S. cerevisiae*. Nevertheless, *S. boulardii* has specific genetic and phenotypic characteristics which confers unique probiotic properties when compared to others *S. cerevisiae*. Its specific resistance to gastrointestinal conditions has been demonstrated *in vitro* and *in vivo*. Beneficial effects on digestive health and performances of *S. boulardii* administrated in humans or livestock animals has been well documented. The modulation of the gut microbiota composition is likely to be involved in the effects exhibited by *S. boulardii* on host. Beyond the impact on the gut microbiota, we focus here on the direct and the indirect effects of *S. boulardii* on the host and highlight some possible modes of action through an integrative analysis.

Data regarding the direct effect of *S. boulardii* in the gut of livestock animals are limited. *In vitro* and animal model studies have shown that *S. boulardii* CNCM I-1079 (SB) exerts direct luminal effects by producing inhibitory compounds against pathogens, such as acetate, and by preserving the intestinal barrier integrity leading to reduced invasion of pathogenic *E. coli* and *Salmonella*. Moreover, SB possesses antioxidant and immunomodulation properties. Indeed, supplementation with SB reduces the production of pro-inflammatory cytokines in the host, while promoting mucosal anti-inflammatory response leading to improved innate immunity response during weaning in calves and piglets.

The integrative analysis of studies performed in rodent models, swine and dogs allowed to highlight links between SB supplementation and host metabolism. Improved insulin sensitivity associated with *S. boulardii* supplementation pinpoints the involvement of the entero-pancreatic axis and suggests important impact on energy metabolism. SB supplementation has also been associated with a modulation of the bile acids profiles suggesting an indirect effect through the gut liver-axis. The interaction between the microbiota and bile acids metabolism is known to be involved in the maintenance of intestinal barrier function, regulation of immunity, of energy metabolism and resistance to pathogens. Moreover, antioxidant and anti-inflammatory properties of SB may be partially due to a modulation of the glycerophospholipid metabolism, as evidenced thanks to metabolomics studies in dog and swine. Finally, a maternal programming effect has been evidenced in swine and canine species.

The better understanding of the link between *S. boulardii* CNCM I-1079 supplementation, microbiota modulation and host metabolism and immunity paves the way for further experiments designed to confirm suggested new modes of action.

WHY MAMMALIAN MILK CONTAINS LACTOSE

Richard Ducatelle, J. Derix, F. Van Immerseel and E. Goossens

Livestock Gut Health Team, Veterinary Medical Faculty, Ghent University, Belgium

richard.ducatelle@ugent.be

Lactose is a unique disaccharide composed of glucose and galactose. It is exclusively found in the milk of mammals and is not found anywhere else in nature. In the intestinal tract of mammalian offspring the expression of the disaccharidase enzyme lactase allows for digestion of the lactose, since lactose itself is not absorbed. We found strong indications that the bacterial pathogen *Clostridium perfringens* (CP) may be one of the important evolutionary selection pressures towards secretion of lactose in mammalian milk. CP is a ubiquitous spore forming gram-positive bacterial pathogen which can be found in the intestinal tract of all mammals. In spite of its impressive arsenal of virulence factors, it rarely causes disease, except under very specific conditions. One such condition is created in the veal industry, where calves are reared on milk replacer up to slaughter age. Considerable numbers of calves are lost due to a syndrome called enterotoxaemia or necro-haemorrhagic enteritis. There is consensus in the literature that the primary cause is CP type A. Nevertheless, the disease cannot be reproduced experimentally by the sole inoculation of calves with CP type A.

We developed an intestinal loop model in anaesthetized calves in which we could reproduce the typical necro-haemorrhagic lesions by injecting CP directly into the lumen of the loops. Any strain of CP was capable of eliciting these same lesions, suggesting that alpha toxin, which is the only major toxin expressed by all CP strains, may be involved in the development of the lesions. This was confirmed in an experiment where we showed that an alpha toxin knock-out mutant was unable to induce the lesions in the intestinal loop model, while in the complemented strain this capacity was largely restored. In an *in vitro* study, we showed that the presence of milk in the culture medium did not inhibit the growth of CP type A, but it did inhibit alpha toxin secretion in a concentration dependent manner. When testing different fractions of the milk it became clear that this phenomenon was induced by the lactose in the milk. *In vivo* we showed that veal calves, which are mainly raised on milk replacer, produce less antibodies against CP alpha toxin than beef calves, which are weaned at an early age and thereafter predominantly are fed with solid feed. Finally, in an *in vivo* experimental study it was shown that treating a lactose rich milk replacer with lactase immediately before administration of the milk allowed the calves to develop an active immunity against alpha toxin.

These data indicate that maternal antibodies together with lactose in milk protect the newborn against the most essential virulence mechanism of CP. In the natural state, young mammals initially suckle very frequently, but after a few days/weeks they start nibbling hard food. This natural behaviour most probably allows for a complete block of alpha toxin production in the early post-natal period, a block which is gradually lifted, in order to allow a smooth transition from passive to active immunity.

SESSION 2
ORAL-GUT MICROBIOME AXIS

ORAL MICROBIOME: A FRIEND OR A FOE?

Egija Zaura

Department of Preventive Dentistry, Academic Centre for Dentistry Amsterdam (ACTA), the Netherlands

e.zaura@acta.nl

Oral microbial ecosystem is exposed to numerous daily perturbations such as toothbrushing and mastication, antimicrobial substances in saliva, in oral care products and foods, fluctuations in pH, oxygen and temperature. Nevertheless, it is incredibly stable. Our previous research has shown that oral microbiome is far more resistant to a single dose of antibiotics than microbiome of the gut. At health, oral microbial communities are in balance and in beneficial symbiosis with the host. If the balance is lost, a dysbiotic microbial community evolves, which enters an antagonistic symbiosis with the host. This may lead to oral diseases or can even have an impact on the general health of the host. For instance, systemically, the oral microbiome has been associated with pregnancy complications, colorectar and pancreatic cancer, neurodegenerative disorders and autoimmune diseases. Locally, once the host-microbiome balance is lost, such as during frequent sugar intake or prolonged neglect of oral hygiene, dental caries or periodontal diseases may develop. This, however, does not occur in all hosts – some individuals are more resilient towards these stressors than others.

THE ORAL-GUT MICROBIOME AXIS IN HEALTH AND DISEASE

Benoît Kunath

Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Luxembourg

benoit.kunath@uni.lu

The human body hosts trillions of microorganisms throughout many diverse habitats with different physicochemical characteristics. Among them, the oral cavity and the gut harbour some of the most dense and diverse microbial communities. While these two sites are physiologically distinct, they are directly connected and can influence each other in several ways. For example, oral microorganisms can reach and colonise the gastrointestinal tract, particularly in the context of gut dysbiosis. However, the mechanisms of colonisation and the role that the oral microbiome plays in causing or exacerbating diseases in other organs have not yet been fully elucidated. I will describe recent advances, including based on integrated multi-omics, in our understanding of how oral and intestinal microbiota interplay in relation to their impact on human health and disease.

GUT-MOUTH AXIS IN PERIODONTITIS

Jun-ichi Nagao[1,2] and Y. Tanaka[1,2]

[1] Section of Infection Biology, Department of Functional Bioscience, Fukuoka Dental College, Japan
[2] Oral Medicine Research Center, Fukuoka Dental College, Japan

jungao@fdcnet.ac.jp

Periodontitis, a leading cause of tooth loss, is strongly associated with the oral pathobiont *Porphyromonas gingivalis*, a member of periodontal pathogenic bacteria. The pathogenesis of periodontitis has been reported to be mediated by host immune responses, especially IL-17A-producing helper T cells, Th17 cells. Th17 cells are the key mediator in inducing gingival inflammation and promoting alveolar bone loss in the oral cavity. However, where and how the Th17-type immune response is induced during the development of periodontitis is not well understood. Here we demonstrate that gut translocation of *P. gingivalis* exacerbates *P. gingivalis*-induced periodontitis with enhanced Th17 cell differentiation. The *P. gingivalis*-responsive Th17 cells are differentiated in Peyer's patches and translocated systemically in the peripheral immune tissues. They are also capable of migrating to and accumulating in the mouth upon oral infection. Development of periodontitis via the *P. gingivalis*-responsive Th17 cells is regulated by the intestinal microbiome, and altering the intestinal microbiome composition with antibiotics affects the development of periodontitis. Our study highlights that the *P. gingivalis*-responsive Th17 cells in the gut-mouth axis and the intestinal microbiome work together to provoke periodontitis.

METAGENOMIC ANALYSIS OF THE GUT AND ORAL MICROBIOMES REVEALS CROSS-COHORT SIGNATURES TO PREDICT PANCREATIC CANCER

Suguru Nishijima

Structural and Computational Biology Unit, European Molecular Biology Laboratory, Germany

nishijim@embl.de

Pancreatic cancer remains one of the most lethal malignant neoplasms, with an overall 5-year survival rate of <5%. Although early detection of this cancer is pivotal for improving clinical outcomes, it is still challenging due to the limited accuracy and specificity of current biomarkers. Recent studies have identified altered gut and oral microbiomes in pancreatic cancer patients, suggesting the microbiomes as potential biomarkers. However, the robustness and accuracy of the microbial signature have not been systematically analysed yet. In this study, we conducted a multinational study to investigate the gut and oral microbiomes of patients with treatment-naïve pancreatic ductal carcinoma (PDAC) patients and non-PDAC controls in Japan, Spain, and Germany. Through comparative metagenomics, we revealed the altered structure of both the gut and oral microbiomes and identified 30 gut and 18 oral species significantly associated with PDAC in the Japanese cohort. These gut and oral microbial signatures showed a high area under the curve (AUC) values of 0.78 to 0.82, respectively. Notably, the prediction model trained on the Japanese gut microbiome also had high predictive ability in Spanish and German cohorts, with respective AUC values of 0.74 and 0.83. Significant enrichments of *Streptococcus* and *Veillonella* spp. and depletion of *Faecalibacterium prausnitzii* were common gut microbial signatures across the 3 cohorts. Furthermore, prospective follow-up data revealed that patients with certain gut and oral microbial species were at higher risk of PDAC-related mortality. Our multinational study revealed consistent gut microbial signatures across independent cohorts, suggesting the feasibility of constructing a global, specific, and reproducible predictive model to screen for PDAC based on non-invasive gut microbiome profiling.

SESSION 3
BENEFICIAL MICROBES IN ANIMAL HEALTH AND NUTRITION

VISUALISING AND QUANTIFYING RUMEN MICROBIOME RESPONSES TO LIVE MICROBIALS AND PREBIOTICS

Greta Reintjes

Microbial-Carbohydrate Interactions Group, University of Bremen, Germany

greintje@mpi-bremen.de

The microorganisms living in the gut have a big impact on our health and nutrition. They play a crucial role in helping us digest food and maintaining our health. To harness the potential of these gut microbes for our benefit, it is essential to gain a deep understanding of how they interact with each other and the substrates that we introduce in our diet, such as prebiotics.

Traditionally, our knowledge of gut microbiomes has relied on metagenomic predictions and laboratory culturing of individual strains. While these approaches provide valuable insight, they fall short of capturing the full picture of what is happening in the gut in a given time and space. To truly comprehend the effect of pre- and pro-biotics, we employed innovative techniques that allowed us to investigate the real-time metabolic behaviours of individual bacterial cells in a complex gut environment. Specifically, we investigated the metabolism of yeast mannan, a prebiotic known to have a significant impact on gut microbiota in rumen microbiomes. By creating a fluorescently labelled yeast mannan, we were able to track how single bacterial cells in a rumen sample were processing yeast mannan. We could identify the specific microbes targeted by our prebiotic and, through cell sorting and sequencing, discover their metabolic potential to use the prebiotic. We discovered that our prebiotic had a significant effect on the microbial community and rumen fermentation. When we added rumen-specific probiotics, we could not see further additive effects.

Our study introduces a new *in vitro* methodology applicable for assessing prebiotics and probiotics in more complex rumen systems and live animals.

RESPONSE OF THE GUT-LIVER AXIS TO STIMULATION DURING EMBRYONIC DEVELOPMENT IN CHICKENS

Aleksandra Dunislawska, E. Pietrzak, A. Beldowska and M. Siwek

Department of Animal Biotechnology and Genetics, Bydgoszcz University of Science and Technology, Poland

aleksandra.dunislawska@pbs.edu.pl

The liver in chicken plays an important role as the endocrine and exocrine gland. This organ is crucial for the host organism because it is involved in a range of metabolic and homeostatic functions. The liver is also one of the largest immune organs. A recent study places the liver in the centre of the intersections between the host and the gut commensal microbiota. The anatomy of the liver provides its close interaction with the gut, where nutrients and the microbiome contribute to the maintenance of a healthy metabolism. Many studies prove a correlation between the intestines and substances penetrating the liver, i.e., gut-liver axis. Even 30% of metabolites in peripheral blood are of bacterial origin, which is why bacteria strongly influence all bodily systems. Research has shown that the composition of the gut microbiota is determined by environmental factors and is most dynamically shaped in the pre-hatch period. Due to this fact, we have developed a technology for programming the composition of the intestinal microbiota of chickens by administering one dose of bioactive substances to the air chamber of the incubated egg on day 12. This technique, called *in ovo* stimulation, involves the administration of a bioactive solution at the stage of embryonic development. The peri-hatching period is crucial for programming the microbiota to enable colonization of the embryo's intestines with beneficial bacteria before hatching. It has been shown that the administration of a single dose of a prebiotic or synbiotic suspension into the egg's air chamber on day 12 of incubation ensures effects that are visible throughout the life of chickens. After *in ovo* administration of the substance, the intestines of the hatched chick are first colonized, followed by the delivery of antigens for the maturation of the immune system and the protection of the intestinal ecosystem, producing products of basic metabolism.

We completed several projects to understand the interaction between gut microbiota and the chicken organism. We aim to describe the molecular mechanisms of the gut-liver axis in the chicken. We have analysed the impact of an early *in ovo* stimulation of chicken microbiota with various bioactive compounds. We have proven that substances administered *in ovo* influence changes in the intestinal bacterial profile. A favourable microbiome profile is also observable on the last day of chicken rearing. The early stimulation of chicken microbiota influenced a range of traits, including hatchability, mortality, and performance among different genotypes of broiler and native chickens. We have shown that *in ovo* stimulation with synbiotics affects the transcriptomic profile of the liver, and the effects depend on the composition of substances. These results were also confirmed at the proteome level. The administration of bioactive substances (prebiotics, probiotics, and synbiotics) affects the expression profile of genes related to metabolism and the immune response. Interestingly, these results are strongly correlated with the methylation level of genes and miRNA activity. Changes in the gut microbiota composition can lead to alternations in this communication, which may ultimately lead to modifications in gene expression driven by epigenetic mechanisms. The results obtained so far also allow to understand the modulatory effects of bioactive substances delivered *in ovo* on the metabolic status of the host and the interactions between microbiota, SCFA, and biochemical pathways in the liver. All data collected in our research can be further developed into solutions for the poultry industry. The bioactive compounds for *in ovo* stimulation in chickens can target specific effects. For example, the challenging environment may need immunomodulatory compounds to prevent diseases. But, in optimal conditions, the pro-metabolic compounds can be used to improve meat quality and shelf life.

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PREBIOTIC GALACTO-OLIGOSACCHARIDE FEED ENHANCES BROILER CHICKEN PRODUCTIVITY AND *SALMONELLA* CLEARANCE

Ian F. Connerton

Division of Microbiology, Brewing and Biotechnology, School of Biosciences, University of Nottingham, UK

ian.connerton@nottingham.ac.uk

Improvements in health, growth performance and the control of zoonotic pathogens are key divers in the production of broiler chickens. The inclusion of prebiotic galacto-oligosaccharides in broiler feed enhanced the growth rate and feed conversion of chickens relative to a calorie-matched control diet. Comparison of the caecal microbiota identified key differences in abundance of *Lactobacillus* spp. Increased levels of *L. johnsonii* in GOS-fed juvenile birds at the expense of *L. crispatus* was linked to improved performance (growth rate and market weight). Quantification of the autochthonous *Lactobacillus* ssp. revealed a correlation between bird performance and *L. johnsonii* abundance. Shifts in the caecal populations of key *Lactobacillus* spp. of juvenile birds primed intestinal innate immunity. The juvenile prebiotic diet was demonstrated to hasten the clearance of *Salmonella enterica* serovar Enteritidis challenge at 20-days-old. Differential abundance *Salmonella*-challenged microbiota identified taxa belonging to the *Negativicutes* class, which were accompanied by increases in the caecal concentrations of propionate and valerate. Greater concentrations of propionate and valerate were detected in chickens fed the galacto-oligosaccharide-supplemented diet in early life, which corresponded with the abundance of the *Acidaminococcaceae* taxon. Deliberate cultivation of these taxa with prebiotic galactooligosaccharide presents a straight-forward, safe and cost-effective intervention against *Salmonella*.

DEVELOPMENT OF PROBIOTIC FEED FOR SALMONIDS WITH IMMUNOMODULATORY POTENTIAL

Dagmar Mudroňová[1], M. Sørensen[2], P. Popelka[1], J. Koščová[1], A. Fečkaninová[1], I. Cingeľová Maruščáková[1], M. Ratvaj[1], N. Chomová[1], J. Mareš[3] and M. Faldyna[4]

[1] Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy, Slovakia
[2] Faculty of Biosciences and Aquaculture, Nord University, Norway
[3] Department of Zoology, Fisheries, Hydrobiology and Apiculture, Mendel University, Czech Republic
[4] Veterinary Research Institute, Czech Republic

dagmar.mudronova@uvlf.sk

Aquaculture is one of the fastest-growing food producing sectors and during the last decade it has become an important economic activity in many countries including inland countries, such as Slovakia. Intensive fish farming is only possible through efficient feeding and high fish concentration, thus increasing the risk of diseases. In addition, repeated handling, temperature changes, poor water quality and poor nutrition contribute to the creation of stress, which causes immunosuppression and thus reduces the fish's resistance to disease. On the other hand, the medicines used to treat or prevent them have a negative effect on meat quality and environment. The aim of our research was, therefore, to develop a product based on autochthonous probiotic bacteria, which would increase the fish's immunity against infections and stress factors. Lactic acid bacteria (LAB) were isolated from the digestive tract of healthy rainbow trout and were tested for their inhibitory activity against serious salmonid bacterial pathogens, for their ability to survive in the digestive tract conditions of fish and in the aquatic environments, and for susceptibility to antimicrobials based on EFSA regulation.

Lactobacillus plantarum R2 Biocenol™ and *Lactobacillus fermentum* R3 Biocenol™, which showed the best properties, were tested on trout intestinal primary cell cultures infected with *Aeromonas salmonicida* subsp. *Salmonicida* and *Yersinia rückeri*. For application in the aquatic environment, two new application forms were developed by applying strains on commercial fish feed with the help of auxiliary substances. Subsequently, continuous and cyclic application was tested in a non-infectious experiment on trout. Both strains were also administered separately and in combination to salmon with induced intestinal inflammation. Finally, probiotic feed was fed to trout, which were subsequently infected with the pathogen *A. salmonicida*. Experiments proved that both strains can positively influence both the immune response and gut microbiota. It was manifested by reduction of diet-induced enteritis in salmon, what was confirmed by increased TGF-β gene expression also in trout. Moreover, after cyclic administration was stimulated gene expression of other immune-related molecules in gut (e.g., CD8, IgM, IL-8, TLP-9) without increase of proinflammatory cytokines (IL-1, TNF-α). The intestinal microbiota was shifted in favor of beneficial LAB. Their testing will continue under large-scale farm conditions on trout farm in Slovakia and on salmon farm in Norway.

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PROBAC-SEQ TECHNOLOGY, A POWERFUL TOOL TO SCREEN NEW SOLUTIONS IMPACTING NECROTIC ENTERITIS TOXIN B AND *C. PERFRINGENS* CYTOTOXICITY

Marion Bernardeau[1,4], J.P. Meisch[2], M.A. Brennan[2], A.Z. Rosenthal[2,3], G. Saxer[2] and K. Gibbs[1]

[1] IFF Danisco Animal Nutrition and Health, the Netherlands
[2] IFF Health and Biosciences, USA
[3] present address: Department of Microbiology and Immunology, UNC Medical School, USA
[4] Normandy University, UNICAEN, ABTE, France

marion.bernardeau@iff.com

Necrotic enteritis (NE) caused by *C. perfringens* (CP) is a reemerging threat to the poultry industry following mounting pressure to reduce antibiotic use. As a means for developing more consistent antibiotic alternatives, the aetiology and characteristics of intestinal challenges must be fully elucidated. Here we show the power of ProBac-seq, a probe-based bacterial single cell sequencing method that analyses transcriptional variation across individual cells within a clonal population, to study interactions of soluble factors produced by probiotic with a pathogen. The CP strain studied (25037-CP01) was isolated from broilers with clinical NE and confirmed for CPA^+ and $NetB^+$. The tested cell-free supernatants (CFS) were harvested from probiotics *Lactobacillus acidophilus* AG01 and *Bifidobacterium animalis* subsp*. lactis* AG02 grown in MRS and tested at 3% AG01 and 2.5 or 5% for AG02. We report the ability of AG01 bacterial suspension (but not AG02), to inhibit 100% of the growth of 25037-CP01 after 24 hours of co-culture. Subsequently, we report the gene expression, production of NetB and the cytotoxicity of CP 25037-CP01, grown in unchallenged conditions and in the presence of the AG01 and AG02 CFSs. Following incubation, CP cells were harvested and prepared for ProBac-seq according to McNulty *et al.* (2023). Western blot (WB) and HT-29 enterocytes were used to assess changes in toxin production and cytotoxicity of CP respectively. AG02 (and not AG01) reduced production of NetB in WB, and cytotoxicity of supernatant from CP was reduced by 60% ($p<0.05$) in the presence of 5 % AG02 CFS. Following analysis of the transcriptomes of single CP cells, we demonstrate cells grown in the presence of 2.5% AG02 CFS have numerically reduced *netB* expression while 5% AG02 CFS significantly reduced the overall *netB* expression compared to control ($p<0.0001$, Welch t-Test). Overall, we report here heterogeneous gene expression in CP populations, with the *netB* toxin gene being predominately expressed by a subset of cells. We further show that AG02 CFS changed overall gene expression and community organization of the CP population. Subsequently, the efficacy of a novel dual probiotic blend (AG01-AG02) was assessed in birds challenged with netB+CP. Results confirm improved bird performance, reduced NE lesions and NE induction.

These *in vitro* results introduce a novel strategy to identify new targets within a population of CP, new mode of action for probiotics. Technology and information released thereof can be applied for the development of antibiotic alternatives like next generation probiotics.

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SESSION 4
BENEFICIAL MICROBES, HUMAN HEALTH & WELL-BEING

THE POTENTIAL OF *DOLOSIGRANULUM PIGRUM* AMBR11 AS BENEFICIAL MICROBE FOR RESPIRATORY HEALTH

Ilke De Boeck[1], E. Cauwenberghs[1], I. Spacova[1], J. Bastiaenssens[1], K. Martens[1,2], S. Wittouck[1], O. Vanderveken[3-5] and S. Lebeer[1]

[1] Research Group Environmental Ecology and Applied Microbiology, Department of Bioscience Engineering, University of Antwerp, Belgium
[2] Allergy and Clinical Immunology Research Group, KU Leuven, Belgium
[3] Faculty of Medicine and health Sciences, University of Antwerp, Belgium
[4] Translational Neurosciences, University of Antwerp, Belgium
[5] Multidisciplinary Sleep Disorders Centre, Antwerp University Hospital, Belgium

ilke.deboeck@uantwerpen.be

The upper respiratory tract (URT) microbiome has an important gatekeeper function by forming a barrier to potential pathogens and modulating immune responses. The current knowledge on the URT microbiome is mainly based on association studies mapping the occurrence and relative abundance of different bacterial taxa. These studies point towards certain beneficial microbiome members, associated with nasal health, such as *Dolosigranulum pigrum*. This bacterium is an understudied member of the lactic acid bacteria, that is mainly found in the human nasal cavity based on data mining of publicly available sequencing data. Its association with the healthy respiratory tract and niche preference for the nasal cavity makes *D. pigrum* a promising candidate as live biotherapeutic product (LBP) for this niche. However, its biology, habitat adaptation, and health-related functions must be further explored.

In this study, we isolated *D. pigrum* strains from the healthy human URT and aimed to obtain insights into its adaptation capacity to the URT via evaluation of its growth and antimicrobial peptides. Furthermore, cocultures experiments with different *in vitro* cell culture models, among others CuFi-1 lung cells and monocyte THP1-Dual monocyte reporter cells, were performed to study its immunomodulatory and barrier enhancing capacity. The latter was also investigated in a mouse model based on measurement of FD-4 passage. Male BALB/c mice were pre-treated endonasally with *D. pigrum* AMBR11 prior to interleukin (IL)-4 application, an agent known to disrupt the epithelial barrier. We could isolate *D. pigrum* AMBR11 from a healthy nose. Growth capacity investigated in several URT-inherent conditions indicated that mucin, as well as supernatant from specific URT bacteria induced an enhanced growth of our isolate. Based on the AMBR11 genome, the genes encoding two putative lantipeptides and 1 putative bacteriocine were predicted. qPCR analysis with designed primers for the predicted genes showed that supernatants of specific URT bacteria promoted the expression of these antimicrobial peptides, and we have identified URT isolates with the most promoting effect. Coculture experiments of *D. pigrum* AMBR11, with and without the presence of supernatants of these specific URT bacteria, with the opportunistic pathogens *Staphylococcus aureus* and *Haemophilus influenzae* are ongoing. Finally, mice that were endonasally pre-treated with *D. pigrum* AMBR11 showed less IL-4-induced nasal epithelial barrier disruption.

In conclusion, *D. pigrum* shows high potential as topical LBP for the respiratory tract among others via its immunomodulatory properties, beneficial interactions with URT microbiome members and positive effects on the airway epithelial barrier.

EXTRACELLULAR VESICLES AND SURFACE LAYER PROTEINS AS THE POST-BIOTIC ACTIVE INGREDIENT OF THE PROBIOTIC BACTERIUM *PROPIONIBACTERIUM FREUDENREICHII* AGAINST COLITIS AND MUCOSITIS

Gwénaël Jan[1], B. Foligné[2], F.L. Rosa Do Carmo[1,3], V. De Rezende Rodovalho[1,3], H. Rabah[1], F. Gaucher[1], V. Azevedo[3] and E. Guédon[1]

[1] INRAE, Research Unit Science and Technology of Milk and Eggs (STLO), Institut Agro, France
[2] Université Lille, Inserm, CHU Lille, Institute for Translational Research in Inflammation (INFINITE) – U1286, France
[3] Institute of Biological Sciences, Federal University of Minas Gerais, Brazil

gwenael.jan@inrae.fr

Gut inflammation constitutes a growing health concern in developed countries. It coincides with dysbiosis, including a lack of anti-inflammatory bacteria. As an example, propionibacteria are lacking in the microbiota of newborns which develop necrotizing enterocolitis. We investigated immunomodulatory properties of *Propionibacterium freudenreichii*. A screening led to the selection of *P. freudenreichii* CIRM-BIA129, a strain inducing high levels of regulatory IL-10 in human PBMCs. Consumption of this strain protected mice from colitis induced either by TNBS or by DSS. It alleviated severity of symptoms, modulated local and systemic inflammation, as well as colonic oxidative stress and epithelial cell damages. It further mitigated severity of mucositis induced by 5-fluorouracyl, preventing weight loss, reducing inflammation and mucosal damages. Mutation of the *slpB* gene, encoding a key surface layer protein, suppressed this immunomodulatory effect and the resulting ΔslpB mutant induced a rather proinflammatory response and failed to prevent mucositis. Accordingly, consumption of *Lactococcus lactis* NCDO 2118 harbouring pXIES-SEC:slpB and expressing the propionibacterial SlpB reduced severity of colitis, lowered weight loss, disease activity index, shortening of the colon length, and histopathological score, compared with mice treated with *L. lactis* NCDO 2118 wild-type strain.

P. freudenreichii was further shown to produce extracellular vesicles (EVs), which mimic the immunomodulatory features of propionibacteria *in vitro* by modulating NF-κB transcription factor activity and IL-8 release in cultured human intestinal epithelial cells (HIEC). Proteomic analysis revealed presence of surface layer (S-layer) proteins, including SlpB, in these EVs. Guanidine treatment of *P. freudenreichii* intact cells leads to extraction of surface proteins, which constitute the S-layer, the outmost structure of bacteria. These extracted proteins also mimic the effect of propionibacteria, inducing IL-10 in immune cells and modulating NF-κB and IL-8 in HIEC.

This work emphasizes the importance of extractable surface proteins, including SlpB, and of EVs, in *P. freudenreichii* probiotic effects. It opens perspectives for the development of probiotic and postbiotic products aimed at decreasing inflammation.

INTERACTIONS OF MICROPLASTICS WITH THE HUMAN GUT MICROBIOTA OF ADULTS AND INFANTS USING *IN VITRO* GUT MODELS

Elora Fournier[1,2], M. Mercier-Bonin[2], L. Etienne-Mesmin[1] and S. Blanquet-Diot[1]

[1] Université Clermont Auvergne, INRAE, UMR 0454 MEDIS, France
[2] Toxalim, Université de Toulouse, INRAE, ENVT, INP-Purpan, UPS, France

elora.fournier@uca.fr

Microplastics (MPs) are recognized as a global threat due to their prevalence in natural environments and food chain. The human gastro-intestinal tract (GIT) is one of the first sections exposed, but to date, the fate and potential adverse effects of MPs in the human digestive sphere remain largely unknown, especially for at-risk populations such as infants. This study aimed to investigate the effects of a chronic ingestion of spherical commercially available MPs of polyethylene (PE), the most manufactured plastic polymer worldwide, on adult and infant microbiota using the Mucosal Artificial Colon. This *in vitro* model recreates the main nutritional, physicochemical and microbial (luminal and mucus-associated microbiota) parameters of gut environment. The impact of gut microbiota-derived metabolites after exposure to PE MPs on the intestinal barrier was evaluated using a co-culture of Caco-2 and mucus-secreting HT29-MTX cells. We reported that PE MP effect on gut microbiota was donor-dependent and resulted in an increased abundance of potential pathobionts, such as *Enterobacteriaceae*, regardless of age conditions. Exposure to PE MPs was associated with a significant decrease in butyrate production in infants, while skatole levels were significantly increased in adults. Conversely, no significant impact of PE MPs on the intestinal barrier, as mediated by changes in gut microbial metabolites, was evidenced.

This pioneering work provided significant insights into the interactions of PE MPs with human gut microbiota and intestinal barrier of two age groups, under relevant human colonic conditions. Next steps will be dedicated to study the impact of more representative MP types (shape, ‘real-life’ aged and/or contaminated particles, etc.) in healthy but also disturbed situations including an altered gut barrier and dysbiotic microbiota (e.g., obesity, inflammatory bowel syndrome, inflammatory bowel diseases).

UNCOVERING THE MECHANISMS OF *LACTIPLANTIBACILLUS PLANTARUM*-MEDIATED TYPE I INTERFERON INDUCTION*:* ROLE OF IMMUNOMODULATORY SURFACE PROTEINS

Selvin Solis[1], J. Gutierrez-Merino[1], C. Maluquer de Motes[1] and S. Mukhopadhyay[2]

[1] School of Biosciences, University of Surrey, UK
[2] Peter Gorer Department of Immunobiology, King's College London, UK

s.solis@surrey.ac.uk

Type I Interferon cytokines (IFN-I) and probiotics administration are strategies currently being explored to reduce inflammation and reverse imbalance of microbial populations in the gut that could otherwise lead to chronic disorders such as inflammatory bowel disease (IBD). It has recently been reported that the probiotic bacterium *Lactiplantibacillus plantarum* (LP) is a potent inducer of IFN-I responses via cytosolic nucleic acid sensors. Therefore, LP is most suited as a therapeutic tool to combat IBD. However, the immunomodulatory mechanisms and, more specifically, the molecules involved in the host-protective responses elicited by LP are not well understood, making them a crucial area of research. In this study, innate immune cells, particularly phagocytes exposed to different strains of LP with different self-aggregative properties were tested for their ability to bind to phagocytes followed by IFN-I induction. This revealed that IFN-I induction positively correlates with the high self-aggregative phenotype of the LP strains and that the removal of cell wall proteins significantly decreases their IFN-I induction ability. To unveil genes encoding relevant cell wall proteins a double genomic approach was also conducted, wherein, mRNA transcripts of LP were analysed and found that expression of certain cell wall adhesins including the well-known mannose specific adhesin (*msa*) was upregulated on high IFN-I inducers and that mutation in these genes generated by subjecting these cells to Ultraviolet (UV) significantly reduced this ability. Furthermore, transformation of a heterologous host with *msa*, was indeed found to increase phagocyte interaction and elicit a higher IFN-I response when compared to the wild type.

These findings highlight the significance of cell wall proteins including aggregation promoting factors and adhesins responsible for self-aggregation and binding to phagocytes, respectively, in enhancing microbe-host interactions. The interplay between these surface proteins seems to be crucial to activate the production of IFN-I cytokines and the levels at which theses cytokines are produced are strain-dependent, potentially leading to different degrees of microbe-host interactions. By understanding these interactions, the specific proteins involved could be manipulated for the design of probiotic therapies against immune-related gut disorders affecting humans and animals.

DAILY LACTOSE SUPPLEMENTATION IN LACTASE NON-PERSISTENT INDIVIDUALS INDUCES COLONIC ADAPTATION AND REDUCES INTOLERANCE SYMPTOMS

Lonneke Janssen Duijghuijsen[1], E. Looijesteijn[2], M. van den Belt[1], B. Gerhard[3], R. Ariens[1], R. Tjoelker[2] and J. Geurts[2]

[1] Wageningen Food and Biobased Research, Wageningen University & Research, the Netherlands
[2] FrieslandCampina, the Netherlands
[3] Biomax Labvantage, Germany

lonneke.janssenduijghuijsen@wur.nl

Globally, about 70 percent of the adult population is lactase non-persistent (LNP), lacking the enzyme required for lactose digestion. The main consequence of intolerance is withholding nutrient-rich dairy foods, while literature shows that many LNPers are able to consume ≥12 grams of lactose, comparable to a 250 ml glass of milk, without experiencing gastrointestinal discomfort. Repetitive consumption of lactose may improve intolerance symptoms even further via the process of colonic adaptation. This study aimed to assess the effects of daily consumption of incremental lactose doses on microbiota composition and function, and intolerance symptoms. 25 healthy adults of Asian origin (age 22-44 years, BMI 19-28 kg/m^2) carrying the LNP genotype and avoiding lactose in their habitual diet were included in this 12-week single-blinded intervention trial. Each participant consumed lactose twice daily, doses being gradually increased from 3 to 6 g, to finally 12 g twice daily, each dose being provided for 4 consecutive weeks. Participants handed in repeated stool samples, and before and after the 12-week intervention participants underwent a 25 g lactose challenge hydrogen breath test. Daily gastrointestinal symptoms and acute intolerance symptoms during the lactose challenge were recorded. There was a significant increase in *Bifidobacterium* upon 12 weeks lactose consumption ($p=0.009$), accompanied by a two-fold increase ($p<0.001$) in faecal β-galactosidase activity. There was a 1.5-fold decrease (AUC; $p=0.01$) in expired hydrogen after the second lactose challenge. A trend was shown towards diminished total symptom score ($p=0.06$) during this second challenge, which was already relatively low during the baseline lactose challenge. Daily consumption of lactose was generally well-tolerated, with mild to no gastrointestinal complaints reported during the intervention.

Repetitive consumption of incremental doses of lactose increases lactose tolerance in LNP individuals via colonic adaptation, most likely through increasing the relative abundance of lactose-fermenting *Bifidobacterium*. Repetitive lactose consumption is well-tolerated and able to reduce expired hydrogen during a 25 g lactose challenge test. Here we show that regular and incremental exposure to lactose in LNP individuals leads to colonic adaptation, without any increase in gastrointestinal symptoms. This lifts the necessity to remove dairy foods completely from the diet.

SESSION 5
FERMENTED FOODS AND HEALTH

FERMENTED FOODS AND HEALTH, AN INDUSTRY PERSPECTIVE

Janneke Ouwerkerk

NIZO food research, the Netherlands

janneke.ouwerkerk@nizo.com

Humans have been using fermentation to produce food for millennia. However, in recent years, there has been a huge resurgence in interest with everyone from home cooks and microbreweries to multinational food companies taking a fresh look at the technology. The attraction of fermentation is clear. It is a natural way to change raw materials into stable, nutritious and tasty foods or beverages. It does not rely on long lists of artificial-sounding ingredients and does not come with the negative image of 'highly processed foods'. Instead, it promotes a natural and artisanal vision of food as it should be. There is also growing evidence that fermented foods can bring health benefits for the consumer. Fermentation can release multiple bioactive compounds that have beneficial effects for human health. Moreover, consuming fermented food has been linked to a more diverse gut microbiota which, in turn, is linked to a healthier gut ecosystem, improved immune system responses and reduced inflammation. Challenging is how to reliably assess if fermented foods lead to health benefits, for instance through changes in the gut microbiome composition and/or metabolism.

As a result of all above, there is a huge amount of innovation happening in fermentation right now. In traditional applications, food producers are looking at ways to improve the process and results of fermentation, for instance by combining different kinds of microbes to produce rich and complex flavour profiles that are hard to reproduce any other way. Meanwhile, exciting new applications for fermentation are opening up beyond the traditional uses of the technology. For example, fermentation is at the forefront of the protein transition and the drive towards more-sustainable food production. It offers unique opportunities to improve the sensory experience and food safety of plant-based proteins. And it can be a direct source of sustainable protein in its own right. For instance, microbial biomass (e.g., brewer's spent yeast, microalgae) has low arable land requirements and can be used for sustainable food production. Thus far, the focus has been on microbial biomass as an alternative protein source. However, other fractions of microbial biomass, in particular the fibre fraction, also have technological functionalities as well as nutritional and health benefits. Exploring these characteristics will add to further valorisation and 'total use' of microbial biomass.

Many of these applications require new fermentation processes based on new feed stocks such as plant-based ingredients that offer a very different environment for microbial growth. They also need new organisms or combinations of organisms to use as cultures. Combining the right organisms with the right substrates from the millions of possible combinations is a huge challenge – and new techniques such as bioinformatics and genetic analysis are key to solving that challenge affordably. In this new world of fermentation, NIZO can support your innovation with confidence. We combine 75 years of research experience in fermentation, protein and food processing, and health with unrivalled infrastructure – including Europe's largest third-party, food-grade pilot plant. NIZO leverages the integrated power of science and technology to support our customers in transforming food and nutrition more successfully, sustainably and faster; ultimately leading to better food and health.

HARNESSING MICROBIOME DATA TO CREATE THE NEXT GENERATION OF FERMENTED FOODS

Paul Cotter

Department of Food Biosciences, Teagasc, Ireland

paul.cotter@teagasc.ie

A tremendous variety of fermented foods are produced by all societies globally. However, there are still many fermented foods that have yet to undergo in-depth microbiome analysis to reveal the diversity of species and strains present therein. An even smaller subset of fermented foods have been the focus of pre-clinical/clinical studies. Despite this, the studies that have taken place show a huge untapped potential. This potential can be achieved through the creation of communities of fermented food microorganisms designed to capture key health promoting, and other, features of fermented foods in a manner that also ensures that highly consistent products can be generated at scale.

FERMENTED DAIRY AND LEGUME-BASED FOOD PRODUCTS: FROM SMART DESIGN OF LACTIC ACID BACTERIA TO INNOVATIVE PRODUCTS

Valérie Gagnaire, F. Guyomarc'h, G. Jan and A. Thierry

INRAE, Institut Agro, Research Unit Science and Technology of Milk and Eggs (STLO), France

valerie.gagnaire@inrae.fr

For reasons linked to lifestyle, health, nutrition and sustainability, our diets are tending to change, in particular by rebalancing animal and plant sources in Western diet. This leads to the development of new food products, particularly fermented ones. Actually, fermentation can confer hedonic, hygienic and probiotic properties to fermented products. Fermented dairy products are well known, while legume-based fermented dairy analogues are less familiar. The development of legume-based fermented products requires a combination of knowledge of the raw material and of bacterial potential, in order to select bacterial strains with appropriate functions.

In our study, new fermented products were developed based on milk, on plant-based analogues, and on the mixes thereof, fermented by lactic acid bacteria and/or propionic acid bacteria, selected on their capability to hydrolyse carbohydrates, proteins and on their immunomodulatory properties. By taking advantage of the functional complementarity between strains, we designed bacterial consortia capable of transforming different raw materials into functional fermented food products, with improved technofunctional and health properties. The development of such fermented products also raises the question of how positive interactions between bacteria can be promoted in order to improve the final qualities of these products.

RAW MILK AND RAW MILK KEFIR FOR THE DIETARY MANAGEMENT OF ALLERGIC DISEASES

B.C.A.M. van Esch[1,2], T Baars[1], S Abbring[1], L van Ooijen[3], Zuomin Zhang[4], P. Dekker[4,5], S. Boeren[5], K. Hetting[4], M. Diks[1], R. Kort[3,6] and **Johan Garssen**[1,2]

[1] Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, the Netherlands
[2] Danone Nutricia Research, the Netherlands
[3] Department of Molecular Cell Biology, Vrije Universiteit Amsterdam, the Netherlands
[4] Food Quality and Design Group, Wageningen University & Research, the Netherlands
[5] Laboratory of Biochemistry, Wageningen University & Research, the Netherlands
[6] Artis-Micropia, the Netherlands

johan.garssen@danone.com

Epidemiological studies have shown an inverse relation between unprocessed cow's milk consumption and the development of asthma and allergies. This protective effect seemed to be abolished by milk processing. Previously, we confirmed the epidemiological findings on asthma and food allergy by showing causality. Current research is aiming at the potency of raw milk kefir to reduce allergic symptoms in models for food allergy. Besides we aimed to characterize the microbial and peptidomic composition of raw milk kefir to address the suppression of allergic symptoms by raw milk kefir in a mouse model for food allergy. Fresh, raw bulk milk was transferred within 1 h after milking, raw or after heating, to a fermentation tank, and kefir was produced after incubation with a defined freeze-dried starter culture. Milk and kefir were sampled before and during fermentation at seven-time intervals. Bacterial and fungal microbiota compositions were determined by V3-V4 16S rRNA and ITS1 amplicon sequencing, respectively. Peptide compositions were determined for the raw and heated milk, and kefir end products made from these milks. Allergy modulating effects of raw milk (Dannwish, Germany) were assessed in a murine allergic asthma model and food allergy model. Kefirs made from either raw or heated milk (100°C; 5 s, the Netherlands) were investigated on their allergy modulating effects in a murine food allergy model. Raw milk was protective in reducing allergic symptoms in models for allergic asthma and food allergy. Effects were preserved after skimming but abolished after pasteurization. Bioactive proteins in the whey fraction of milk are partly responsible for the protective effects. The raw milk used for kefir production contained low numbers of bacteria, less than 10^3 colony forming units per ml. In both kefirs made from either raw or heated milk, we identified amplicon sequence variants identical to those in the starter, matching the bacteria *Lactococcus lactis*, *Streptococcus thermophilus*, *Leuconostoc* and the yeast *Debaromyces*. In raw milk kefir, additional sequence variants of *L. lactis* and the yeasts *Pichia* and *Galactomyces* could be identified, which were absent in heated milk kefir. Analysis of peptide compositions in kefirs indicated that the number and intensity of peptides drastically increased after fermentation. Heating of milk negatively affected the diversity of the peptide composition in kefir. In contrast to kefir made from heated milk, raw milk kefir suppressed the acute allergic skin response to the food allergen ovalbumin in sensitized mice. These effects coincided with differences in the T-cell compartment.

In conclusion, unprocessed raw cow's milk reduces allergic symptoms in a murine model for food allergy and allergic asthma. Raw milk kefir reduced food allergic symptoms. The presence of *L. lactis* and the yeasts *Pichia* and *Galactomyces* combined with a higher diversity in peptides in raw milk kefir might underlie the food allergy protective effect of raw milk kefir.

SESSION 6
BENEFICIAL MICROBES AND THE GUT-BRAIN AXIS

MODULATION OF THE MATERNAL MICROBIOTA AND BEHAVIOURAL EFFECTS IN THE OFFSPRING

Daniel Radford-Smith

Department of Pharmacology, University of Oxford, UK

daniel.radford-smith@pharm.ox.ac.uk

Maternal obesity disturbs brain-gut-microbiota interactions and induces negative affect in the offspring, but its impact on gut and brain metabolism in the offspring (F1) are unknown. Here, we tested whether perinatal intake of a multispecies probiotic could mitigate the abnormal emotional behaviour in the juvenile and adult offspring of obese dams. Untargeted NMR-based metabolomic profiling and gene-expression analysis throughout the gut-brain axis were then used to investigate the biology underpinning behavioural changes in the dams and their offspring. Prolonged high-fat diet feeding reduced maternal gut short-chain fatty acid abundance, increased markers of peripheral inflammation, and decreased the abundance of neuroactive metabolites in maternal milk during nursing. Both juvenile (postnatal day [PND] 21) and adult (PND112) offspring of obese dams exhibited increased anxiety-like behaviour, which were prevented by perinatal probiotic exposure. Maternal probiotic treatment increased gut butyrate and brain lactate in the juvenile and adult offspring and increased the expression of prefrontal cortex *PFKFB3*, a marker of glycolytic metabolism in astrocytes. *PFKFB3* expression correlated with the increase in gut butyrate in the juvenile and adult offspring. Maternal obesity reduced synaptophysin expression in the adult offspring, while perinatal probiotic exposure increased expression of brain-derived neurotrophic factor. Finally, we showed that the resilience of juvenile and adult offspring to anxiety-like behaviour was most prominently associated with increased brain lactate abundance, independent of maternal group.

Taken together, we show that maternal probiotic supplementation exerts a long-lasting effect on offspring neuroplasticity and the offspring gut-liver-brain metabolome, increasing resilience to emotional dysfunction induced by maternal obesity.

GUT MICROBIOME COMPOSITION AND FUNCTIONALITY IMPACT THE RESPONSIVENESS TO A DAIRY-BASED PRODUCT CONTAINING GALACTO-OLIGOSACCHARIDES FOR IMPROVING SLEEP QUALITY IN ADULTS

G.A.M. Kortman[1], E.R. Hester[1], A. Schaafsma[2], J. Mulder[1], L. Mallee[2] and **Arjen Nauta**[2]

[1] NIZO food research, the Netherlands
[2] FrieslandCampina, the Netherlands

arjen.nauta@frieslandcampina.com

Sleep quality has been linked to gut microbiota composition and function through the microbiota-gut-brain axis. Therefore, one strategy to improve sleep quality could be through the modulation of the gut microbiome. The effect of an intervention with a dairy-based product (DP) containing protein and galacto-oligosaccharides (GOS) on gut microbiota composition and function was assessed in a study with 70 healthy adult subjects with moderate sleep disturbances [1]. The study showed a beneficial effect of DP over placebo on sleep quality after 14 days of intervention. Associations of the gut microbiota with sleep quality and with response/non-response to the DP treatment were revealed by shotgun metagenomics sequencing of faecal DNA samples, and subsequent analyses of microbiota taxonomy and generic functionality. A database of manually curated gut-brain modules (GBMs) [2] was applied to analyse specific microbial functions/pathways that have the potential to interact with the brain. A small effect of the DP treatment on gut microbiota composition and function was observed, which could be attributed to several microbiota species. As interindividual variance in microbiota composition could have given rise to a heterogenous responsiveness of the subjects in the intervention group, we zoomed in on the differences between responders and non-responders. Strikingly, there was a compositional difference between responders and non-responders at baseline regarding improved sleep quality. Significant differences were also observed when examining the microbial pathway profiles of responders and non-responders.

Thus, on basis of the microbiome functionality analysis, we found leads with respect to the effectiveness and potential underlying mechanisms of mode of action in sleep improvement that could support future nutritional interventions to aid sleep improvement.

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EARLY-LIFE GUT MICROBIOTA AND NEURODEVELOPMENTAL OUTCOMES

Rochellys Diaz Heijtz

Department of Neuroscience, Karolinska Institutet, Sweden

rochellys.heijtz@ki.se

It is now widely recognized that the microorganisms inhabiting our gastrointestinal tract–the gut microbiota–modulate many aspects of host development and physiology, including the modulation of brain development and behaviour. Growing evidence from preclinical research, cross-sectional clinical studies, and preliminary microbiota-focused intervention studies implicates the gut microbiota as a potential key susceptibility factor in neurodevelopmental and psychiatric disorders, such as autism spectrum disorder (ASD). ASD is a group of heterogenous neurodevelopment conditions, defined by the presence of social communication and interaction challenges in conjunction with restricted, repetitive behaviours and atypical sensory processing. Many individuals with ASD also experience gastrointestinal and immune dysfunction. Currently, there are no approved drugs for treating the core symptoms of ASD. Although the aetiology remains poorly understood, it is widely recognized that genetic and environmental factors and their interactions contribute to ASD phenotypes. One such risk factor is having a sibling with ASD, with studies consistently demonstrating a higher prevalence among siblings and in families with a history of ASD.

Using a prospective longitudinal study design, we recently investigated the developmental profile of the faecal microbiota and metabolome in infants with and without a family history of ASD (in first- or second-degree relatives) across the first 3 years of life [1], a critical period when the gut microbiota and brain are both undergoing rapid development. Our study revealed distinct gut microbial and metabolic developmental profiles of typically developing infants with and without family history of ASD during the first 3 years of life. These differences were more pronounced at 5 months of age and characterized by lower abundance of beneficial *Bifidobacterium* species and GABA, and by an increased abundance of *Clostridium* related species in the faecal samples of infants with a family history of ASD (i.e., infants at elevated-likelihood (EL) of developing ASD). Interestingly, changes in gut microbiota composition and functionality preceded atypical neurodevelopmental trajectories of infants in the EL group, supporting a possible role for the gut microbiota in emerging behavioural variability later in life.

In this presentation, I will discuss potential microbiome-focused strategies to promote healthy development of infants at EL of ASD.

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DISORDERS OF THE GUT-BRAIN AXIS AS A TARGET FOR PROBIOTICS: INSIGHT INTO DEVELOPMENTS OF THE INDUSTRY

Cato Wiegers

Athena Institute, VU Amsterdam, the Netherlands

c.wiegers@vu.nl

The past years have been characterized by a rise in prevalence of mental and neurological disorders, which is causing a high burden on society. Unfortunately, adequate interventions are not always available, if they exist at all. However, as an increasing body of literature demonstrates, the gut-brain axis may provide a new angle for the development of (microbial) clinical modalities. Due to the intricate bi-directional signalling between the brain, the gut, and its microbiota, it may be beneficial to focus on interventions that target the gut microbiota, such as probiotics.

While several studies have already shown promising effects of probiotics for mental and neurological conditions such as Alzheimer's disease, autism spectrum disorder, depression and anxiety, the status of the field is not entirely clear. Besides published articles, a great deal of information regarding the potential of probiotics for gut-brain related disorders can be found in registered clinical trial records and intellectual property such as patents. Together, these documents can provide valuable insight into the development of the probiotic industry. Therefore, we investigated the state of the art of probiotics and their potential as clinical modalities for gut-brain-associated indications by gaining insight into patents and clinical trials that have been applied for and executed since 1999. After the identification of 137 mental, neurological, and gastrointestinal disorders, clinical trial and patent databases were queried for each indication. A total of 565 patents and 390 clinical trials were found, focusing on probiotic applications for 83 indications. Since the start of the 21st century, the highest numbers of patents and clinical trials were related to primary neuropsychological, affective (depression, anxiety) and cognitive disorders, neurodegenerative and/or inflammatory brain disorders (Alzheimer's disease, Parkinson's disease, amongst others), and gastrointestinal disorders (irritable bowel syndrome). The locations where the most patents and clinical trials were registered included China, the United States, and Iran.

Overall, the state of the art of probiotics as clinical intervention modality is showing an increasing interest from the industry as reflected by the number of registered patents, as well as from academia, reflected by registration of clinical trials. However, it appeared that clinical trials showed a slightly slower growth compared to patents. This could indicate a stagnation in the transition from academic knowledge to the development of new probiotic products. In turn, this may have implications for the future implementation of probiotics as clinical modalities for gut-brain-associated indications.

PLENARY SESSION
THE BATTLE: (NON-)SENSE OF COMMERCIAL MICROBIOME TESTING

THE RELEVANCE OF MICROBIOME TESTING FOR THE CONSUMER

Eline Klaassens

BaseClear, the Netherlands

eline.klaassens@baseclear.nl

An overview of microbiome testing kits and their applications will be presented with their benefits for consumers. Several case studies are presented showcasing the use of microbiome information in different subjects together with diet and lifestyle data. We will touch upon expectations for the future of these kind of kits. We will finish with an overall concluding remarks on the relevance of microbiome testing for the consumer, which is open for discussion.

…versus…

IS MICROBIOME TESTING GENUINELY HELPFUL FOR THE CONSUMER?

Karen Scott

The Rowett Institute, University of Aberdeen, UK

k.scott@abdn.ac.uk

There is no doubt that we are now capable of analysing the microbiome of every individual on the planet – if we so desired and had sufficient funding. The question therefore is not ‘Can we do it?’ but rather ‘Why should we do it?’. There is huge variation in the microbiota between every individual, and despite years of effort we still cannot categorically state what composition a healthy microbiome should have. Probably because there is no single specific answer. We have learnt that there are differences between the microbiome in healthy individuals compared to those who have some specific diseases, but only in a few cases have causal links been made to specific bacterial genera. In this part of the discussion, I will question whether finding out about one’s microbiome is merely interesting, or actually useful.

VITAMINS AND EFFECTS ON THE GUT MICROBIOME

Robert E. Steinert, M. Sadaghian and A. Rehman

Health, Nutrition & Care (HNC), dsm-firmenich, Switzerland

robert.steinert@dsm-firmenich.com

The gut microbiome is a complex ecosystem of microorganisms including bacteria, viruses, protozoa and fungi, all of which have been shown to be crucial to human health and well-being. Among other factors such as genetic background, environment, age and health condition, diet is the main determinant of the composition and function of the gut microbiome and its interaction with the host. As contributors to a healthy diet, supplements including pre- or probiotics provide promising strategies for targeted modulation of the gut microbiome. However, this influence is not limited to traditional biotics only, instead recent discoveries suggest that vitamins when, e.g., delivered to the colon in adequate quantities, may beneficially support gut microbial ecosystems. The available data indicate highly interdependent networks of microorganisms constantly trading B-vitamins (e.g., riboflavin, niacin) as essential microbial nutrients to stimulate growth, to provide detoxification of inhibitory molecules or to reduce reactive oxygen species (ROS).

In this talk, I will briefly review the current *in vitro* and clinical evidence related to some of the effect of a selection of vitamins, including riboflavin (vitamin B2), ascorbic acid (vitamin C) and niacin (vitamin B3) on the gut microbiome including mechanisms of action and the possible impact on host health.

POLYPHENOL-RICH EXTRACTS FROM OLIVE LEAVES MODULATE GUT MICROBIOTA COMPOSITION AND METABOLISM IN THE *IN VITRO* MICROCOLON MODEL AND SHOW PROTECTIVE EFFECTS ON THE INTESTINAL BARRIER FUNCTION

Guus A.M. Kortman, I. van Alen, M. Beerthuyzen, E. Lucas-van de Bos, S. van Schalkwijk, G. Staring, S. Jacobs, E.R. Hester, T.P.M. Scheithauer and A. Hartog

NIZO food research, the Netherlands.

guus.kortman@nizo.com

Olive production creates around 4.5 million tonnes of leaves each year. Although rich in bio-resources, only around 0.2% is currently used for extracts. The European OLEAF4VALUE project has been setup to develop a valorisation system for olive leaves. It aims to extract high value bioactive compounds (polyphenols, triterpenoids, essential oils, lipids, lignocellulose) and assess their health benefits. Polyphenols can act as prebiotics on the gut microbiota, and they are indicated to possess antioxidant activity and to suppress inflammation in intestinal cells.

Olive leaf extracts and pure polyphenols were screened for effects on gut microbiota composition and activity in the NIZO MicroColon technology, which is a high-throughput *in vitro* screening model constructed based on human colon-like culture media. Experiments with and without spiking of the pathogen *Clostridioides difficile* into the healthy adult faecal inoculum were performed. Microbiota composition was analysed by qPCR and 16S rRNA gene sequencing. Microbial metabolism was analysed by short-chain fatty acid analysis (HPLC) and polyphenols metabolites analysis by UHPLC-MS/MS. Spent MicroColon supernatant, containing the fermentation derived metabolites was applied to a co-culture model that combines the human intestinal epithelial cell line Caco-2 with the human monocyte/macrophage cell line THP-1, to assess effects on barrier function and immune modulation. Results show that both microbial composition and metabolism can be modulated by olive leaf extracts and pure polyphenols, and that they are being metabolised. Oleuropein enriched extracts (20% and 70%) stimulated, e.g., *Megasphaera* (putatively beneficial) growth. Notably, growth of the intestinal pathogen *C. difficile* was shown to be inhibited by several extracts and pure polyphenols. The oleuropein enriched (20%) extract showed the strongest effect on *C. difficile* inhibition and did not affect beneficial bifidobacteria abundance. Pure polyphenols quercetin and rutin remarkably stimulated the production of butyrate, but without a substantial increase in the relative abundance of typical butyrate producers. Cell culture experiments with differentiated Caco-2 monolayers and THP-1 cells in co-culture, showed protective effects of some extracts/pure polyphenols and MicroColon spent supernatant on recovering intestinal barrier function after an inflammatory challenge.

In conclusion, olive leaf extracts tested show gut microbial modulatory properties, with potential beneficial effects on the host. More in-depth analyses, such as metatranscriptomics analysis and polyphenols metabolites analysis, are ongoing to better understand the mechanisms, and to substantiate the effects. In addition, more (modified) olive leaf extracts and combinations will be assessed in these models soon.

PREBIOTICS FOR THE ATHLETE AND ACTIVE CONSUMER – THE EVIDENCE AND CURRENT STATE OF PLAY

Neil Williams

Sport Health and Performance Enhancement Research Centre, Nottingham Trent University, UK

neil.williams@ntu.ac.uk

Respiratory and gastrointestinal infections can limit an athlete's ability to train and compete. Although athletes may not be susceptible to more infections than the general population, infections typically cluster around important periods of increased training, competition, and travel. This talk will highlight the prevalence of illness in athletes, and how appropriate nutrition strategies that harness the gut microbiota could be used to improve immune tolerance to respiratory and gastrointestinal infections and help manage respiratory disease in athletes and the active individual (asthma, EIB). The proposed interaction between the gut microbiota and respiratory health (often termed the gut-lung axis) in athletes will be highlighted. We will focus on the latest evidence supporting the use of probiotics and prebiotics. There are now numerous studies reporting positive improvements in athlete's health – in particular, a reduction in the risk for upper respiratory symptoms (including infections), asthma, and exercise-induced bronchoconstriction following probiotic and prebiotic interventions – and the talk will critically discuss the existing evidence and apply it into sporting practice.

An important message that the audience will be able to take away from this talk is that sports nutrition practices are not only fuelling the athlete, but also impacting on their gut microbiota and subsequently athlete health.

HUMAN MILK OLIGOSACCHARIDES, INFANT GUT MICROBIOME AND HEALTH: RESULTS FROM THE HELMi BIRTH COHORT

Dollwin Matharu[1], A.J. Ponsero[1], M. Lengyel[2], A. Meszaros-Matwiejuk[2], K.-L. Kolho[1,3], W. de Vos[1,4], D. Molnar-Gabor[2] and A. Salonen[1]

[1] Human Microbiome Research Program, Faculty of Medicine, University of Helsinki, Finland
[2] dsm-firmenich, Denmark
[3] Children's Hospital, University of Helsinki and HUS, Finland
[4] Laboratory of Microbiology, Wageningen University & Research, the Netherlands

dollwin.matharu@helsinki.fi

Early life exposures such as breastfeeding play an important role in shaping infants' health and gut microbiota. Via breastfeeding the infant is exposed to the human milk oligosaccharides (HMOs) that represent the 3rd most abundant solid component in human milk after lipids and lactose. The human body lacks enzymes to digest HMOs, but they can be metabolized by certain gut bacteria, leading to the release of bioactive products with potential beneficial effects on the immune system and brain development.

The HMO composition in breast milk depends on several factors, including the maternal secretor (*FUT2*) genotype. However, the complex crosstalk between HMOs, infant gut microbiota, maternal factors, and child health is still not well understood. Our project is based on the large Health and Early Life Health (HELMi) birth cohort (NCT03996304), leveraging HMO profiles and infant gut metagenome data from a subset of 350 families. In this ongoing study, we explore the associations between the HMO composition, infant microbiota composition and functions, and extensive questionnaire data on maternal factors and early life exposures, as well as child growth and health, focusing on infectious and allergic diseases. HMOs were profiled from breast milk samples collected 3 months postpartum and were analysed for associations with longitudinal microbiota profiles from infant stool samples generated at the age of 3, 6, and 12 months, and child health followed up until 2 years. We determined the absolute and relative abundances of 14 HMOs in the breast milk samples. A vast majority of infants were breastfed over the entire microbiome follow-up period (12 months). In this cohort, 87% of mothers were *FUT2* secretors, producing milk with the dominant fucosylated HMO, 2'-fucosyllactose (2'-FL). Our analysis indicates strong links between the HMO profiles and background and maternal variables, including the season of milk collection and parity. The associations to infant gut microbiome were weaker, and many were only identified when analysed on a birth-mode specific manner. No consistent findings linking the mother's *FUT2* secretor status to child health outcomes were observed in this cohort. Although the HMO composition differs between mothers, our results, in concordance with earlier studies, suggest that most if not all mothers' milk is sufficient in supporting infant gut microbiota.

This study enhances our understanding of infant nutrition, microbiota-mediated health outcomes, and growth. Our findings have implications for advanced nutritional strategies to support infant health and growth.

MODIFICATIONS OF THE RAFFINOSE FAMILY OLIGOSACCHARIDES PROFILE IN PEAS BY PLANT BREEDING INFLUENCE THE HUMAN GUT MICROBIOTA STRUCTURE AND FUNCTION

Aryana Zardkoohi[1-3], T. Rayner[2], A. Bell[1], C. Domoney[2] and N. Juge[1]

[1] Gut Microbes and Health, Quadram Institute Bioscience, UK
[2] Department of Biochemistry and Metabolism, John Innes Centre, UK
[3] Medical School, University of East Anglia, Medical School, UK

aryana.zardkoohi-burgos@quadram.ac.uk

The raffinose family oligosaccharides (RFOs) are a group of galacto-oligosaccharides stored in the seeds of legumes with important biological effects on plant fitness such as resistance to drought or environmental stressors. Here we evaluated the role of RFOs in peas on human health using *Pisum sativum* lines, a commercial cultivar, Cameor; a mutant lacking a major raffinose synthase enzyme (rfs) and lacking RFOs, BCFN 1551, obtained through fast-neutron mutagenesis; and a line obtained through Targeting Induced Local Lesions in Genomes (TILLING) with intermediate depletion of RFOs. LC-MS analyses confirmed a significant reduction in RFOs on both mutant lines compared to the commercial cultivar in both flour and extracts. Using shotgun metagenomics, we showed that supplementation of faecal samples from human donor with individual raffinose and pea flour from Cameor led to increased *Bifidobacterium* population, associated with health-promoting properties. Untargeted metabolomics analysis and LC-MS analysis of short-chain fatty acids of the supernatant revealed an increase in metabolites such as 12-ketolithocholate in the batch fermentations supplemented with BCFN 1551 fermentation, while no major differences were observed in total SCFA or acetate irrespective of the material used for supplementation, pea flour or whole cell extracts. Gas production was monitored using the ANKOM RF gas production system. The cumulative pressure was highest for the fermentation of RFO-containing pea flour, either from Cameor cultivar or the TILLING mutant.

These results showed that the deletion of *rfs* and reduction in RFOs within pea seeds influenced the human gut microbial distribution and metabolic profile after fermentation. Work is on-going to test the effects of peas with variable levels of RFOs on gut barrier function using human intestinal organoids gown on transwells or gut-on-chips.

EFFECTS OF SYNBIOTICS IN CHILDREN AND ADOLESCENTS WITH OBESITY

Ener Cagri Dinleyici

Department of Pediatrics, Faculty of Medicine, Eskisehir Osmangazi University, Türkiye

enercagri@gmail.com

The modification/restoration of gut microbiota has the potential to serve as a therapeutic target for the purpose of reducing energy storage in the host. While a definitive causal link between gut microbiota, diet, and obesity has to be proved, existing research indicates that the use of probiotic, prebiotic, synbiotic, or postbiotic supplements with the goal of enhancing the composition and diversity of the microbiota may yield beneficial outcomes for gut health. The International Scientific Association for Probiotics and Prebiotics (ISAPP) defined synbiotics as “a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host”.

The conventional approach to addressing childhood obesity involves implementing a dietary regimen that restricts energy consumption and promoting physical activity to enhance energy expenditure. Dietary interventions with probiotics, prebiotics or synbiotics aimed at correcting disruption of the gut microbiota observed in obesity or following imbalanced diets may provide health benefits by facilitating weight loss and maintenance. These interventions may confer health benefits by promoting weight reduction and sustaining weight management. Previous studies have demonstrated that the consumption of some probiotics and prebiotics leads to alterations in the microbiota composition, reductions in body weight and fat mass, enhancements in lipid profiles, improvements in fasting glucose and insulin levels, and reductions in inflammatory markers. Numerous investigations have been done to examine the potential therapeutic benefits of probiotics and prebiotics in the context of obesity management and their impact on the gut microbiota composition. However, it is worth noting that the majority of these studies have primarily focused on adult populations. The existing body of research on the impact of synbiotics on childhood obesity is currently constrained in scope. The majority of research conducted on the impact of probiotics and synbiotics on obesity mostly focuses on anthropometric measurements, lipid parameters, and non-alcoholic fatty liver disease. However, there is a limited amount of literature available that specifically examines the effects of these interventions on the composition of intestinal microbiota, particularly in children. Our recent study shown that a 12-week intervention of synbiotic supplementation led to significant alterations in the makeup of the gut microbiota, alongside notable improvements in body mass index values. Following a 12-week synbiotic intervention, a notable increase in the abundance of the genera *Prevotella* and *Dialister* was seen in the synbiotic group as compared to the baseline measurements. A rise in the abundance of *Ruminococcus albus* and *Ruminococcus flavefacine* species was seen, while a decline in the abundance of *Eubacterium dolichum* species was observed when compared to the baseline period. The potential factors contributing to variations in gut microbial community dynamics over time could potentially be elucidated by the administration of synbiotic supplements, potentially in conjunction with body mass index. There is an established association between the human gut microbiota and metabolic disease; however, further investigation is needed to determine the temporal sequence of events, specifically if alterations in the intestinal microbiota precede the onset of inflammation or vice versa. The efficacy of pro-/synbiotics is contingent upon several factors, including the specific strain, the quantity of colony forming units, and the timing of application. It is important to note that the outcomes observed with one strain or preparation cannot be generalized to other strains.

Numerous hypotheses have been put up pertaining to the processes by which pre-/pro-/synbiotics may potentially mitigate weight gain or weight loss in individuals with obesity. The observed effects include the reduction of inflammation, reinforcement of the intestinal epithelial barrier, prevention of bacterial translocation, modulation of intestinal enzyme activity, impact on neuroendocrine and immunological functions, inhibition of energy storage and food intake, decrease in dietary cholesterol absorption, prevention of bile acid reabsorption in the small intestines, and alleviation of intestinal inflammation. There is ongoing research indicating that novel therapeutic strategies targeting the microbiota have promise for addressing obesity.

BEYOND HOLOBIONT: MICROBIOME INTERCONNECTEDNESS THROUGHOUT ENVIRONMENTS

Tanja Kostić

Center for Health and Bioresources, AIT Austrian Institute of Technology GmbH, Austria

tanja.kostic@ait.ac.at

'Microbiomes are in, on and all around us!'. Microbiomes have crucial roles in maintaining life on earth. For example, marine microbiomes produce most of the oxygen we breathe and are indispensable in carbon sequestration and nutrient cycling. Soil microbiomes fix nitrogen and methane, enabling fertilisation and greenhouse gas mitigation effects. The human gut microbiome has clear links with human health, and similarly, plant and animal microbiomes have important roles in plant and animal health.

Although we are starting to understand that microbiomes in different systems are interconnected, there is still a poor understanding of microbiome transfer and connectivity across systems. Current understanding, future perspectives, unresolved issues, and needs pertaining to the microbiome R&D field will be discussed in this presentation.

SOIL MICROBIOME AND SOIL HEALTH AS BASIS OF CROP QUALITY AND GUT HEALTH

Emilia Hannula

Institute of Environmental Sciences, Leiden University, the Netherlands

s.e.hannula@cml.leidenuniv.nl

Soils are unexplored hotspots of (hidden) biodiversity. One spoonful of soil can contain hundreds of species that are connected to each other in complex webs of interactions. The soil biota performs many important ecosystem functions such as nutrient and carbon cycling, they shape the soil structure, and interact with plants that bridge the belowground with aboveground. The most notable of these ecosystem functions is provisioning of food.

Long-term intensive agricultural land management has been focused on crop productivity. Yet this often comes at an ecological cost: it leads to simplification of soil food webs and to reduced microbial diversity which negatively affects many soil functions, including nutrient and carbon cycling, resistance to disturbances such as drought and plant growth and health. Reduced soil microbial biodiversity can also have important consequences for plant-associated microbiomes, which can thereby impact plant nutritional quality and also, according to preliminary plant-studies, how plants taste. On the other hand, it is known that food microbiome and food quality affect gut microbiome and hence gut health. However, there are only handful of studies that have evaluated this whole cycle ranging from soil health to gut health.

In this talk I will first present the concepts of soil health, crop quality and gut health and then discuss the recent findings on the topic of linking soil health with gut health directly through transfer of microbes via plants but also indirectly through changes in crop quality. I will also go through the advancements of methods needed to study transfer of microbes but also to measure chemical quality. I will end by calling for interdisciplinary collaborations to find answers to the burning questions related to this field.

DOES THE (AGRICULTURAL) SOIL MICROBIOME AFFECT HUMAN HEALTH?

Harro Bouwmeester

Plant Hormone Biology lab, Swammerdam Institute for Life Sciences, University of Amsterdam, the Netherlands

h.j.bouwmeester@uva.nl

The soil microbiome, or rather the microbes that are recruited from the soil by plant roots, are increasingly recognized as the second genome of plants, a valuable resource for functions that help plants survive under adverse environmental conditions, such as protection against pathogens and herbivores and providing difficult to get nutrients. In return, plants provide a niche for these microbes, with food and protection. In the Gravitation programme, MiCRop (www.microp.org), we study this relationship, in wild and cultivated crops, with the aim to make better use again of this second genome in a more sustainable agriculture that uses less inputs. Indeed, during millions of years of evolution plants have evolved mechanisms to make optimal use of this mutualistic relationship with the microbiome. For example, plants secrete a complex blend of thousands of molecules into the rhizosphere. The composition of this blend is highly plastic and is strongly affected by environmental conditions, but with a few exceptions, we do not have a clue of the biological significance of most of these molecules. The exceptions, however, paint a picture of enormous complex and intricate relationships. This is illustrated by the strigolactones, a cry for help signal of plants to call in help from AM fungi and Rhizobium, symbiotic organisms that are essential in the acquisition of phosphorus and nitrogen by plants. Indeed, the production of strigolactones is upregulated under P and N deficiency, supporting their ‘cry for help’ role.

During the past 5,000 years of crop domestication and the green revolution, however, one could argue, we have increasingly selected against the dependency of plants on the microbiome and replaced the latter by pesticides and fertilizer. This has resulted in a less efficient colonization of plant roots by these beneficial micro-organisms, as well as a lower diversity, and this likely extends to the products that we harvest from these crops. Moreover, there are indications that the lowered root microbial diversity also results in a lower nutritional value of agricultural products, which may negatively affect human health.

Whether the soil microbiome, through the agricultural products produced on that soil, indeed affects human health is a complex question with many factors involved. There is the community of micro-organisms that is present in an agricultural field (the inoculum), there is the plant host (with its genetic make-up) that is grown on this field and the agricultural inputs such as fertiliser and pesticides that a farmer uses, and then there is the agricultural product harvested from this field and how it is consumed by humans. In NWO-KIC funded project, MicroHealth, we will for the first time experimentally establish the connection between the impact of agriculture on the soil and plant quality/microbiome and the consequences for the health of humans. We will achieve this by studying the impact of farming strategies on the soil and crop microbiome and nutritional composition; through advanced data analysis this will be coupled to human health through an intervention study in the HELIUS cohort tracing microbiome changes and its relation to health status; to maximise impact we investigate opportunities and conflicts in policies and stakeholder-networks and microbiome-related health practises at the household level.

POSTER ABSTRACTS

POSTER INDEX

P8 DEVELOPMENT OF NEW APPLICATION FORM OF PROBIOTIC FEED MIXTURE FOR AQUACULTURE AND EVALUATION OF ITS CHARACTERISTICS

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[1]Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Košice, Slovakia; [2]Department of Pharmaceutical Technology, Masaryk University, Czech Republic; [3]Department of Pharmaceutical Technology, Pharmacognosy and Botany, University of Veterinary Medicine and Pharmacy in Košice, Slovakia; [4]Department of Food Hygiene, Technology and Safety, University of Veterinary Medicine and Pharmacy in Košice, Slovakia; [5]Research Institute for Animal Production, National Agricultural and Food Center, Slovakia

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[1]Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Luxembourg; [2]Department of Naturopathy, Charité Clinical Center, Germany; [3]Department of Translational Science, Movement Disorders Center, Paracelsus-Elena-Klinik, Germany; [4]Department of Life Sciences and Medicine, University of Luxembourg, Luxembourg

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[1]Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, the Netherlands; [2]Danone Nutricia Research, the Netherlands; [3]Department of Pathology and Medical Biology, University Medical Center Groningen, the Netherlands

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[1]Department of Bromatology, University of Belgrade, Serbia; [2]Department of Bromatology, Univrsity of Banja,Bosnia and Herzegovina; [3]Department of Medical Biochemistry, University of Belgrade, Serbia; [4]Department of Cell and Tissue Biology; University of Belgrade, Serbia

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[1]Department of Emergency, Hospital Universitario Infanta Leonor, Spain; [2]Department of Pediatrics, Hospital Universitario Vithas Madrid La Milagrosa, Spain; [3] Probisearch S.L.U, Spain

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[1]INRAE, Institut Agro Rennes-Angers, UMR 1253 STLO, France; [2]INRAE, Institut agro Rennes Angers, UMR1348 PEGASE, France

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[1]Animal Nutrition Group, Department of Animal Sciences, Wageningen University & Research, the Netherlands; [2]Adaptation and Physiology Group, Department of Animal Sciences, Wageningen University & Research, the Netherlands; [3]INRAE, AgroParisTech, UMR Génétique Animale et Biologie Intégrative, Université Paris-Saclay, France; [4]Idena, France

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[1]Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy in Košice, Slovakia; [2]Department of Biology and Physiology, University of Veterinary Medicine and Pharmacy in Košice, Slovakia; [3]National Agriculture and Food Centre, Research Institute for Animal Production, Slovakia

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[1]Department of Microbiology, Nutrition and Dietetics, Czech University of Life Sciences Prague, Czech Republic; [2]Institute of Animal Physiology and Genetics of the Czech Academy of Sciences, Czech Republic; [3]Laboratory of Gnotobiology, Institute of Microbiology, Czech Academy of Sciences, Czech Republic

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[1]Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy, Slovakia; [2]Department of Food Hygiene, Technology and Safety, University of Veterinary Medicine and Pharmacy, Slovakia; [3]Department of Pharmaceutical Technology, Pharmacognosy and Botany, University of Veterinary Medicine and Pharmacy, Slovakia; [4]Department of Zoology, Fisheries, Hydrobiology and Apiculture, Mendel University, Czech Republic; [5]Research Institute for Animal Production, National Agricultural and Food Centre, Slovakia

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[1]School of Pharmaceutical Sciences, University of São Paulo, Brazil; [2]Food Research Center, University of São Paulo, Brazil; [3]Chair of Analytical Food Chemistry, Technical University of Munich, Germany; [4]Chair of Microbiology, Technical University of Munich, Germany

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[1]School of Pharmaceutical Sciences, University of São Paulo, Brazil; [2]Food Research Center, University of São Paulo, Brazil; [3]School of Pharmaceutical Sciences of Araraquara, São Paulo State University, Brazil

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[1]University of Paris Saclay, INRAE, Micalis, France; [2]Department of Plant Sciences, Wageningen University & Research, the Netherlands; [3]UK Centre for Ecology & Hydrology, UK; [4]Living Systems Institute, College of Life and Environmental Sciences, University of Exeter, UK; [5]Ynsect, France; [6]Department of Plant and Environmental Sciences, University of Copenhagen, Denmark

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[1]Microbiome, Nutrition and Health Research Unit, Institute of Agrochemistry and Food Technology, Excellence Center Severo Ochoa-Spanish National Research Council (IATA-CSIC), Spain; [2]Department of Medicine, Autonomous University of Madrid, Spain

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[1]Department of Advanced Convergence, Handong Global University, Korea; [2]Departamento de Alimentos e Nutrição Experimental, Universidade de São Paulo, Brazil; [3]Department of Advanced Convergence, Handong Global University, Korea; [4]Food Research Center, Departamento de Alimentos e Nutrição Experimental, Universidade de São Paulo, Brazil; [5]Center for Research and Development in Agrifood Systems and Sustainability, Escola Superior de Tecnologia e Gestão, Instituto Politécnico de Viana do Castelo, Portugal

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[1]ProBacLab and Laboratório de Microbiologia de Alimentos, Departamento de Alimentos e Nutrição Experimental, Universidade de São Paulo, Brazil; [2]Food Research Center, Departamento de Alimentos e Nutrição Experimental, Universidade de São Paulo, Brazil

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[1]Kemin Animal Health and Nutrition, Kemin Europa N.V., Belgium; [2]BioLizard, Belgium; [3]BIOBIX, Department of Data Analysis and Mathematical Modelling, Ghent University, Belgium

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[1]Yili Innovation Center Europe, the Netherlands and [2]Yili Innovation Center, China

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[1]Department of Biotechnology, Jožef Stefan Institute, Slovenia; [2]Faculty of Pharmacy, University of Ljubljana, Slovenia; [3]Department for Microbiological Research, National Laboratory of Health, Environment and Food, Slovenia; [4]Department of Microbiology, University of Maribor, Slovenia

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[1]Yakult Europe, the Netherlands; [2]University of Copenhagen, Denmark; [3]ILVO, Belgium; [4]Maastricht University, the Netherlands; [5]University of Leeds, UK; [6]ADM, UK; [7]Novozymes, Germany; [8]UKSH and University of Lubeck, Germany; [9]Caelus Health, the Netherlands; [10]Cryptobiotix, Belgium; [11]ILSI Europe, Belgium; [12]WUR, the Netherlands; [13]IFF, Finland

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SACCHAROMYCES CEREVISIAE: MULTIFACED APPLICATIONS IN ONE HEALTH AND THE ACHIEVEMENT OF SUSTAINABLE DEVELOPMENT GOALS

Nathalie Ballet[1], S. Renaud[2], H. Roume[1], F. George[1], P. Vandekerckove[1], M. Boyer[1] and M. Durand-Dubief[1]

[1]Discovery & Front-End Innovation, Lesaffre Institute of Science & Technology, Lesaffre International, France; [2]Efor, France

n.ballet@lesaffre.com

Through a comprehensive analysis of existing literature, we explore the multifaceted roles of *Saccharomyces cerevisiae* (SC) in the One Health paradigm. This holistic health model, which emerged in response to the avian flu H5N1 outbreak, promotes an integrated perspective on the well-being of humans, animals, and the environment, aligning seamlessly with the United Nations' Sustainable Development Goals (SDGs). Traditionally a cornerstone in baking and brewing, SC has revealed its versatile nature by serving as an eco-friendly alternative to industrial fertilizers and chemical biocontrol agents, while also demonstrating bioremediation capabilities for toxic metals. In the sphere of animal health, SC boosts growth and immune function, serving as a sustainable alternative to antibiotic-heavy feed regimens. In human health, SC goes beyond its traditional use as a flavour additive to act as a beneficial probiotic, assisting in the treatment of Inflammatory bowel diseases (IBDs) and candidiasis. In summary, *Saccharomyces cerevisiae* is a versatile microorganism that plays a significant role in advancing the One Health initiative and fulfilling the SDGs, thereby opening new pathways for sustainable health practices and environmental conservation.

P2

POSITIVE EFFECTS OF A NEW DESIALIZING PROBIOTIC BLENDS IN THE OUTCOME OF CANINE CHRONIC ENTEROCOLITIS

G. Rossi[1], A. Gavazza[1], M. Cerquetella[1], D. Olivero[2], G. Pengo[3], L. Galosi[1], F. Carnevali[4] and **Lucia Biagini**[1]

[1]School of Bioscience and Veterinary Medicine, University of Camerino, Italy; [2]BiEsseA scil S.r.l. Laboratorio Analisi Veterinarie, Italy; [3]Clinica Veterinaria San Antonio, Italy; [4]Centro Ricerche Casaccia, Italy

lucia.biagini@unicam.it

Chronic enterocolitis is a very common clinical problem in dogs and cats. This pathological condition involves many causes, but nutrition can represent, in addition to a primary triggering factor, a possible complication when the pathology is already underway. Neu5Gc is indeed synthesized from its N-acetyl precursor (Neu5Ac) by cytidine-5′-monophospho-N acetylneuraminic acid hydroxylase (CMAH), absent in humans, and polymorphic in dogs. The study evaluates the possible role of N-glycolyl-neuraminic acid (Neu5Gc) (xenosialization) dietary absorption in the induction/sustentation of dog lymphoplasmacytic enterocolitis and the effect on the clinical signs of a new probiotic blend (FSG6822®) administered for a period of 60 days. Neu5Gc in stools of 10 healthy and 10 enteropathic dogs, enrolled on the basis of the clinical and histopathological diagnosis of chronic enterocolitis, was evaluated by ELISA, before and after 60 days of administration of probiotic FSG6822® rich in live bifidobacteria with sialic acid cross-feeding activity. Neu5GC was highlighted with specific antibody (Creative Diagnostic, DMABH-C003). At the moment of dog's enrolment, the distribution of desializing bacteria in faecal material was performed using two different sequencing techniques for different regions of the 16S rRNA gene (healthy vs enteropathyc). Neu5Gc histologically expressed in enterocolic biopsies and shedded in faeces was associated with severe colitis ($p<0.005$), and at T0, the faecal microbiota of enteropathic dogs revealed a higher prevalence of *Clostridiales* and *Bacteroidales* ($p=0.0011$), without difference of *Bifidobacteria*, compared with controls. In enteropathic dogs, 60 days of *FSG6822*® administration, reduced Neu5Gc fecal shedding and clinical signs. To conclude, in dogs, the severity of colitis correlates with Neu5Gc and with an increase in *Clostridiales* and *Bacteroidales*. In the absence of an increase in desializing bifidobacteria this predisposes to xenosialitis. Actually, FSG6822® probiotic mixture shows good levels of reduction in faecal elimination of Neu5Gc and could reduce xenosialization.

P3

IN OVO ADMINISTRATION OF ESPECIALLY TREATED INTESTINAL CONTENT OF ADULT CHICKENS ENRICHED WITH A HIGH BIFIDOBACTERIAL SPECIAL BLEND, CAN REDUCE POSTNATAL COCCIDIOSIS, ENTERITIS AND MORTALITY IN CHICKEN

Lucia Biagini, L. Galosi, S. Mari, V. Grifantini, A. Roncarati and G. Rossi

School of Bioscience and Veterinary Medicine, University of Camerino, Italy

lucia.biagini@unicam.it

Coccidiosis is one of the major parasitic diseases in the commercial broiler industry due to its high mortality and morbidity rate, resistance to existing anti-coccidial drugs and limited effectiveness of commercial vaccination. Several studies describe reduction of oocysts shedding and improvement of enteric lesions in *Eimeria* spp. experimental infected chickens, thanks to probiotics administration. 150 embryonated eggs were divided into three treatment groups of 50 eggs with 3 replicate cages of 15 birds each. At day 18 of incubation, eggs were subjected to *in ovo* inoculation into amniotic fluid of the following mixtures: UFC $1x10^5$ of probiotic bacteria (Slab51®) in 0.05 ml of reconstituted Marek disease vaccine (PROB group); UFC 50 of freeze dried and tyndallized product obtained from the intestinal contents of healthy adult chickens (In Ovo patent ®) enriched with live probiotic bacteria UFC $1x10^5$ (FSG 6822®) in 0.05 ml of reconstituted Marek disease vaccine (TYND group); 0.05 ml of reconstituted Marek disease vaccine (CON group). After hatching, all chickens were raised for 42 days using the same facility, separated by nets. Stool samples were collected weekly from each replicate to assess oocyst shedding using the McMaster chamber. At 42 days of age, 5 chickens per replicate were sacrificed by cervical dislocation to collect intestinal samples for histopathological evaluation using a gravity score (0-4) for intestinal coccidial lesion. Results showed the presence of oocyst in faeces of TYND group from day 14 while absent in the other groups. The number of oocyst shedding then progressively decreased from the next sampling up to the end in the TYND group, while an exponential increase in oocyst counting was observed in the PROB and CON groups from day 14 to the end of the trial (day 42). These results are in agreement with the histopathological analysis where, in the different intestinal tracts, more severe lesions are detected in the CON group, associated with widespread inflammation. Although not subjected to experimental infection, these results suggest that chickens treated *in ovo* with In Ovo patent ® mixture were more resistant to natural environmental infection. Early oocysts shedding could be due to an early infection, following *in ovo* administration, which mimics a form of vaccination supported by the stimulation of the immune system. These results are preliminary and will have to be further accredited by an experimental infection.

P4

HUMAN MILK METABOLITES ARE BIOACTIVE AND CAN MODULATE GUT PHYSIOLOGY: *IN VITRO* STUDY IN A PLURICELLULAR MODEL OF INTESTINAL EPITHELIUM

Sarah Blanchet[1], M. Bostoën[1], V. Romé[1], S. Even[2] and S. Blat[1]

[1]Institut NuMeCan, INRAE, INSERM, Université Rennes, France; [2]UMR STLO, INRAE, Institut Agro, France

sarah.blanchet@inrae.fr

Human milk (HM) is associated with major short- and long-term health benefits for infants. However, infant formulas (IF), substitutes for HM, are widely used for infant nutrition. Although these IFs meet the nutritional needs of newborns, they are devoid of many bioactive compounds present in HM, such as immunoglobulins, hormones and a multitude of metabolites. HM metabolites include short chain fatty acids (SCFAs) (butyric acid, acetic acid, propionic acid), polyamines (putrescine, spermine, spermidine), tryptophan derivatives (indole, indole-lactic acid, kynurenine) as well as GABA and lactate. These metabolites are known to be produced within the gastrointestinal tract by the intestinal microbiota and to have effects on the host physiology but their relevance in HM has not been studied so far. The objective of our study was, therefore, to decipher the effects of HM metabolites on intestinal physiology using an *in vitro* pluricellular and polarized model of intestinal epithelium, including Caco-2 (as enterocytes), HT-29 MTX (as goblet cells), NCI-H716 (as enteroendocrine cells) and M cells. HM metabolites were studied at concentrations close to those found in HM, which is quite low. Their effects on trans-epithelial electric resistance (TEER) and on the expression of genes involved in the intestinal barrier, immune, antioxidant, endocrine and digestive functions were analysed. SCFAs strongly modulated different intestinal functions, particularly the immune one with a significant downregulation of genes coding for IL-8, MyD88 and TFF3. They also modulated genes encoding tight junctions, as did GABA and polyamines, upregulating CLDN3, TJP1 and CLDN4, respectively, and downregulating CLDN1 for SCFA and polyamines and CLDN7 for GABA. In parallel, SCFAs significantly increased TEER, highlighting a potential reinforcing effect on the epithelial barrier while polyamines and GABA had no effect on TEER. Finally, SCFAs, GABA and lactic acid modulated the expression of some transporters involved in nutrition, such as MCT1, GLUT1 and SGLT1, respectively. In conclusion, our results demonstrated for the first time that HM metabolites, despite their low concentration, are able to impact the intestinal barrier physiology, inviting us to consider their implementation in IF for a better mimicry of HM health benefits.

P5

INTESTINAL EPITHELIAL BARRIER INTEGRITY: IMPLICATIONS FOR HOST HEALTH AND MICROBIAL INFLUENCE

Nikoleta Boteva[1], I. Mukhopadhya[2], J.C. Martin[1], K.P. Scott[1] and S.W. Gratz[1]

[1]Gut Microbiology Group, Rowett Institute, University of Aberdeen, UK; [2]Institute of Medical Sciences, University of Aberdeen, UK

nikoleta.boteva@abdn.ac.uk

The maintenance of the intestinal epithelial barrier integrity is crucial for sustaining host homeostasis. Preserving functions such as active and selective nutrient absorption, impermeability, and the physical barrier for microorganisms and microbial products, the intestinal epithelial cell layer has paramount importance for maintaining host health. Many diseases and conditions including colon cancer, inflammatory bowel disease, diabetes mellitus, autism, non-alcoholic steatohepatitis, celiac disease, necrotizing enterocolitis, systemic inflammation, and septic complications are linked to epithelial disruption and barrier loss. Epithelial barrier repair capacity has important implications in maintaining barrier function. The influence of the gut microbiota on intestinal epithelium regeneration is well known. Different gut microbiome-derived metabolites can modulate epithelial cell proliferation. The main microbial fermentation end-products – acetate, butyrate, propionate, and lactate – are well-known inducers or repressors of epithelium cell physiological pathways. This study aimed to assess the capacity of key members of the human gut microbiome to alter the epithelium repair. An *in vitro* epithelial scratch assay with the human intestinal cell line Caco-2 was performed to assess the impact of commensal human gut bacteria on epithelium repair. Members of *Lachnospiraceae*, *Coriobacteriaceae*, *Prevotellaceae*, and *Bifidobacteriaceae* families showed taxa-specific influences on epithelium repair, not necessarily correlated to the type and quantity of the short-chain fatty acids produced. Some distinct patterns of epithelium repair were observed to be strain specific. This study utilises a relatively simple assay to demonstrate differential effects of bacterial supernatants on the repair of the gut epithelium, that are independent of short-chain fatty acid presence. **Acknowledgements**. This work was sponsored by Probi AB.

P6

A POPULATION-BASED STUDY OF THE HUMAN FAECAL MICROBIOME ACROSS AGES IN THE NETHERLANDS

David Boverhoff[1,2], J. Kool[1], R. Pijnacker[1], S. Shetty[1,2], Q.R. Ducarmon[3], G. Zeller[3], S. Sie[1], A.C. Mulder[1], F. van der Klis[1], E. Franz[1], L. Mughini-Gras[1,4], D. van Baarle[1,2] and S. Fuentes[1]

[1]Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), the Netherlands; [2]Virology & Immunology Research, Department of Medical Microbiology and Infection Prevention, University Medical Center Groningen, the Netherlands; [3]Structural and Computational Biology Unit, European Molecular Biology Laboratory, Germany; [4]Institute for Risk Assessment Sciences, Utrecht University, the Netherlands

david.boverhoff@rivm.nl; susana.fuentes@rivm.nl

The human gut microbiome has been associated with numerous disease and health parameters. With high inter- and intra-individual variation, human gut microbiome diversity and composition is impacted by many environmental exposures and host factors (e.g., genetics), many of which remain unclear. Here, we described the faecal microbiome viewed across ages (0-89 years old) in a population-wide and ethnically representative cross-sectional study of the Netherlands (n=3,692) using 16S rRNA gene sequencing. This approach allowed us to validate earlier findings (infant – adult trajectories) in the context of less explored influences, such as culture-related factors, but also identify new associations. Microbiome diversity, composition and functionality were analysed and associated with participant exposures (e.g., human and animal contact) and other characteristics (e.g., age, sex, and socio-economic status) collected through comprehensive questionnaires. Microbiome diversity showed a steep rise in the first years of life, peaking at approximately 10 years of age. Additionally, composition was markedly different in early life, with an initial dominance of *Actinobacteriota* in the first year of life, transitioning to *Firmicutes* and *Bacteroidota* dominated profiles in the years after. Variables with a significant impact on the adult microbiome (25-87 years) were mainly cultural (country of birth, ethnical background, origin of father and mother), explaining the highest proportion of variance in both diversity and community structure. These results were corroborated by shotgun metagenomic sequencing on a subset of samples (n=95, ages 20-74 years), both in composition and functionality. In addition, obesity-related factors showed an association with microbiome composition and functionality, such as acetate metabolism and species contributing to different metabolic modules. In conclusion, we found both known (e.g., medication use) and novel associations (e.g., sex across age and ethnical background across the Netherlands) with microbial diversity and composition, which will allow us to define a baseline faecal microbiome across ages in a Western-like population of heterogenous background. This will aid the definition of a healthy microbiome throughout the lifespan, which is key for designing microbiome-related therapeutic interventions.

P7

TRANSCRIPTOMIC RESPONSE OF *SACCHAROMYCES CEREVISIAE* CNCM I-1077 IN RUMEN FLUID INCUBATIONS REVEALS UNIQUE METABOLIC ADAPTATION AND CAPACITY OF INTERACTION WITH KEY FIBROLYTIC BACTERIAL SPECIES FROM RUMEN MICROBIOTA

L. Guillot[1,2], L. Dunière[1,2], E. Forano[2] and **Frédérique Chaucheyras-Durand**[1,2]

[1]Lallemand SAS, France; [2]UCA-INRAE UMR MEDIS, France

fchaucheyrasdurand@lallemand.com

Live yeasts are fed to ruminants with the aim to improve rumen function with measured benefits on animal health and performance. It has been reported that the effects may differ according to the yeast strain considered. In addition, if their effects on rumen microbiota have been quite well described, the mechanisms by which yeast strains may produce various effects are still poorly understood. Therefore, we carried out gene expression microarray studies to investigate the behaviour of *Saccharomyces cerevisiae* (Sc) CNCM I-1077 strain by measuring differential gene expression when incubated in rumen conditions (filtered rumen fluid, strict anaerobiosis, 39°C) compared to laboratory conditions (YPD medium, aerobiosis, 30°C). In a second step, we compared gene expression profile of three strains of *S. cerevisiae* incubated in rumen conditions. Finally, we analysed the transcriptome of *S. cerevisiae* I-1077 in a co-culture *in vitro* model with *Fibrobacter succinogenes* S85, a major fibre degrading rumen bacteria strain. When incubated in rumen conditions for 10 h, 395 genes were significantly differentially expressed by Sc I-1077 (I log2FC I>2) compared to laboratory conditions. The most overexpressed genes were related to stress response and sugar transport notably nutrient alternative to low levels of glucose, whereas the most underexpressed were related to amino acid metabolism. Discriminant analysis of gene expression data allowed to clearly separate the three yeast strains incubated in the same ruminal conditions. The most expressed genes in Sc I-1077 were related to metabolism with significant differences in log2 intensity for genes involved in carbohydrate metabolism, in particular hexose and pentose, and in stress response (heat, oxidative and osmotic stress). In the co-culture with *F. succinogenes* S85, Sc I-1077 transcriptome was oriented as well towards sugar metabolism and stress response, and an overall benefit of the addition of Sc I-1077 was measured on *F. succinogenes* fermentation end products. Taken together, our data suggest that Sc I-1077 reacts to its environment by adapting its metabolic activity to rumen conditions. Different strains of *S. cerevisiae* have different transcriptomic profiles highlighting a strain-specific behaviour in the rumen environment which can thus trigger different effects. The metabolic capacity of Sc I-1077 allows the strain to interact with key rumen bacteria leading to further improvement in hydrolytic and fermentative activities of rumen microbiota.

P8

DEVELOPMENT OF NEW APPLICATION FORM OF PROBIOTIC FEED MIXTURE FOR AQUACULTURE AND EVALUATION OF ITS CHARACTERISTICS

Natália Chomová[1], A. Franc[2], S. Pavloková[2], M. Sondorová[1], D. Mudroňová[1], A. Fečkaninová[3], P. Popelka[4], J. Koščová[1] and R. Žitňan[5]

[1]Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Košice, Slovakia; [2]Department of Pharmaceutical Technology, Masaryk University, Czech Republic; [3]Department of Pharmaceutical Technology, Pharmacognosy and Botany, University of Veterinary Medicine and Pharmacy in Košice, Slovakia; [4]Department of Food Hygiene, Technology and Safety, University of Veterinary Medicine and Pharmacy in Košice, Slovakia; [5]Research Institute for Animal Production, National Agricultural and Food Center, Slovakia

natalia.chomova@student.uvlf.sk

Infectious diseases are currently one of the most serious problems in aquaculture. The use of antimicrobial agents for the therapy and the prophylaxis of diseases in large-scale fish farms potentiates the emergence and spread of bacterial resistance. Substances of natural origin (probiotics, humic substances, etc,) appear to be promising candidates for prevention of disease and transmission of bacterial resistance. Therefore, the aim of this work was to develop new economically available and technologically undemanding coating technology for pellets containing verified probiotics. Applied probiotic strain *Lactiplantibacillus plantarum* R2 Biocenol™ (CCM 8674) was selected based on already tested selection criteria and presence of plantaricin-related genes. At first, the commercial pellets were coated with colloidal silica (Aerosil® 200) which created hydrophilic surface and increased surface area. Second coating layer consisted of hydrogel dispersion formed from starch and concentrated probiotic suspension. Prepared probiotic feed was evaluated for various physical characteristics (weigh uniformity, friability, density, hardness) and compared to the uncoated cores and coated control without probiotic layer. Pellets were subjected to the examination of morphology employing scanning electron microscopy. Investigation of the viability of probiotic strain were performed during eleven months at 4°C and at 22°C by the plate count method. *In vitro* release kinetics of probiotic bacteria in water and artificial gastric juice were also tested. Chemical analysis and nutrient content were determined in uncoated cores and probiotic pellets to compare quality of pellets. Coating of pellets did not worsen any of physical properties, on the contrary, several of them were ameliorated after the process. Results showed that probiotic pellets could be stored at 22°C for short time period (approximately 3 months). However, storage at 4°C ensure stable number of living probiotic bacteria even up to 10 months. Determined release of probiotics had rising trend with sufficient number of living bacteria and it was gradual throughout the tested period (24 h) into both environments without any bigger differences. Only slight differences were noted in the composition of basal and probiotic feed, moreover some nutrients were in higher concentrations in probiotic feed (e.g., rough fibre, valine, leucine, lysine). Thus, newly developed coating technology is able to improve properties of commercial pellets and is suitable for aquaculture. This assumption was already tested during *in vivo* experiment with rainbow trout *(Oncorhynchus mykkis*) during which high acceptance of probiotic feed was noted. **Acknowledgments**. This work was supported by the Slovak Research and Development Agency under the contract no. APVV-19-0234.

P9

DEVELOPMENT OF A SYNBIOTIC MIXTURE TO DECREASE INTESTINAL NEURO-INFLAMMATION AND RESTORE SHORT-CHAIN FATTY ACID PRODUCTION IN PARKINSON'S DISEASE PATIENTS

Charlotte De Rudder[1], L. Grandmougin[1], B. Hansen[1], C.C. Laczny[1], C. Jäger[1], D. Liebscher[2], S. Schade[3], A. Michalsen[2], B. Mollenhauer[3] and P. Wilmes[1,4]

[1]Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Luxembourg; [2]Department of Naturopathy, Charité Clinical Center, Germany; [3]Department of Translational Science, Movement Disorders Center, Paracelsus-Elena-Klinik, Germany; [4]Department of Life Sciences and Medicine, University of Luxembourg, Luxembourg

charlotte.derudder@uni.lu

Parkinson's Disease (PD) is primarily known as a central nervous system disorder, yet it could start in the gut. Gastro-intestinal symptoms precede motor symptom onset with years to decades, and evidence supports PD initiation by enteric inflammation and changes in gut microbial composition (i.e., reduced fibre-degrading bacteria) and function (i.e., reduced short chain fatty acid (SCFA) production). Based on observed structural and functional microbiome shifts, we aim to develop a synbiotic, a combination of probiotic bacteria and a prebiotic fibre, to decrease intestinal (neuro-)inflammation and to increase SCFA production. Bacterial species were selected based on their metabolic properties and reduction in PD patients' gut microbiota compared to healthy individuals. An *in silico* safety screening (PathoFact) of the resulting mixture was performed for antibiotic resistance genes, virulence factors, toxins, and for genes for proteins known to interfere with PD medication. Effects of the synbiotic on epithelial barrier function (transepithelial electrical resistance), inflammation (IL-8 release), cell viability (alamarBlue assay, CASY cell counter), and cytotoxicity (lactate dehydrogenase) were assessed in intestinal epithelial cell (IEC) culture models, in co-cultures of IEC and induced pluripotent stem cell (iPSC)-derived enteric neurons (EN) in tissue culture inserts, and in neuroHuMiX, a gut-on-chip model enabling studying microbial-IEC-EN interactions. Our results show that the synbiotic mixture did not cause inflammation or cytotoxicity in IEC and IEC-EN co-cultures in tissue culture inserts, nor affected viability. The synbiotic mixture increased epithelial barrier function ($p<0.05$) in an IEC model, yet in IEC-EN co-cultures, this increase was not significant. In follow-up experiments, the synbiotic's effect on neurotransmitter release will be examined. Furthermore, probiotic SCFA production will be tested, and the synbiotic's effect on SCFA production will be tested in PD patients' and healthy individuals' faecal cultures. This can result in a mechanistically supported synbiotic for prophylactic treatment in at-risk individuals, and mitigation of intestinal symptoms and disease progress in PD.

P10

SYNBIOTICS, A PROMISING APPROACH TO REDUCE THE EXACERBATED ALLERGIC AIRWAY IMMUNE RESPONSES IN OFFSPRING MATERNALLY EXPOSED TO CIGARETTE SMOKE

Ali Dehghani[1], L. Wang[1,3], J. van Bergenhenegouwen[1,2], J. Garssen[1,2], G. Folkerts[1] and S. Braber[1]

[1]Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, the Netherlands; [2]Danone Nutricia Research, the Netherlands; [3]Department of Pathology and Medical Biology, University Medical Center Groningen, the Netherlands

a.dehghani@uu.nl

Air pollution remains one of the biggest and most immediate environmental threats to human health. Environmental cigarette smoke (CS) exposure serves as a primary contributor to household air pollution in both developing and developed countries. Exposure to air pollution has detrimental effects on human health, starting during early pregnancy and persisting throughout childhood and adolescence, ultimately contributing to the development of severe respiratory diseases. Considering the importance of immune development in early life, the present preclinical study investigated the effects of synbiotic supplementation on allergic asthma symptoms in house dust mite (HDM)-sensitized and challenged pups maternally exposed to CS. Pregnant dams were exposed to either CS or air during pregnancy and lactation. After weaning, offspring received a control or synbiotic diet, and were intranasally sensitized and challenged with HDM to induce allergic asthma. After the HDM challenges, the lung function, bronchoalveolar lavage (BAL) cell counts, T cell subsets in the lungs and antigen-specific serum immunoglobulins were measured in the offspring. In addition, the composition of the gut microbiome was analysed. In HDM-sensitized and challenged offspring of CS-exposed dams, lung resistance and antigen-specific serum immunoglobulins (IgE and IgG1) were significantly higher compared to the PBS-treated group. Synbiotic supplementation effectively mitigated this increase in lung resistance and elevated immunoglobulin levels. HDM-sensitized and challenged offspring of air-exposed dams showed increased numbers of eosinophils in BAL fluid, while maternal CS exposure further enhanced this inflammatory response in the offspring. Synbiotic supplementation significantly reduced the eosinophil numbers in BAL fluid of HDM-treated offspring from air and CS-exposed dams. Furthermore, Th2 cell activation was higher in HDM-allergic offspring born to CS-exposed mothers, and synbiotic supplementation tended to decrease the infiltration and activation of Th2 cells. Additionally, synbiotic supplementation altered the composition and richness of the gut microbiome, favouring the presence of beneficial microbes, such as *Bifidobacterium* and *Akkermansia*. In conclusion, early life synbiotic supplementation can exert a beneficial influence on allergic asthma by reducing the exacerbated allergic airway responses, and altering the diversity and composition of the gut microbiome in HDM-challenged offspring maternally exposed to CS. These findings suggest the potential of synbiotics as an immediate and safe clinical strategy for the management of allergic asthma in context of maternal air pollution exposure.

P11
SKINBAC™ CARE: EXPLORING THE POTENTIAL OF BIOTICS IN SKIN HEALTH

Giovanni Deusebio, A. Visciglia, A. Bissone, D. Zogno, S. Allesina, A. Amoruso and M. Pane

Probiotical Research S.r.l., Italy

g.deusebio@probiotical.com

The human skin is a complex organ that provides a protective barrier against the external environment, preventing the invasion of pathogens and fending off chemical and physical assaults, as well as the unregulated loss of water and solutes. Different microorganisms, including bacteria, fungi and viruses, colonize the skin and their interactions with the skin itself and its environment have been the aim of the cosmetics and dermatology fields. The microbiome of healthy skin varies depending on age and body area, with differences in sebaceous, moist, and dry areas. Lifestyle, ultraviolet light (UV) exposure and presence of skin disorders also impact the skin microbiome. Recently, increasing evidence suggests topical application of probiotics and postbiotics as potential care product annotating them considerable benefits for skin. Aim of this work was the evaluation of safety and efficacy, *in vitro* and *in vivo*, of Skinbac™ strains, to unravel their potential in skin health. Heat-treated (HT) strains *L. salivarius* Skinbac™ SB04*, L. reuteri* Skinbac™ SB02*, B. breve* Skinbac™ SB03, collectively known as Skinbac™ Care, were assessed. *In vitro* experiments were conducted using normal human epidermal keratinocytes (NHEK). Safety was determined by MTT and LDH assays, while efficacy was evaluated by measuring aquaporin3 (AQP3), claudin4 (CLND4) and occluding (OCLN) expression, the reduction of reactive oxygen species (ROS), the modulation of cytokines (e.g., TNF-α, IL-6, IL-8 and IL-23), and pathogen inhibition. Skinbac™ Care was then incorporated as unique active ingredient in a cream and tested to evaluate trans-epidermal water loss (TEWL) after single application, lenitive and restoring efficacy on skin experimentally damaged by sodium lauryl sulfate (SLS), and efficacy in preventing the skin damage induced by the irritating SLS. Data reveal that Skinbac™ Care was safe for NHEK allowing the possibility to use it for topical application. *In vitro*, Skinbac™ Care has resulted effective in increasing AQP3 expression (+18% vs. untreated cells – $p<0.05$), in reducing ROS (-53% vs. untreated cells – $p<0.05$), in increasing CLDN4 (+12% vs. damaged cells – $p<0.05$) and OCLN (+11% vs damaged cells), in pathogen inhibition by reducing *Staphylococcus aureus* growth and biofilm, and in modulating cytokines. *In vivo*, statistically significant improvements have been obtained in TEWL after single application and in skin barrier damage. In conlusion, all the collected results demonstrate that Skinbac™ Care has different beneficial skin properties as important role in the maintenance of barrier regulation and integrity, and moisturizing, antioxidant and antipathogen effects suggesting their potential in skin health.

P12

EFFECTS OF PROBIOTIC SUPPLEMENTATION IN OBESE WOMEN – CORRELATIONS BETWEEN ANTHROPOMETRIC PARAMETERS AND SELECTED EPIGENETIC MARKERS

Brizita Djordjevic[1], N.D. Ivanovic[1], N. Okuka[2], N. Milinkovic[3] and K. Velickovic[4]

[1]Department of Bromatology, University of Belgrade, Serbia; [2]Department of Bromatology, Univrsity of Banja,Bosnia and Herzegovina; [3]Department of Medical Biochemistry, University of Belgrade, Serbia; [4]Department of Cell and Tissue Biology; University of Belgrade, Serbia

brizitadjordjevic@gmail.com

In the last decade, the gut microbiota has been identified as one of the influential environmental factors that play a critical role in the development and progression of obesity and obesity-related diseases. Research on probiotics has made progress in identifying the precise molecular mechanisms underlying the health-promoting properties of probiotics, which are still poorly understood. In particular, the results of several studies suggest that epigenetic influences may be mediators of the interaction between host and microbiome or probiotics. The main objective of this study is to investigate the effects of a new probiotic product on the expression of certain miRNAs crucial for regulating inflammation and adipogenesis, and to examine if there is correlation with miRNA expression and anthropometric parameters. Twenty overweight/obese women participated in a randomized, placebo-controlled, double-blind study and were randomly divided into two groups: the intervention group (daily one capsule containing *Lactobacillus plantarum* 299v (DSM9843), *Saccharomyces cerevisiae* var. *boulardii* and 40 mg octacosanol; n=12) and the placebo group (n=8). Changes in miRNA expression and anthropometric parameters were assessed before and after the 12-week intervention period. After the intervention, positive correlation was found between the miR-24-3p expression and total cholesterol (r=-0.667, p=0.05), and BMI positively correlated with miR-155-5p expression (r=-0.597, p=0.05). Namely, patients whose BMI were lower had lower miR-155-5p expression, and patients whose TC levels were lower had lower miR-24-3p expression. In conclusion, although this study has limitations, particularly the relatively small sample size that restricts the generalization of the results, our results provide a new perspective for evaluating the beneficial effects of a new probiotic formulation intended for the prevention and treatment of obesity.

P13

EFFECT OF A NEW PROBIOTIC FORMULATION ON THE EVOLUTION OF COVID-19 PATIENTS AND THEIR IMMUNOLOGICAL PROFILE

S. Iglesias López[1], A. López-Escobar[2], V. Ortiz Heras[1], M. Sánchez Pérez[1], I Cuevas-Gómez[3], N. Cárdenas[3], I. Espinosa-Martos[3], S. Manzano[3] and **Sergio Esteban**[3]

[1]Department of Emergency, Hospital Universitario Infanta Leonor, Spain; [2]Department of Pediatrics, Hospital Universitario Vithas Madrid La Milagrosa, Spain; [3] Probisearch S.L.U, Spain

sergio.esteban@probisearch.com

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly transmissible virus that have caused a global outbreak of the pandemic disease COVID-19. Few treatments have been identified to improve infection resolution. However, studies have shown that probiotics can modified the immune response against respiratory virus. A multicenter, randomized, double-blind, placebo-controlled clinical study was conducted in adults diagnosed with COVID-19. The objective of this study was to evaluate the effectiveness and tolerance of the probiotic strain *Ligilactobacillus salivarius* PS7 on SARS-CoV-2 infection resolution and the evolution of its main immunological markers. Patients were treated for 28 days with 1 capsule/day containing 10^9 CFU of *Ligilactobacillus salivarius* PS7 or placebo. Blood and nasopharyngeal samples were collected at the beginning of treatment, and after 7 and 28 days of treatment. Anti-SARS-CoV-2 specific antibodies (IgG and IgM) and the main immunological markers were assessed in blood sample using ELISA and multiplex technology. Additionally, the presence of SARS-CoV-2 in nasopharyngeal samples was evaluated by qPCR. Thirty-eight patients completed the study, among them, 20 were treated with the probiotic strain and 18 with placebo. No differences were detected in anti-SARS-CoV-2 specific antibodies levels between both groups. However, the SARS-CoV-2 positivity rate by qPCR after 28 days of treatment was lower in patients treated with the probiotic formulation ($p<0.05$). Furthermore, patients treated with the probiotic strain had a higher concentration of cytokines responsible for the activation of the immune system, such as IL-15, IP-10 and MCP-1 ($p<0.05$) after one week of treatment. After 28 days, patients of the probiotic arm showed lower levels of IL-5 and a decrease in the levels of IL-17. In conclusion, the probiotic supplementation of COVID-19 patients with *Ligilactobacillus salivarius* PS7 showed immunomodulatory capacity and produced an early resolution of SARS-CoV-2 infection compared to placebo treatment.

P14
CANADIAN FERMENTED FOOD INITIATIVE

Yi Fan[1], K. Sampsell[2], C. Marcolla[1], R.A. Reimer[2], B. Willing[1] and J. Burton[3]

[1]University of Alberta, Canada; [2]University of Calgary, Canada; [3]Western University, Canada

fan5@ualberta.ca

Fermented foods, known for their potential health benefits, have deep historical and cultural roots in Canada influenced by diverse indigenous and immigrant communities. Yet, our understanding of their roles in Canadian society remains incomplete. This project covers three primary areas of fermented foods in Canada. *Advancing fermented foods research*. We conducted an extensive exploration of Canadian research on fermented foods and their health benefits. Our approach involves a comprehensive literature review synthesizing insights from Canadian and global studies. While dairy products like cheese and yogurt received substantial attention, we identify gaps warranting further investigation. Notably, we highlight emerging areas like botanical fermented foods (e.g., kimchi, kombucha, and plant-based dairy alternatives) offering rich research opportunities. Further, while some studies analysed the gut/fermented food microbiome, few assessed live-organisms, impeding the identification of beneficial microbes and their health impact. *Assessing the educational and commercial landscape*. We evaluated the courses of all 16 Canadian universities offering dietetic degrees. Across 11 universities, we identified 31 courses with content related to fermented food or microbial fermentation, indicating that most students in these programs are likely to acquire knowledge of fermented foods during academic training. Furthermore, we conducted a broad assessment of Canada's fermented foods market by surveying non-specialty grocery stores nationwide. It revealed that many global fermented foods are available in Canada with some exceptions such as soured cassava and fermented yam. In contrast, when assessing food-based dietary guidelines worldwide, most countries including Canada, prioritize fermented dairy products leaving limited standards for the diverse fermented foods. *Closing knowledge gaps and promoting awareness*. To bridge knowledge gaps between beneficial microbes, fermented foods and the reported human health outcomes, we are working towards establishing standardized workflow for future fermented food research and development. Employing high-throughput technology and bacterial culture, we profiled microbial communities and metabolites in commercial kombucha and kefir, aiming to unravel the connection between microbes, metabolites and human health. Also, a comprehensive nation-wide human survey was designed to gauge public knowledge, consumption habits, and perceptions of fermented foods in Canada, with a focus on assessing the potential for further product development and knowledge dissemination. Our ultimate goal is to provide insights to benefit all Canadians including consumers, researchers, healthcare providers, and policymakers. Empowering stakeholders with evidence-based information, we seek to unlock the full potential of fermented foods to enhance the health and well-being of the Canadian populace through informed adoption, research, education, and product development.

P15

POST-MILKING APPLICATION OF A *LACTICASEIBACILLUS PARACASEI* STRAIN ON THE TEAT SKIN AS A NEW MICROBIAL STRATEGY TO PREVENT BOVINE MASTITIS

Coralie Goetz[1], J. Cuffel[1], L. Rault[1], P. Poton[2], S. Philau[2], G. Boullet[2], A. Mottin[2], J. Orinel[2], M. Boutinaud[2] and S. Even[1]

[1]INRAE, Institut Agro Rennes-Angers, UMR 1253 STLO, France; [2]INRAE, Institut agro Rennes Angers, UMR1348 PEGASE, France

coralie.goetz@agrocampus-ouest.fr

Bovine mastitis is an inflammation of the mammary gland generally due to an intramammary infection. Prophylactic treatments mostly rely on post-milking teat disinfection with chemical products such as iodine. However, the latter are questioned due to a possible irritation of the teat skin but also dissemination of residues in milk and environment. Besides, the antibiotics commonly used to treat mastitis are unfortunately not entirely effective and may contribute to the risk of dissemination of antimicrobial resistance, inviting us to explore alternative solutions. Here, a microbial strategy to prevent mastitis was explored, aiming to improve the barrier effect of the teat microbiota, through the application of a lactic acid bacteria (LAB) strain. Firstly, the impact of *Lacticaseibacillus paracasei* CIRM BIA 1542 application on the teat skin was explored on 23 Holstein cows in mid-lactation. Treatment (LAB, iodine or no treatment) was applied twice a day post-milking on the 4 quarters of healthy animals for 15 days. Blood, milk samples and teat skin swabs were collected at day 1, 8, 15 and 26 to evaluate the LAB treatment impact at the microbial, immune and physiological levels. Data were analysed using an analysis of variance based on mixed models. *L. paracasei* CIRM BIA 1542 was transiently present on teat skin and in foremilk during the 15 days of treatment, but not in the cisternal milk. Total microbial population on teat skin, in foremilk and cisternal milk was significantly increased in LAB-treated cows compared with iodine-treated cows ($p<0.05$). However, no pathogen was found in cisternal milk. Ongoing analyses will evaluate the impact on teat skin and in foremilk microbiota using metataxonomic. In addition, LAB treatment did not trigger any major inflammatory response in the mammary gland: no significant impact was observed on milk somatic cell scores, although interleukin 8 released in milk tended to be slightly higher in LAB-treated cows compared with others. Finally, LAB treatment had no impact on the functionality and the integrity of the mammary epithelium, as revealed by no significant effect on milk yield and composition, nor on the mammary epithelial cell exfoliation rate into milk or the milk Na^+:KA^+ ratio. Altogether, these results indicate that a topical treatment with *L. paracasei* CIRM BIA 1542 is safe with regard to mammary gland physiology and immune system, while impacting its microbiota, inviting us to further explore its effectiveness for mastitis prevention.

P16

STRAIN SCREENING AND APPLICATION OF VITAMIN K2-PRODUCING MICROORGANISMS IN FERMENTED DAIRY PRODUCTS

Fabio Grasso[1], C. Bär[1], B. Walther[1], U. von Ah[1], R. Portmann[1], E. Binz[1], S.C. Ganal-Vonarburg[2], S. Christensen[2] and G. Vergères[1]

[1]Agroscope, Switzerland; [2]Department of Visceral Surgery and Medicine, Bern University Hospital, University of Bern, Switzerland

fabio.grasso@agroscope.admin.ch

Fermented foods have gained popularity in recent years due to their distinct flavour profiles and multiple health benefits. Among the different bioactive substances found in fermented foods, vitamin K2 (menaquinone) has emerged as a crucial factor in improving human health. This study presents an in-depth exploration of Agroscope's strain collection and application of vitamin K2-producing microorganisms in the production of fermented dairy products. The primary objective of this study was the identification of microorganisms capable of synthesizing vitamin K2 during the fermentation process and to investigate which form of vitamin K2 is produced by which strain. To achieve this goal, the genomes of bacteria were screened to explore the individual potential of each bacterial strain to produce vitamin K2. Subsequently, the strains were evaluated phenotypically for their vitamin K2 production and best-performing strains were selected for application in fermented dairy products. With the goal to design a framework for enhancing vitamin K2 production in fermented dairy products, which will eventually lead to the development of functional foods with improved nutritional profiles, this work also elucidates the key parameters influencing vitamin K2 production, such as fermentation conditions, substrate selection, and strain-specific variations. In conclusion, our work illuminates the promising potential of microorganisms that produce vitamin K2 in the field of fermented food production. It also highlights differences in the form of vitamin K2 produced by different strains and strain combinations, which will be investigated in a next step in mouse and organoid-based studies for variations in bioavailability, thus providing a roadmap for the development of functional and health-promoting fermented foods by optimizing strain selection, fermentation conditions, and application tactics, thereby contributing to the ever-evolving field of nutrition and food science.

P17

THE FRUIT OF LIFE: USE OF A POMEGRANATE EXTRACT TO SUPPRESS THE MICROBIAL PRODUCTION OF PROTHROMBOTIC TMA

Julia Haarhuis, P. Day-Walsh, M. Gamal El-Din, E. Shehata, B. Peck, S. Saha and P. Kroon

Quadram Institute Bioscience, UK

julia.haarhuis@quadram.ac.uk

High plasma levels of trimethylamine N-oxide (TMAO) can cause prothrombotic effects in humans [Zhu *et al.*, 2017. Circulation 135: 1671-1673]. L-carnitine is a major dietary precursor of TMAO, which is metabolised by the gut microbiota to γ-butyrobetaine (γ-BB) and finally to trimethylamine (TMA). In the liver, TMA is converted to TMAO. Interventions that have been suggested to reduce the microbial production of TMA include broad-spectrum antibiotics [Wang *et al.*, 2015. Cell 163: 1585-1595], but long-term use of antibiotics can cause loss of beneficial gut microbiota. The aim of this study was to investigate the potential of a polyphenol-rich pomegranate extract to inhibit TMA production from the dietary precursor L-carnitine by gut microbiota.*In vitro* fermentation models were inoculated with 1% faecal inoculum from different healthy donors (n=4, ClinicalTrials.gov, NCT02653001), 2 mM L-carnitine, and a pomegranate extract (5.7, 11.4, or 22.8 mg/ml). Batch fermentations were carried out under colonic conditions (anaerobic, pH 6.66-7.12, 37°C). Additionally, a 96-well plate-format fermentation model [Iglesias-Carres *et al.*, 2021. Nutrients 13: 1466] was carried out in an anaerobic cabinet at 37°C to screen the effect of the main pomegranate polyphenols: ellagic acid, punicalagin and gallic acid. Samples were collected from both models over 48 hours. The concentrations of L-carnitine, γ-BB, and TMA were quantified in the samples using LC-MS/MS. The pomegranate extract inhibited the metabolism of L-carnitine to γ-BB and TMA in a dose-dependent manner, showing higher L-carnitine concentrations at 10 and 12 h after inoculation for all doses ($p<0.001$) and at 20 h for the highest dose ($p<0.01$). After 48 h, TMA production was significantly inhibited for all pomegranate doses ($p<0.01$). Upon screening of the isolated polyphenols, punicalagin could completely abolish TMA production from L-carnitine at all time points ($p=0.008$, compared with the control at 48 h). In conclusion, we demonstrated that the pomegranate extract and punicalagin suppressed L-carnitine conversion to γ-BB and TMA. This may subsequently reduce the levels of the prothrombotic TMAO. Prospectively, a pharmacokinetic human study will be performed to investigate the potential for the pomegranate extract to suppress TMAO production from L-carnitine.

P18
MOLECULAR CHARACTERIZATION OF PROTEINS ENCODED IN THE INTESTINAL MICROBIOTA THAT MIMIC THE DDP4/CD26 FUNCTION

Paula Hernández-Calderón and A. Benítez-Paez[1]

Host-Microbe Interactions in Metabolic Health Laboratory, Principe Felipe Research Center (CIPF), Spain

phernandez@cipf.es

The gut microbiota has gained recognition for conditioning human health and serving as the basis for the development of innovative therapeutic approaches. Nevertheless, disease causality is poorly explored, and massive association studies are strongly influenced by functional redundancy. In this study, we adopted a targeted functional metagenomics and gene-centered approach to explore the presence and impact of DPP4-like activity in the intestinal microbiota on host metabolism and health. Our hypothesis proposed is that the intestinal microbiota encodes DPP4-like activity, which could significantly influence host metabolism and overall health outcomes linked to glucose metabolism, control, satiety and behaviour. We discovered that the bacterial enzyme DPP4-like shares a similar ability to inactivate human GLP-1 and other enteroendocrine hormones and neuropeptides. Furthermore, we tested the inhibitory capacity of different gliptins, inhibitors of the human DPP4 used as pharmacological therapy in type-2 diabetes (T2D), to demonstrate their role in inhibiting the bacterial DPP4-like enzyme. Our results strongly suggest bacterial DPP4-like enzymes escape gliptin inhibition leaving an open highway to study the multiple implications this could have in the treatment and evolution of T2D. Our research line is promising to delineate either the molecular basis of metabolic dysfunction in obesity and pave the way to develop novel therapeutic approaches focused on tackling this particular microbial functional mimicry usurping control of metabolic circuits in the host.

P19

DAIRY STARTERS AS '2-IN-1' PROBIOTIC MICROORGANISMS FERMENTING FOODS AND MODULATING GUT MUCOSAL IMMUNITY

N. Illikoud[1], M. Mantel[1,2], M. Rolli-Derkinderen [2], V. Gagnaire [1] and **Gwénaël Jan**[1]

[1]INRAE, Institut Agro, STLO, France; [2]Université de Nantes, Inserm, TENS, The Enteric Nervous System in Gut and Brain Diseases, IMAD, France

gwenael.jan@inrae.fr

The gut microbiota plays a key role in the regulation of mucosal immunity and of the function of the intestinal barrier. In line with this, dysbiosis of the gut microbiota is associated with rupture of mucosal immune homeostasis, leading to inflammatory bowel diseases (IBD). IBD can cause acute clinical response and the existing treatments may be responsible for severe side effects. In a context of growing prevalence of these diseases, probiotic bacteria, including a new generation of intestinal probiotics, can maintain intestinal homeostasis, mitigate inflammation, and promote health. Surprisingly, little is known about the impact of fermented dairy products, and of the bacteria they contain, on gut inflammation, while they represent our main source of live and active dietary bacteria. Indeed, they provide, through our daily diet, a high number of bacteria whose effects on mucosal immunity deserve attention. Among bacteria ingested in fermented dairy products, *Streptococcus thermophilus*, *Lactobacillus delbrueckii*, *Lactobacillus helveticus*, *Lactococcus lactis* and *Propionibacterium freudenreichii* are on top, as they are ingested in high concentrations (close to 10^9 per gram of product) in fermented milks or cheeses. Selected strains, within each of these bacterial species, are reportedly able to down-regulate the inflammatory response *in vitro* and to mitigate the severity of colitis *in vivo*. This oral communication will give an overview of the potential immunomodulatory effects of these main dairy starters. It will further explore studies dealing with fermented dairy products containing these starters, in a context of inflammation. A better knowledge of these strains, of corresponding fermented foods, and of their effects, opens avenues for the development of targeted functional fermented foods.

P20

DIET COMPOSITION INFLUENCED PROBIOTIC AND POSTBIOTIC EFFECT ON BROILER CAECAL MICROBIOTA

Samuel C.G. Jansseune[1-4], F. Blanc[3], W.H. Hendriks[1], J. van Baal[1], Aart Lammers[2], A. Mahieu[4], M. Aoun[4], M.-H. Pinard van der Laan[3] and F. Calenge[3]

[1]Animal Nutrition Group, Department of Animal Sciences, Wageningen University & Research, the Netherlands; [2]Adaptation and Physiology Group, Department of Animal Sciences, Wageningen University & Research, the Netherlands; [3]INRAE, AgroParisTech, UMR Génétique Animale et Biologie Intégrative, Université Paris-Saclay, France; [4]Idena, France

samuel.jansseune@idena.fr

Microbiota play a key role in gut health of chickens. Its composition and functionality are affected by feed ingredients and may be modulated by probiotics (Pro) and postbiotics (Post). This study aimed to assess the effects of 2 diets (standard [SD], challenge [CD]) on the effects of a lactobacilli-based Pro and Post on broiler caecal microbiota and metabolome. Following a 2×3 randomised block design, day-old Ross male broilers (n=1368) were fed 1 of 2 diets (SD, CD) and 1 of 3 additives (control, Pro, Post) from day 1 to 42 with 6 pen replicates per treatment. SD and CD were formulated to contain identical levels of nutrients, with CD formulated to be richer than SD in non-starch polysaccharides. Caecal microbiota composition was assessed by 16S rRNA gene sequencing for 3-4 broilers/pen at day 14 and 35, respectively. Variations in α- and β-diversity indexes were analysed by ANOVA and for contrasts. Operational taxonomic unit (OTU) abundances were compared by differential abundance analyses. At day 14, Observed and Chao1 α-diversity indexes were higher for Pro compared to control in SD, and lower for Post compared to control in CD ($p<0.05$). At day 35, no effects of additives were observed, while the latter indexes were lower for CD compared to SD. At day 14, Bray and Jaccard β-diversity indexes were affected by diet only. However, at day 35, these indexes showed an interaction effect, with a significant difference for Post CD vs. control CD and control SD vs. control CD. At day 14, 2 OTU were differentially abundant (DA) for Pro and Post compared to control for SD, whereas 6 and 17 OTU, respectively, were DA for Pro and Post compared to control for CD. At day 35, Pro and Post had no DA OTU compared to control for SD. By contrast, for the CD, Pro and Post had 37 and 44 DA OTU compared to control, respectively. Four and 86 OTU were DA between the SD and CD controls at day 14 and 35, respectively. In conclusion, Pro and Post modified broiler caecal microbiota in an age and diet dependent manner. In particular, their effects were more pronounced at day 35 in the CD. Pro and Post effects on microbiota are in line with their effects observed for broiler growth, since Pro and Post increased body weight in CD without effects in SD. This highlights the importance of diet composition to assess Pro and Post effects on gut microbiota.

P21

PROBIOTIC SUPPLEMENTATION OPTIMIZES GUT MICROBIOTA COMPOSITION AND IMPROVES INTESTINAL MORPHOLOGY OF WEANED PIGLETS

I. Cuevas-Gómez, J. de Andrés, N. Cardenas, I. Espinosa-Martos and **Esther Jiménez**

Probisearch S.L.U., Spain

esther.jimenez@probisearch.com

Post-weaning diarrhoea in piglets remains an important cause of economic losses for swine producers. Feed supplementation with probiotics is one of the alternatives to antibiotics that can be considered to reduce the impact of such gastrointestinal disease. The aim of the present study was to evaluate the effect of *Ligilactobacillus salivarius* PS21603 supplementation on the intestinal structure and the gut microbiota composition of weaned piglets. Safety and tolerance of *L. salivarius* PS21603 were previously evaluated in a 28-days study using 384 weaned piglets (28±2 days old and 7.5±1.5 kg) divided in three treatment groups: (i) T1, basal diet + *L. salivarius* PS21603 10^9 CFU/day; (ii) T2, basal diet + *L. salivarius* PS21603 10^7 CFU/day; (iii) and T3, basal diet (control group). For the present study, 16 piglets per treatment group were randomly selected and faecal samples were collected on day 0 (weaning) and day 28 of study. At the end of study, three males and three females per treatment were euthanized. Intestinal morphometric values were measured after necropsy. Faecal counts of *E. coli* were evaluated by culture techniques, and faecal microbiota composition was assessed by high-throughput sequencing. All data were analysed and compared between treatment groups. The results of the present study showed that supplementation with *L. salivarius* PS21603 increased the intestine length of piglets from T1 group of treatment and the villous height:crypt ratio of piglets from T2 group of treatment ($p<0.05$). In addition, piglets from T1 and T2 had lower faecal *E. coli* counts than T3 at the end of study ($p<0.05$). Moreover, supplementation with *L. salivarius* PS21603 modulated gut microbiota through a more optimal composition, reducing *Escherichia* and increasing *Bifidobacterium* relative abundance in piglets from T1 ($p<0.05$). Therefore, the strain *L. salivarius* PS21603 has shown probiotic properties to be used as feed additive in the pig industry, along with good hygiene and farm management practices, for the prevention and/or treatment of post-weaning diarrhoea in piglets.

P22

THE EFFECT OF *ENTEROCOCCUS FAECIUM* AL41 ON INTESTINAL MUCOSAL PARAMETERS IN BROILER CHICKEN

Viera Karaffová[1], R. Szaboová[2], R. Herich[1], Z.Ševčíková[1], V. Revajová[1], R. Žitňan[3] and E. Hudec[1]

[1]Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy in Košice, Slovakia; [2]Department of Biology and Physiology, University of Veterinary Medicine and Pharmacy in Košice, Slovakia; [3]National Agriculture and Food Centre, Research Institute for Animal Production, Slovakia

viera.karaffova@uvlf.sk

The use of antibiotics in livestock is the main reason for the development of resistant bacterial strains, including pathogens with zoonotic potential. While feeding probiotic bacteria, including *Enterococcus faecium*, into the feed may represent a promising solution. In this study, we investigate the effect of *Enterococcus faecium* AL41 on the gene expression of selected molecules of mucosal immunity (immunoglobulin A, mucin-2, insulin like growth factor 2, olfactomedin 4, occludin, claudin, lumican) and mucus production (all parts of the small intestine) in broilers. Eighty broiler chicks were divided into groups control and *E. faecium* AL41 (birds were inoculated with AL41 for 7 days) group. The experiment lasted 11 days. Changes in transcription and relative expression of selected genes were measured by real-time PCR on a LightCycler 480 II (Roche) using specific primers according to a predefined program. The relative expression of target genes was normalised to an average Cq value of the reference genes (glyceraldehyde 3-phosphate dehydrogenase). The amount of produced mucus was determined by the ELISA assay. Our results showed that the administration of *E. faecium* AL41 had a significant stimulatory effect on the relative expression of all tested molecules ($p<0.05$; $p<0.01$; $p<0.001$) as well as on the dynamics of mucus production in the chicken intestine. We assume that administration of *E. faecium* AL41 may have a beneficial effect on the intestinal mucosa of broiler chicken. **Acknowledgements**. This research was funded by the Slovak Research and Development Agency (APVV-21-0129) and the Grant agency for Science of Slovakia VEGA č. 1/0098/22 from the Ministry of Education, Science, Research and Sport of the Slovak Republic.

P23
GUT MICROBIOTA MODULATORY CAPACITY OF FERMENTED KETCHUP

Kübra Küçükgöz[1,2]

[1]Department of Food Gastronomy and Food Hygiene, Institute of Human Nutrition Sciences, Warsaw University of Life Sciences (SGGW), Poland; [2]Centre for Healthy Eating & Food Innovation, Maastricht University, the Netherlands

kubra_kucukgoz@sggw.edu.pl

This study aimed to assess the impact of fermented beetroot ketchup enriched with *Lactobacillus johnsonii K4* and non-fermented beetroot ketchup, formulated with tomato concentrate, beetroot, spices, and vinegar, on pooled faecal microbiota obtained from healthy adults. The influence of these products on the composition and functionality of the gut microbiota was investigated within a validated dynamic *in vitro* model of the colon, the TNO Intestinal Model (TIM-2). Predigested and freeze-dried ketchup products were implemented as a single 60 g dose after the starvation phase of experiment, and the potential probiotic strain *Lactobacillus johnsonii K4* was added for three days, and a carbohydrate mixture of standard ileal effluent medium (SIEM) was used as control. Through our analysis, we identified 21 taxa that exhibited statistical significance (q-value <0.2) when comparing ketchup samples to control samples. Within these taxa, significant patterns emerged, providing insights into their potential implications for human health. Specifically, the ketchup samples demonstrated an increase in the butyrate-producing taxa *Faecalibacterium*, *Blautia*, *Ruminococcaceae*, *Ruminiclostridium* 6, and *Anaerostipes*. There was also a reduction in *Desulfovibrio* and *Escherichia-Shigella,* both potentially pathogenic species. These findings show that beetroot ketchup has the potential to positive influence the gut microbiota composition.

P24
LACTOBACILLUS GASSERI HN910: A PROMISING PROBIOTIC FOR ACNE THERAPY

Chaewon Lee[1], L. Pei[2] and C. Sung Huh[3,4]

[1]WCU Biomodulation Major, Department of Agricultural Biotechnology, Seoul National University, Korea; [2]SZCO Cosmetic, Korea; [3]Graduates School of International Agricultural Technology, Seoul National University, Korea; [4]Research Institute of Eco-friendly Livestock Science, Institute of Green Bio Science and Technology, Seoul National University, Korea

lhw37@snu.ac.kr

Acne, along with eczema, pruritus, and fungal skin diseases, constitutes one of the four most prevalent dermatological conditions. Its occurrence is notably high among women (9.81%) compared to men (8.96%), irrespective of age. Traditional treatments frequently involve antibiotics such as macrolides, clindamycin, and tetracycline; however, antibiotic resistance and adverse effects pose considerable impediments. Probiotics have emerged as a promising alternative due to their potential to modulate the skin microbiome and exert beneficial effects. Within the confines of this investigation, we isolated and identified 246 isolates from the epidermis of 16 healthy female utilizing a high-throughput screening technique, with a specific focus on lactic acid bacteria (LAB). Among these isolates, *Lactobacillus gasseri* HN910 (HN910) stood out due to its remarkable competitive exclusion attributes, which encompass antioxidant, antimicrobial, anti-quorum sensing, and antibiofilm properties. HN910 exhibited high sensitivity to antibiotics, evident by its minimum inhibitory concentration (MIC) surpassing the EFSA standard, while successfully passing safety assessments. Moreover, it displayed an industrially advantageous growth curve and featured a distinctive fermentative and enzymatic profile. Additionally, we explored the potential utility of HN910 lysate, acquired through cellular disintegration via a Microfluidizer, for cosmeceutical purposes. HN910 lysate exhibited no significant impact on the viability of keratinocytes while demonstrating the capacity to suppress the inflammatory response in macrophages. To further comprehend the genetic makeup of HN910, its whole genome sequence was analysed and compared with *Lactobacillus gasseri* ATCC 33323, a type strain of the same species. The genome size of HN910 (1.86 Mbp) was smaller than that of ATCC 33323 (1.89 Mbp), and there was also a difference in functional analysis. The findings of this study suggest that HN910 functions as both a probiotic and a postbiotic, offering potential benefits in improving acne-prone skin. The identified antioxidant, competitive exclusion activities and anti-inflammatory effects further support the potential of HN910 as a novel therapeutic approach for acne.

P25

SEX-RELATED CHANGES IN EARLY-LIFE ON MICROBIOTA, GUT PERMEABILITY AND ILEAL GENE EXPRESSION IN BROILER CHICKENS

E. Arevalo Sureda[1], N. Smeets[2], **Jeroen Maertens**[2] and N. Everaert[1]

[1]Nutrition and Animal Microbiota EcoSystems Laboratory, Department of Biosystems, KU Leuven, Belgium; [2]Kemin Europa NV, Belgium

jeroen.maertens@kemin.com

In the poultry industry, both sexes are reared together despite their differences in growth and endocrine system. There is the need for research on sex-related differences for optimisation of health and performance. Hence, the aim of this study was to investigate differences between females and males in microbiota, gut permeability, and ileal gene expression in broiler chickens in early life. One-day-old chicks Ross 308 (144 females and 144 males) were divided into four pens each and monitored until 21 days under optimal health conditions, consuming a diet rich in protein and non-starch polysaccharides (NSP) from wheat and rye. Ileal tissue and content samples were collected, and *in vivo* gut permeability was assessed using FITC-dextran-4kDa. Sampling occurred at 1 and 3 days (mixed intestinal content), and at 7, 14, and 21 days (ileal content). Analysis included body weight, microbiota (V1-V9 16sRNA sequencing), gut permeability, and ileal gene expression (high-throughput qPCR analysis). As expected, weight showed sex differences at 21 days. Mixed intestinal microbiota showed that all α-diversity indexes increased markedly, and β-diversity showed significant differences between 1 and 3 days, indicating that microbiota colonisation occurred. Ileal microbiota showed a peak in α-diversity at 14 days that significantly decreased at 21 days, when sex-related differences appeared, with lower α-diversity in males. Also, β-diversity showed a trend for sex-related differences at 3 and 7 days. Gut permeability showed a decrease with age and a sex effect at 21 days, with higher permeability in females. Gene expression showed sex-related differences at different ages. At 7 days, differences were observed in genes related to barrier function: expression of fatty-acid-binding-protein-2 (FABP2) gene was increased in males, whereas the expression of junctional-adhesion-molecule-3 (JAM3) and occludin genes was higher in females. At 14 days, the cytochrome P450 (CYP450) gene, enzyme that processes xenobiotics, showed an effect of sex, with higher expression in males. At 21 days, the genes related to nutrient transport and absorption, Solute Carrier Family 2 Member 5 (SLC2A5), a fructose transporter, and SLC7A9 (cationic amino acid transporter), showed higher expression in males. Age was not a main factor of interest of the study, but many of the investigated genes showed significant effects of age. Differences in microbiota and gene expression could be observed between sexes soon after hatching. Microbiota, permeability and expression of nutrient-transport related genes showed differences at 21 days, coinciding with the sex-related growth curve divergence. Thus, sex differences should be considered from early life when optimizing health and performance.

P26

DISCOVERY OF NOVEL ANTIMICROBIAL COMPOUNDS BY INVESTIGATING THE GENETIC POTENTIAL OF LACTIC ACID BACTERIA

Thibaut Maeyens, J. Dillen, E. Cauwenberghs and S. Lebeer

Research Group Environmental Ecology and Applied Microbiology, Department of Bioscience Engineering, University of Antwerp, Belgium

thibaut.maeyens@uantwerpen.be

In the near future, humankind will be faced with major challenges concerning antibiotic resistance, necessitating immediate action to avert a surge in casualties caused by antibiotic-resistant pathogens. To address this pressing issue, innovative treatment strategies are urgently required, with microbiome therapeutics like live biotherapeutic products and probiotics showing great potential. In our ongoing research efforts, two promising probiotic candidates have emerged: *Lactobacillus crispatus* isolate 815 and *Lactobacillus casei* AMBR2 isolated from the vagina and upper respiratory tract (URT) respectively. When compared to other isolates, both strains showed an exceptionally strong ability to inhibit niche-specific pathogens. Therefore, these strains hold promise for treating and/or preventing site-specific conditions. However, before these strains can be employed in therapeutic applications it is crucial to gain insights into the underlying mechanisms behind their beneficial properties. Specific antimicrobial ribosomally synthesized and post-translationally modified peptides (RiPPs) were postulated to contribute to the superior antipathogenic activity of these strains. To test this hypothesis, genome mining was used to screen their genomes for the presence of specialized metabolite clusters as these often produce antimicrobials (e.g., bacteriocins). Confirming our suspicion, each strain possessed unique RiPPs, potentially underpinning their superior inhibitory capacity. *L. crispatus* isolate 815 contained a novel class IV lanthipeptide cluster, a class with only few characterized members. Subsequent *in silico* characterization of the cluster led to the identification of three potential propeptides and the associated lanthipeptide-processing enzyme, along with predictions for their cross-links and structures. To validate the antimicrobial activity of these peptides, they were expressed in a heterologous host, *Escherichia coli*, facilitating their purification and subsequent characterization. Notably, all evaluated lanthipeptides demonstrated significant inhibitory activity against key vaginal pathogens. Similarly, the *L. casei* AMBR2 genome revealed distinct RiPP clusters harbouring propeptides and processing enzymes for which further characterization of these peptides and their activity, could support the use of this strain as a live biotherapeutic product for several respiratory tract diseases. Collectively, our findings underscore the potential of these strains and their respective specialized metabolites to be employed as antimicrobial therapies in the battle against antibiotic resistance.

P27

COMPARISON OF HUMAN MILK OLIGOSACCHARIDE PROFILES OBTAINED BY LC-MS^2 OR XCGE-LIF ACROSS THREE COHORTS

Luc Marée[1,2], K.A. Dingess[1], M. Mank[1] and B. Stahl[1,3]

[1]Danone Nutricia Research, the Netherlands; [2]Faculty of Health Medicine and Life Sciences, Maastricht University, the Netherlands; [3]Department of Chemical Biology and Drug Discovery, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, the Netherlands

l.maree@student.maastrichtuniversity.nl

Human milk oligosaccharides (HMOs) contribute to the early life gut microbiome development. Based on Secretor and Lewis genes, 4 different milk types with different HMO expressions can be formed. A study from Kenyan infants found geographical difference in *Bifidobacterium longum* subsp. *infantis (B. infantis)* abundance in Africa (>80%) versus Europe (<20%) and correlations between the prevalence of bacterial species and individual HMO expression and milk types. Different HMOs have been associated with gut microbiomes with differing protection against certain pathogens. For instance, milk type 3 has been associated with high abundance of *Bifidobacterium pseudocatenulatum* and low abundance of the opportunistic pathogen *Klebsiella pneumoniae*. Variation in protection provided by the gut microbiome against pathogen pressure and inhibition of the growth of certain pathogens by HMOs possibly drive the global difference in milk type distribution and individual HMO expression. These results led us to further investigate the difference in HMO profiles between African and European cohorts to unravel the prebiotic effects of HM based on geography. In the current study, we performed a comparative analysis of HMO profiles based on individual HMOs and distribution of milk types from Africa and Western Europe with differing analytical methodologies (targeted LC-MS^2 and xCGE-LIF). Further difference in data normalization vs transformation were compared to determine the best approaches to combine different data formats. Comparability of these different techniques paves the way for new insights into the role various HMO expressions have on the observed associations of geographical differences and infant gut microbiomes. The analysis concluded that HMO measurements and milk type determination is comparable between different methodologies. Unexpectedly, HM type III (Secretor + and Lewis genes -) was predominant in HM of women from Central Africa (40%) and Kenya (22%) relative to Western Europe (7%) and was enriched in 2′-FL. Significant difference between individual HMO expression was observed between the 3 cohorts. These results demonstrate that, if milk typing is consistent and data is transformed, individual HMO analysis and milk type determination is comparable across distinct global cohorts even when different analytical methods are used. The pooled data from these cohorts reveal new insights into the HMO profiles from African countries relative to those from Western Europe. Geographical variation in individual HMO expression and milk type distribution can partly explain the observed geographical differences in infant gut microbiomes.

P28
GUT BACTERIAL AND IMMUNE COMMUNITIES' ROLE ON DEPRESSION

Eva M. Medina-Rodriguez[1,2]

[1]Psychiatry, Dr. Peset Hospital – FISABIO, Spain; [2]Institute of Agrochemistry and Food Technology, Spanish National Research Council (IATA-CSIC), Spain

emaria.medina@fisabio.es

Depression is a leading cause of disability worldwide which affects 280 million people in the world, as stated by the World Health Organization, and strongly contributes to the overall global burden of disease. The existing drug-based treatments for depression are ineffective in a great number of patients and the search for alternative treatments is an important need. Utilization and/or modification of the microbes that populate the intestine is emerging as a new potential avenue to treat psychiatric disorders. However, current studies looking for specific bacterial taxa (phylum, genera or species) associated with depression show variable results and, therefore, linking a unique microbial signature to depression seems to be complicated. Moreover, the microbiome does not act alone. The microenvironment where the different bacteria are located in the host's intestine, including the epithelial cells and different immune components, seems to have a key role on mental health as well. The main objective is to study stress-linked depression/anxiety-like behaviours associated to different bacterial communities and the associated immune components in order to identify new beneficial bacteria or immune targets to combat depression. Exposure to chronic stress (social defeat) induces depression/anxiety-like behaviours which are measured by different tests (social interactions test, force swimming test, tail suspension test, open field activity) in the presence of different bacterial communities in the gut. Different intestinal bacterial communities mediate different stress-linked outcomes and this is associated with changes in the immune system. In conclusion, specific bacterial communities are able to induce or reduce the deleterious effects of stress on depression onset and these effects are linked to the host immune system, which mediates the gut-brain communication. The therapeutic potential of specific bacterial combinations will contribute to reduce the clinical and societal burden of depression.

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DEFINED PIG BACTERIAL MIXTURE WITH A PROTECTIVE EFFECT OF GNOTOBIOTIC PIGLETS INFECTED WITH *SALMONELLA* TYPHIMURIUM

Nikol Modrackova[1], K. Horvathova[1], V. Neuzil-Bunesova[1], C. Mekadim[2], I. Splichal[3], A. Splichalova[3], J. Mrazek[2] and E. Vlkova[1]

[1]Department of Microbiology, Nutrition and Dietetics, Czech University of Life Sciences Prague, Czech Republic; [2]Institute of Animal Physiology and Genetics of the Czech Academy of Sciences, Czech Republic; [3]Laboratory of Gnotobiology, Institute of Microbiology, Czech Academy of Sciences, Czech Republic

modrackova@af.czu.cz

Salmonella Typhimurium is one of the commonly spread enteric pathogens causing enterocolitis in warm-blooded animals worldwide, mainly detected within livestock farms. Salmonellosis signs are expressed mainly as diarrhoea, vomiting, and fewer, but it can cause life-threatening conditions in weakened individuals associated with exhaustion of the host organism and death. In order to prevent salmonellosis outbreaks or reduce ongoing illness, it is desirable to find new agents from which the host organism could benefit as prevention or that could contribute to a milder course of the disease. Multi-strain probiotics are one of the promising adepts, mainly if the microbial strains are host-specific. To find a potential protective effect against *S.* Typhimurium infection of this bacterial community, we have previously prepared a defined pig bacterial mixture consisting of nine strains, specifically *Bacillus* sp., *Bifidobacterium animalis* subsp. *lactis*, *B. porcinum*, *Clostridium sporogenes*, *Lactobacillus amylovorus*, *L. paracasei* subsp. *tolerans*, and three *Limosilactobacillus reuteri* strains, which have exhibited anti-*Salmonella* activity, ability to aggregate, adherence to epithelial cells, and bile and acid tolerance, have been without mutual inhibition and classified as safe without pathogenic phenotype and resistance to antibiotics *in vitro*. The cultivation and amplicon sequencing analyses were combined to evaluate the ability to colonize gnotobiotic piglets' gut and protect the host against *Salmonella in vivo*. The bacterial mixture successfully colonized the gut and was stable till the end of the *in vivo* experiment. Although there was no significant *Salmonella*-positive elimination from the gut environment, it seems the administered potentially probiotic mixture contributed to the protection of the infected piglets because of slowed delayed infection manifestation without translocations of *Salmonella* cells to the blood circulation compared to the control group. In conclusion, the suggested potential probiotic mixture has a promising potential in pigs' production for its colonization stability and protective nature, but advanced immunological tests are necessary. **Acknowledgements**. The research was funded by grant 21-15621S of the Czech Science Foundation and was supported by METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2023064) including access to its facilities.

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SCREENING OF BIFIDOBACTERIA VS. CLOSTRIDIAL ENDOSPORE-FORMERS IN DEVELOPING INFANT GUT MICROBIOTA

Vera Neuzil-Bunesova[1], E. Ingribelli[1], N. Modrackova[1] and C. Schwab[2]

[1]Department of Microbiology, Nutrition and Dietetics, Czech University of Life Sciences Prague, Czech Republic; [2]Department of Biological and Chemical Engineering, Aarhus University, Denmark

bunesova@af.czu.cz

The phylogenetic coevolution of the host and its commensal microbiota developed a mutualistic relationship between the microbial inhabitants and the host metabolic functions and health. A mode of delivery, nutrition, and antibiotic application are the most important factors that influence the microbial colonization of the gastrointestinal tract and postnatal development. Facultative anaerobes are pioneer colonizers that create conditions for the subsequent onset of strict anaerobes, such as bifidobacteria and clostridia. Genus *Bifidobacterium* is dominating taxa of infants, mainly vaginally delivered and breastfed. Moreover, bifidobacteria are known for their antagonistic effect on clostridia. Anyway, clostridia also significantly belong to the first colonizers of the newborn gut. However, the up today presented outputs mainly focus on clostridial infections, typical for infants born by C-section, premature infants, and children of mothers who were on antibiotic therapy. Only a few studies dealt with endospore-forming clostridia in the microbiota of infants without obvious health complications. Therefore, we focused on microbial culture-dependent screening of bifidobacteria and clostridia in faecal samples. Our long-term monitoring of mammalian faecal samples, especially of infants, clearly indicates that mupirocin-based media in combination with acetic acid allow clostridial detection in faeces without or with low quantitative occurrence of bifidobacteria. Therefore, a methodology pipeline using chemical or heat treatment in combination with culture-dependent analysis was used to determine the occurrence of endospore-formers simultaneously with the quantification of the vegetative cells of bifidobacteria and clostridia. Infant faecal samples are a source of many clostridial endospore-formers belonging to the *Clostridiaceae, Lachnospiraceae, Oscillospiraceae,* and *Peptostreptococcaceae* families. Species belonging to the *Clostridiaceae* family are mainly abundant in the initial colonization of infants. As soon as bifidobacteria develop and dominate in the infant microbiota, only bifidobacteria are detected on mupirocin-based media, even though clostridia are still present, which confirms their determination by a cultivation-based pipeline using chemical or heat treatment. Moreover, the *in vitro* testing of obtained isolates showed that bifidobacteria and clostridia are both naturally resistant to mupirocin, which cannot be said about sensitivity to acids, where higher concentrations of acetic acid, as well as lactic and formic acids, have been proven to inhibit clostridial growth. Bifidobacterial presence and metabolites in the infant microbiota can regulate the quantitative occurrence of vegetative cells of clostridia. **Acknowledgement**s. The research was supported by the Ministry of Education, Youth and Sports of the Czech Republic by the grant LUAUS23014 and by METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2023064) including access to its facilities.

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IMMUNOMODULATORY EFFECTS OF AN AUTOCHTHONOUS PROBIOTIC-ENRICHED FEED AND FEEDING REGIME ON RAINBOW TROUT

Marek Ratvaj[1], I. Cingeľová Maruščáková[1], P. Popelka[2], A. Fečkaninová[3], J. Koščová[1], N. Chomová[1], J. Mareš[4], O. Malý[4], R. Žitňan[5] and D. Mudroňová[1]

[1]Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy, Slovakia; [2]Department of Food Hygiene, Technology and Safety, University of Veterinary Medicine and Pharmacy, Slovakia; [3]Department of Pharmaceutical Technology, Pharmacognosy and Botany, University of Veterinary Medicine and Pharmacy, Slovakia; [4]Department of Zoology, Fisheries, Hydrobiology and Apiculture, Mendel University, Czech Republic; [5]Research Institute for Animal Production, National Agricultural and Food Centre, Slovakia

marek.ratvaj@student.uvlf.sk

This work aims to develop an alternative to antimicrobial medication in form of probiotic feed that would protect fish without negative effects on human health and the development of antimicrobial resistance in pathogens. During our previous research, several lactic acid bacteria were isolated from the intestinal tract of rainbow trout, and their probiotic potential was tested (inhibiting growth of pathogens, antimicrobial resistance, and survivability in intestinal conditions) *in vitro*. Two strains showed the best overall performance in these tests and were then studied further. *Lactobacillus plantarum* R2 Biocenol™ was studied *in vivo* on rainbow trout in the present study. Fish in the experiment were divided into three groups depending on their feed regime. The first experimental group (CON) was fed our probiotic feed continually during the whole duration of the experiment. The second probiotic group (CYC) received the same probiotic feed although with a three-week break during which it received only commercial feed. Lastly, a control group (CTRL) was included in the experiment that received just the commercial feed that did not contain probiotics. Sampling took place on the 4th, 7th, 9th, and 11th week. Relative gene expression was measured in the head kidney and gills using qPCR, and the plate count method was used to assess the number and constitution of bacteria in the intestine. Dynamics in the change of expression of selected molecules were studied in both organs. Generally, in a cyclically fed group, there was an initial increase of gene expression (4th week) and then a decrease in expression after the break in probiotic feeding (e.g., immunoglobulin M, transforming growth factor β, tumour necrosis factor α, interleukin 8) and after two weeks (9th week) an increase was observed over control. After 4 weeks of receiving probiotic feed, both experimental groups showed approximately similar levels of gene expression. The continually fed group did show an initial increase of gene expression during the first sampling but over the next samplings, the increase over control was not significant. It should be noted that changes of expression in the head kidney and gills were not identical in several genes (e.g., CD4, immunoglobulin M). These results show that our probiotic feed has a better stimulating effect on immunity when administered at the proper time and not continually, while continual feeding with probiotic feed does not harm the fish organism. **Acknowledgments**. This work was supported by grant APVV-19-0234.

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SOLUBLE COMPOUNDS FROM GRAPE BY-PRODUCTS AS BOOSTERS FOR FOLATE (VITAMIN B9) PRODUCTION BY *STREPTOCOCCUS THERMOPHILUS* TH-4 AND *BIFIDOBACTERIUM LONGUM* SUBSP. *INFANTIS* BB-02

A.C. Candelaria Cucick[1,2], L. Obermaier[3], B.D.G.M. Franco[1,2], M. Ehrmman[4], M. Rychlik[3] and **Susana Marta Isay Saad**[1,2]

[1]School of Pharmaceutical Sciences, University of São Paulo (USP), Brazil; [2]Food Research Center, University of São Paulo, Brazil; [3]Chair of Analytical Food Chemistry, Technical University of Munich, Germany; [4]Chair of Microbiology, Technical University of Munich, Germany

susaad@usp.br; anaclara.candelaria@usp.br

Grape pomace is a fruit by-product that can be reincorporated into the food chain and explored to boost folate (vitamin B9) production during microbial fermentation, avoiding an immense nutritional value loss from the disposal. This study evaluated the effect of different fractions of a grape by-product water extract (GBPE) on folate production by two commercial folate-producing strains (*Streptococcus thermophilus* TH-4 and *Bifidobacterium longum* subsp. *infantis* BB-02). The GBPE was obtained by a hot-water extraction, and the resulting extracts were fractionated by centrifugation and ultra-filtration (Vivaspin®, Sartorius, Goettingen, Germany) with different molecular weight cut-offs (MWCO:10 and 30 kDa). The resulting permeate (<10kDa) and the concentrated (>10kDa) fractions were collected and added (10% v/v) to a dairy matrix comprised of a 50/50 blend of pasteurized whey and milk. The tested folate-producing strains were added to these mixtures in co-culture, and the supplemented dairy matrices were submitted to fermentations in a bioreactor (BioStat®, Sartorius, Goettingen, Germany) at 37°C for 24 h, under automatically controlled pH (5.5). Fermentations were also performed with the whole GBPE (10% v/v) added to the dairy matrix. Before use, each strain was activated in proper culture media, centrifuged, washed, and resuspended in a sterile saline solution. The amount of folate in each fermented dairy matrix after 24 h was determined by LC-MS/MS, in triplicate. The hot-water extraction proved to be successful in extracting the soluble fibres (0.59±0.09 g/100 ml) and phenolic compounds from the grape by-product (0.82±0.04 GAE mg/ml GBPE). Results indicated that the amount of folate was higher in the fermented dairy matrices supplemented with the larger fractions (>10 kDa=136±45.4 µg/100 g and >30 kDa=182.7 1±0.65 µg/100 g) when compared to the whole grape by-product (114.0±12.8 µg/100 g) and to the smallest fraction (103.2±15.2 µg/100 g). These results suggest that larger compounds (>10 kDa or >30 kDa) present in GBPE are more effective than smaller compounds for increasing folate production by the tested strains in fermented dairy matrices after addition of these fractions. Possibly these more effective compounds are phenolics attached to soluble fibres, but further experiments are required to support these findings. **Acdknowledgements**. This study was supported by grants FAPESP 2013/07914-8 and 2018/12190-2, BMBF 031B0875 and CAPES 88887.473569/2020-00 and 88887.694241/2022-00.

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EVALUATION OF FERMENTED MILK WITH CASHEW (*ANACARDIUM OCCIDENTALE*) BY-PRODUCT USING THE SIMULATOR OF THE HUMAN INTESTINAL MICROBIAL ECOSYSTEM (SHIME®)

M.E. Herkenhoff[1,2], I.U. Dantas de Medeiros[1,2], L.H. Grotto Garutti[1,2], M.K. Salgaço[3], K. Sivieri[3] and **Susana Marta Isay Saad**[1,2]

[1]School of Pharmaceutical Sciences, University of São Paulo (USP), Brazil; [2]Food Research Center, University of São Paulo, Brazil; [3]School of Pharmaceutical Sciences of Araraquara, São Paulo State University (UNESP), Brazil

susaad@usp.br

Fruit by-products are raw materials that may currently demonstrate nutritional or functional significance. The by-product of cashew (*Anacardium occidentale*) has already exhibited its suitability as a viable matrix for probiotic strains, in addition to possessing prebiotic potential. This study aimed to assess the probiotic, prebiotic, and functional attributes of fermented milk with cashew by-product (CB), prepared with the starter *Streptococcus thermophilus* ST-M6® and the probiotic strain *Lacticaseibacillus paracasei* subsp. *paracasei* F19®. Using a dynamic *in vitro* simulator of the passage of food through the human gastrointestinal tract – the simulator of the human intestinal microbial ecosystem (SHIME®), formulations with 2.5% CB (test formulation, TF) and without CB (control formulation, CF) were evaluated. The strains behaviour in the formulations and other bacterial groups of interest were assessed. Populations of microorganisms of certain groups of the intestinal microbiota, as well as the starter and probiotic strains were determined by PMA-qPCR. Metabolites produced in the SHIME® reactors fermentation associated with nitrogen balance were also determined, along with antioxidant and phenolic compounds, and short-chain fatty acids. CB was selectively fermented by the probiotic strain *Lacticaseibacillus paracasei* subsp. *paracasei* F19® and the starter *Streptococcus thermophilus* ST-M6®. The two formulations had positive effects on the microbiota reconstitution in the SHIME®, resulting especially in a decrease in *Clostridium* and γ-*Proteobacteria* phyla and in an increase in *Lactobacillu*s spp. and *Bifidobacterium* spp. The presence of CB during the inoculation of TF was adequate to significantly ($p<0.05$) boost phenolic compounds and antioxidant activity. Fermented milk with and without CB represented an alternative for a new food product with an improved functional profile and the presence of CB together with the strains tested lead to a potentially synergistic synbiotic product.
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IMPACT OF PROBIOTICS AND EGG WHITE ON *TENEBRIO MOLITOR* GROWTH, MICROBIAL COMPOSITION AND PATHOGEN INFECTION

Carlotta Savio[1,2,6], P. Herren[3,4,6], A. Rejasse[1], A. Rios[5], A. Bruun-Jensen[6], A. Lecocq[6], J.J.A. van Loon[2] and C. Nielsen-Leroux[1]

[1]University of Paris Saclay, INRAE, Micalis, France; [2]Department of Plant Sciences, Wageningen University & Research, the Netherlands; [3]UK Centre for Ecology & Hydrology, UK; [4]Living Systems Institute, College of Life and Environmental Sciences, University of Exeter, UK; [5]Ynsect, France; [6]Department of Plant and Environmental Sciences, University of Copenhagen, Denmark

carlotta.savio@inrae.fr

The industrial rearing for feed and food purposes of the yellow mealworm, *Tenebrio molitor,* on agricultural by-products may expose larvae and adults to entomopathogens used as biocontrol agents in crop production. Bacterial spores/toxins or fungal conidia from species such as *Bacillus thuringiensis* or *Metarhizium brunneum*, respectively, could affect the survival and growth of the insects. Therefore, the aim of this study was to investigate the potential benefits of a mealworm diet supplemented with probiotic bacteria or dried egg white, on larval development, survival and gut microbiome assemblage. Two probiotic bacterial species, *Pediococcus pentosaceus* KVL B19-01 and *Lactobacillus plantarum* WJB were added to wheat bran feed with and without dried egg-white as an additional protein source, directly from egg hatching. Larvae with a body mass of 20 mg were exposed during 72 h to *B. thuringiensis, M. brunneum* or their combination. Larval survival and growth were recorded for 14 days and the bacterial microbiota composition was analysed by 16S rDNA sequencing pre-pathogen exposure and at day 3 and 11 after inoculation with the pathogens. Increased growth and survival rate were observed for *T. molitor* larvae reared on feed supplemented with *P. pentosaceus*, conferring increased survival in case of co-infection. No significant impact of egg white on larval growth was recorded, while a minor effect of deactivated *Lb. plantatrum* was found in absence of pathogens. At day 14, the bacterial community composition of the larvae was similar in all treatments, indicating that the probiotic strain did not establish at a detectable level in the insect, however, its transient presence improved larval performance.

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COMPARISON OF GUT MICROBIOTA BETWEEN DIABETIC AND NON-DIABETIC OBESE MONGOLIAN

Akari Shinoda[1], S. Demberel[2], D. Jamyan[2], T. Lkhagvajav[2] and J. Nakayama[1]

[1]Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Japan;
[2]Institute of Veterinary Medicine, Mongolian University of Life Sciences, Mongolia

akshinoda800@gmail.com

People in Mongolia have a unique dietary habit largely depending on dairy products in summer and wheat and meat in winter. This dietary habit may relate to a high obesity ratio (20.60%. Furthermore, in recent years, food westernization has progressed in association with changes in gut microbiota and the number of obese people is markedly increasing. However, the population of type 2 diabetes mellitus (T2DM=10.4%) is relatively small despite the high obesity rate. We suspected that the traditional Mongolian diet and gut microbiota influenced by diet may play a key role in this Mongolian paradox. To answer the paradox, we aimed to elucidate the mechanism of how their gut microbiota interacts with diet and host health and suppresses the development of T2DM. In this study, faecal samples were collected from non-T2DM obese subjects(n=43) and T2DM obese subjects(n=31) in a rural site (Bulgan) as well as an urban site (Ulaanbaatar). In addition, a dietary questionnaire was conducted for each subject. To clarify the intestinal bacteria composition and functional capability, the amplicon sequencing targeting the V3-V4 region of the 16S rRNA gene was performed and therewith whole-genome metagenomic sequencing was performed for 9 subjects from each group. In addition, faecal short-chain fatty acids (SCFAs) and bile acids were measured. *Faecalibacterium* and *Anaerostipes*, known as butyrate producers, were significantly decreased in T2DM obese subjects, as has been reported in T2DM patients. The link between *Anaerostipes hadrus* and butyrate synthesis was also found in the whole-genome metagenome sequence. Furthermore, the decreases in acetate and total SCFA level were observed in the stool of the T2DM obese group. Taken together with the known anti-diabetic and anti-inflammatory functions of SCFAs, it is suspected that gut microbiota derived SCFAs play a role to maintain glucose homeostasis in obese Mongolian.

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PROTEOLYTIC AND FUNCTIONAL CHARACTERIZATION OF *LACTIPLANTIBACILLUS PLANTARUM* STRAINS FROM ARTISANAL BRAZILIAN CHEESE CAPABLE OF REDUCING COW MILK CASEINS' ALLERGY

Antonio Diogo Silva Vieira[1,2], M.A. Cavalcante de Albuquerque[2] and B.D. Gombossy de Melo Franco[1,2]

[1]Department of Food and Experimental Nutrition (FBA), School of Pharmaceutical Science, University of São Paulo, Brazil; [2]Food Research Center (FoRC), University of São Paulo, Brazil

antdiogovieira@gmail.com

Food allergies are a global public health problem, affecting more than 10% of the population and 8% of children worldwide. Cow's milk is one of the most important foods responsible for allergic reactions. Studies have shown that antigenicity of allergenic proteins in milk can be reduced during fermentation by proteolytic lactic acid bacteria (LAB) capable to cleave antigen-sensitizing epitopes. In this study, two proteolytic strains of *Lactiplantibacillus plantarum* (QSC472 and QSC507), isolated from Brazilian artisanal cheeses and lacking known virulence genes, were further evaluated for their caseinolytic activity, biotechnological and functional properties, and level of reduction of milk caseins immunoreactivity after fermentation. The tests were performed in a nonproliferative cell system (NPCS) with casein as substrate, followed by SDS-PAGE. The following tests were performed: (i) effect of temperature (30, 37 and 42°C) and pH (5.5, 6.0, 6.5, 7.0 and 7.5) on the proteolytic activity; (ii) type of proteinases produced, determined using specific proteolytic activity inhibitors (EDTA, iodoacetic acid and PSMF); (iii) tolerance to 5 and 10% (m/v) NaCl and sucrose in skimmed milk (SM) at 37°C for 24 h; (iv) survival to simulated gastric and enteric conditions; (v) cell hydrophobicity of the strains; and (vi) reduction of immunoreactivity of milk caseins after hydrolysis, measured by the RIDASCREEN®FASTcasein test. Results indicated that higher hydrolysis of caseins was observed pH 6.5 at 37ºC for both strains, and proteases were inhibited after treatment with EDTA, indicating that they mainly belonged to the group of metalloproteases. Additionally, the tested temperatures did not affect growth ($p>0.05$) but affected the final pH and titratable acidity of the fermented SM for both strains ($p<0.05$). Tolerance to NaCl or sucrose in SM varied according to the strain. The populations of both strains were reduced ($p<0.05$) in the gastric and enteric simulated conditions, but strain QSC507 was statistically equal to *L. rhamnosus* LGG (LGG) used as control. The cell hydrophobicity of the strain QSC507 was ($p<0.05$) higher than the control (LGG). The immunoreactivity of caseins was reduced (>80%) by both strains ($p<0.05$) when compared to the control casein solution. These results indicate that the two strains, especially *Lactiplantibacillus plantarum* QSC507, present properties that give evidence to their potential application for production of hypoallergenic dairy products. **Acknowledgements**. Funding by FAPESP Processes 2018/24061-2 and 2013/07914-8.

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CHRISTENSENELLA MINUTA ALLEVIATES PSYCHIATRIC AND CARDIAC ALTERATIONS INDUCED BY EARLY LIFE STRESS IN MICE

María Tamayo[1,2], A. Agustí[1], G.V. Molina-Mendoza[1], M.C. Cenit[1], V. Tolosa-Enguis[1], A. Flor-Duro[1], V. Rossini [1] and Y. Sanz[1].

[1]Microbiome, Nutrition and Health Research Unit, Institute of Agrochemistry and Food Technology, Excellence Center Severo Ochoa-Spanish National Research Council (IATA-CSIC), Spain;
[2]Department of Medicine, Autonomous University of Madrid, Spain

mtamayo@iata.csic.es

Suffering some type of adversity early in life has been shown to increase both vulnerability to negative neuropsychiatric disorders and to cardiometabolic diseases. Early life stress (ELS) is associated with a sustained activation of the HPA axis, reflected in elevated cortisol levels, and of the immune system. The gut microbiota is considered to play an important role in the control of the stress response through the regulation of immune and neuroendocrine routes of the gut-brain axis. In turn, modulation of the composition of gut microbiota represents a potential intervention strategy to prevent or reduce the sequels of ELS. In this study, we have used a murine model of depression induced by ELS (social defeat) in adolescent mice to prove whether the administration of a commensal bacterium strain isolated in our group from a healthy human subject of the species *Christensenella minuta* is able to reduce the long-term consequences of chronic stress exposure in this critical period of life. For that purpose, mice were exposed to chronic social defeat for 10 days. The bacterium was administered for 21 days previous to the social defeat and until the end of the study. Hereafter, we conducted behavioural tests and sacrificed the animals to obtain biological samples. After the social defeat, the stress-group showed decreases in body weight and increases in liver and spleen weights. *C. minuta* normalized the spleen weight up to control levels. The bacterium also restored social behavioural alterations by enhancing stress resilience and reversed the depressive and the anxiety-like behaviour induced by stress. Regarding cardiovascular implications, stress increased the oxidative stress in heart, impairing gene expression of pro- and anti-oxidative enzymes and inducing increased DNA damage, that could ultimately lead in cardiac function disruption and heart failure. *C. minuta* administration reduced DNA damage induced by oxidative stress through increases in gene expression of major antioxidant enzymes in cardiac tissue and reductions in the expression of pro-oxidants. *C. minuta* also reduced the chronic high levels of corticosterone in plasma and the proinflammatory cytokines and chemokines (TNFα, CCL2) in plasma and splenic ILC1 and ILC2, which could mediate the behavioural and cardiac effects. In conclusion, the oral administration of *C. minuta* reduces cardiac and behavioural sequels induced by ELS, likely through the regulation of the HPA-axis dysfunction and the inflammatory status.

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PREBIOTIC PROPERTIES COMPARISON OF PLANT-BASED SOLUBLE FIBRES OBTAINED WITH VARIOUS TECHNOLOGIES USING THE *EX VIVO* SIFR® TECHNOLOGY

Clémentine Thabuis[1], S. Deyaert[2], A. Baudot[2], C. Perreau[1] and P. Van Den Abbeele[2]

[1]Nutrition and Health R&D, Roquette, France; [2]Cryptobiotix, Belgium

clementine.thabuis@roquette.com

There are two categories of soluble fibres: the viscous fibres, such as pectin, beta-glucans and the non-viscous fibres, such as resistant dextrin's (RD), polydextrose (PDX), inulin (IN) and fructo-oligosaccharids (FOS). IN and FOS are plant-based fructose polymers, extracted from chicory roots and Jerusalem artichoke. RD and PDX are also plant-based as the starting material is either starch or glucose syrup. Contrary to IN, RD and PDX are glucose polymers with various molecular weights. Their fibre/non-digestible properties are due to the presence of specific osidic linkages in their structure that are not hydrolysed by endogenous enzymes in the proximal intestine. The pattern of these specific linkages depends on the production technology. In the present study, we aimed at investigating the combined effect of molecular weight and osidic linkage pattern (i.e., technology: polycondensation or dextrinification) on gut microbiota (composition and metabolism) using a high-throughput *ex vivo* technology to mimic colon. Using the SIFR® (systemic intestinal fermentation research) technology, prebiotic properties of various glucose-based fibres were compared to a negative control (blank) and positive control (IN). The SIFR® is an *ex vivo* bioreactor-based technology, owing to its throughput, which allowed to evaluate the impact on the gut microbiota of 6 human healthy adults, a key aspect given the importance of interpersonal differences on the response to interventions. All fibres (2 samples from dextrinification and 4 from by polycondensation) underwent an oral, gastric and small intestinal digestion procedure, followed by an absorption simulation and colonic fermentation. Key fermentations parameters (pH, gas, SCFA/bCFA) and microbial composition (quantitative 16S rRNA gene profiling) were analysed at 0 and 48 h. Within 48 h, the positive control IN displayed prebiotic properties in line with recently published clinical data. Fermentative parameters allowed clustering of the samples using principal component analysis (PCA) by fibre production technology. Among glucose polymers, those obtained by polycondensation were fermented by the gut microbiota inducing a pH decrease in the fermenters, and the production of the beneficial metabolites that are short-chain fatty acids (SCFA), but the fermentation and prebiotic effects were stronger with the samples obtained by dextrifinication. According to recent publications, the SIFR® technology is now clearly considered as a predictive characterization technology. Consequently, it represents a powerful tool to compare efficiently and precisely the prebiotic properties of glucose polymers that can be structurally close but not identical, to guide industries in the design of new plant-based fibres with health benefits.

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FERMENTED BULGARIAN VEGETABLES, SOURCE OF BACTERIOCINOGENIC AND ANTIOXIDANTS PRODUCING LACTIC ACID BACTERIA

R. Rwubuzizi[1], K. Ordonho Carneiro[2], W.H. Holzapfel[3], B.D. Gombossy de Melo Franco[4], M. Vaz-Velho[5] and **Svetoslav Dimitrov Todorov**[1,2,4,5]

[1]Department of Advanced Convergence, Handong Global University, Korea; [2]Departamento de Alimentos e Nutrição Experimental, Universidade de São Paulo, Brazil; [3]Department of Advanced Convergence, Handong Global University, Korea; [4]Food Research Center, Departamento de Alimentos e Nutrição Experimental, Universidade de São Paulo, Brazil; [5]Center for Research and Development in Agrifood Systems and Sustainability, Escola Superior de Tecnologia e Gestão, Instituto Politécnico de Viana do Castelo, Portugal

todorov@usp.br; slavi310570@abv.bg

Lactiplantibacillus plantarum ST01BG, ST07BG, ST10BG and ST15BG, *Latilactobacillus curvatus* ST02BG, *Lacticaseibacillus paracasei* ST04BG, *Pediococcus pentosaceus* ST05BG, *Leuconostoc mesenteroides* ST06BG and *Enterococcus faecium* ST11BG were isolated from home-made fermented vegetables from North-West Bulgaria and identified by biochemical, physiological and biomolecular analysis, including partial 16S rRNA sequencing. The strains were designated as bacteriocin producers, and the expressed antimicrobials partially characterized. The bacteriocins were effective in inhibiting different strains of *Listeria* spp., *Enterococcus* spp. (including vancomycin resistant enterococci) and *Staphylococcus* spp. These strains can be considered as safe, based on the evaluation of haemolytic activity, production of biogenic amines, mucin degradation, antibiotic susceptibility/resistance, and gelatinase enzyme production. Moreover, the strains can be considered as potentially beneficial based on their stability and survival under simulated gastrointestinal tract conditions (stomach and duodenum), the production of diacetyl and specific levels of hydrophobicity. Recorded levels of DPPH in the studied strains ranged between 70.63% for *P. pentosaceus* ST05BG and 36.96% for *Lp. plantarum* ST10BG. Even between the three evaluated *Lp. plantarum* strains, DPPH values varied (62.53% for strain ST01BG, 61.14% for ST07BG and ST10BG), indicating that antioxidant properties are strain specific. The studied strains ferric ion chelating activities with the highest recorded for *Lp. plantarum* ST07BG (59.10%), and the lowest chelation activity for *Lp. plantarum* ST15BG (33.00%), compared to the positive control, ethylene diamine tetra-acetic acid (EDTA) (1 mg/ml) (82.27%). In our study, evaluated strains demonstrated variable hydroxyl radical scavenging activities with highest levels ranging from 94.5% for *Lp. plantarum* ST01BG to 64.90% for *E. faecium* ST11BG and the lowest of only 29.60% for *Lc. paracasei* ST04BG, as compared to the control, ascorbic acid, with results of 98.34%. The recorded superoxide anion radical scavenging activity was higher for *Lp. plantarum* ST15BG (51.61%) and lower for *P. pentosaceus* ST05BG (11.39%). *Lp. plantarum* ST15BG (83.65%) and *E. faecium* ST11BG (29.80%) showed the highest and lowest anti-lipid peroxidation values respectively. Antioxidant properties were found to be strain specific. The beneficial attributes (antimicrobial and antioxidant) of these cultures to fermented food products may enable the reduction of chemical additives in line with consumers' demand for more natural and chemical-free food commodities. Combination between production of antimicrobial proteins (bacteriocins) and antioxidants can be considered as interesting application scenario for the selection of new starter cultures with multiple beneficial properties.
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ENTEROCOCCUS FAECIUM BACTERIOCINOGENIC STRAINS ARE PREDOMINANT AND WIDELY REPRESENTED IN SURFACES FROM A DAIRY PRODUCTION ENVIRONMENT

J.M. Scafuro Lima[1,2], M. Landgraf[2], B.D. Gombossy de Melo Franco[2], U.M. Pinto[2] and **Svetoslavi Dimitrov Todorov**[1,2]

[1]ProBacLab and Laboratório de Microbiologia de Alimentos, Departamento de Alimentos e Nutrição Experimental, Universidade de São Paulo, Brazil; [2]Food Research Center, Departamento de Alimentos e Nutrição Experimental, Universidade de São Paulo, Brazil

todorov@usp.br

Lactic acid bacteria (LAB) play an important role in the cheese-making process, being responsible for curd acidification and cheese ripening. Besides their fermentation importance for the formation of the final product, LAB can contribute to cheese biopreservation associated to the production of different antimicrobial metabolites, including bacteriocins. Moreover, LAB may have a leading role in the biocontrol of pathogens, including controlling their biofilm formation. The aim of the current study was to prospect LAB from different surfaces from the cheese production environment under the hypothesis that specific bacteriocinogenic strains, with activity against *Listeria monocytogenes*, can be predominant in artisanal cheese production facilities. Swabs were collected from the surfaces of an artisanal cheese production facility at a producer located in the state of São Paulo. Based on the preliminary screening for bacteriocinogenic LAB, from initial 21 selected isolates with antimicrobial potential, 15 were confirmed to be producers of bacteriocins expressing proteinaceous antimicrobial compounds, which are stable at temperature treatments between 8ºC and 100ºC for 60 min and to 121ºC for 15 min, in addition to pH ranging from 2.0 to 10.0 and to the presence of 1% NaCl, SDS, and Tween 80. Preselected isolates were confirmed as safe (γ-haemolytic, not presenting antibiotic resistance and mucus degradation properties and no proteolytic or gelatinase enzyme activity). Selected isolates of interest were differentiated by rep-PCR and further identified according biochemical, physiological, and biomolecular criteria, including 16S rRNA partial gene sequencing, as belonging to the species *Lactococcus garvieae* (1 strain) and *Enterococcus faecium* (14 isolates, grouped into 3 clusters). We highlight that 10 isolates (out of a total of 15) associated with different environments (worktables, cheese mold, ripening wooden shelfs) were identified as representative of the same *E. faecium* strain which was the most predominant bacteriocinogenic LAB obtained within the current study. One representative from this predominant *E. faecium* cluster, named ST01JL, was further evaluated regarding its bacteriocinogenic properties. Bacterial growth of this isolate reached levels corresponding to OD_{600nm} of 2.28. The acidification, resulting in a pH decrease from 6.26 to 4.44, and the production of bacteriocin against *L. monocytogenes* ATCC7644 was observed, with a bacteriocin activity of 25,600 AU/ml detected between 15-24 h, was assessed by cultivating *E. faecium* ST01JL in MRS broth at 37ºC for 24 h. Moreover, addition of cell free supernatant from *E. faecium* ST01JL to actively growing *L. monocytogenes* ATCC7644 cultured at 37ºC resulted in growth inhibition over 12 h period. Alternative biocontrol strategies based on the use of bacteriocinogenic LAB could serve as an important tool to address safety issues in cheese dairy environments aligning with current trends in society, industry, and academia, which seek to replace traditional preservation methods that employ common chemical agents. **Acknowledgments**. Financial support to the Food Research Center from FAPESP (2013/07914-8); CAPES (Cota Institucional - Demanda Social nº 88887.688643/2022-00).

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EFFECT OF *BACILLUS* SP. PB6 (PTA-6737) ADMINISTRATION ON THE INTESTINAL MICROBIOME OF BROILER CHICKENS IN FOUR INDEPENDENT BELGIAN FARMS

Karen Vermeulen[1], S. Taelman[2,3], S. De Smet[1], N. Smeets[1], S. Kirwan[1], A. Wealleans[1] and F. Nuyens[1]

[1]Kemin Animal Health and Nutrition, Kemin Europa N.V., Belgium; [2]BioLizard, Belgium; [3]BIOBIX, Department of Data Analysis and Mathematical Modelling, Ghent University, Belgium

karen.vermeulen@kemin.com

The probiotic potential of *Bacillus* sp. PB6 (PTA-6737) for poultry is well established with studies demonstrating several modes of action, including immune stimulation, antimicrobial activity, and performance enhancement. The aim of the current research was to elucidate the effects of drinking water administration (10^8 CFU/ml) of *Bacillus* sp. PB6 on the caecal microbiome of broilers. Both a control house (CTRL) and probiotic-treated (BAC) house were considered. The probiotic was supplemented from day 0 till slaughter age. At the age of 10 and 28 days, 10 to 14 randomly chosen male birds were euthanized, intestinal content was collected and (intestinal) health was scored. Sampling took place at four different commercial farms in the northern part of Belgium to account for inter-farm variability. In general, all birds in the present study could be considered relatively healthy and no significant differences could be noted between BAC and CTRL birds related to dysbiosis score and bodyweight. A significant improvement in footpad scores was seen in BAC birds (0.868 CTRL vs. 0.396 BAC, p=0.04). The supplementation of *Bacillus* sp. PB6 to the drinking water significantly affected caecal microbial composition as compared to control birds. On day 10, 24 out of 6,070 ASVs, and 6 on day 28 were found to be differently abundant as a result of probiotic administration. Amongst these were on day 10, e.g., significant decreases in relative numbers of an ASV identified as *Bifidobacterium pullorum* (log2 FC -2.7, p<0.001), two ASVs belonging to the *Bacteroides* genus (ASV1215 log2 FC -4.7, p=0.002, ASV1272 log2 FC -4.3, p=0.018) and an ASV appointed to the *Lactobacillus* genus (log2 FC -2.7, p<0.001). On day 28, e.g., an ASV identified as *Bacteroides vulgatus* was significantly more abundant in BAC birds (log2 FC 3.2, p=0.02) while another ASV belonging to the same genus was significantly decreased (log2 FC -3.1, p=0.03). These numbers indicate that the impact was highest in younger broilers when the intestinal microbiome still under development. The nature of the difference varied with farm and depended greatly on the challenges presented at the farms. For example, one of the farms suffered temporarily from water leakage, whereas in one of the other farms problems occurred with ventilation and building up of ammonia levels. These challenges may have introduced room for improvement. To conclude, the supplementation of *Bacillus* sp. PB6 to the drinking water of broilers could significantly affect the caecal microbial composition as compared to control birds.

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INVESTIGATIONS OF BENEFICIAL PROPERTIES OF STRAIN *LACTOBACILLUS CRISPATUS* LCR04

Annalisa Visciglia, C. Morazzoni, G. Deusebio, A. Amoruso and M. Pane

Probiotical Research srl, Italy

a.visciglia@probiotical.com

The vaginal microbiota in healthy women of reproductive age predominatly comprises diverse Lactobacillus species, including *Lactobacillus gasseri, Lactobacillus crispatus, Lactobacillus jensenii,* and *Lactobacillus iners.* Specifically, according to studies, the profile of each female vaginal microbiome can be classified into six community state types (CSTs). *Lactobacillus*, especially *L. crispatus*, *L. gasseri*, *L iners*, and *L. jensenii,* is predominant in CST-I, II, III, and V, *Streptococcus* and *Prevotella* dominate in CST IV-A, and *Atopobium* is highly prevalent in CST IV-B. Notably, these Lactobacillus species appear to be uniquely adapted for dominance in the vaginal niche since other types of lactobacilli are not observed. In this context, we evaluated the probiotic properties of *L. crispatus* LCR04 (DSM 33487), a strain from the Probiotical S.p.A. collection, with a focus on its relevance to vaginal health through various research methods. Initially, bioinformatic analysis was performed to assess the presence of functional genes associated with vaginal wellness within the genome of probiotic LCR04. Interestingly, it has been obtained high identity percentage for key vaginal genes. Then, probiotic properties of LCR04 were investigated through *in vitro* experiments. Firstly, MTT and LDH assays confirmed safety of LCR04 tested both on Caco-2 cells, used as model of intestinal barrier, and VK2-E6E7 cells, used as vaginal model. Then, the capacity of LCR04 to inhibit *C. albicans* and *G. vaginalis* growth was investigated both as direct competition of probiotic and pathogen strains through antimicrobial assays as well as in eukaryotic context using VK2/E6E7 and reconstituted human vaginal epithelium (RHVE) models. Both models were infected with *C. albicans* and treated with LCR04 in order to verify if LCR04 could restore the damage of pathogen. Interestingly, by these analyses, it has been obtained that LCR04 showed strong antipathogen activity both against *G. vaginalis* and *C. albicans*. Collectively, our study highlights the significant probiotic potential of LCR04, positioning it as a promising candidate for non-invasive adjuvant therapy and a potential prevention strategy for the management of vaginal infections.

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INTERACTIONS BETWEEN *STAPHYLOCOCCUS AUREUS* AND *LACTIPLANTIBACILLUS PLANTARUM* IN AGR QUORUM SENSING

Weizhe Wang, S. Fulaz, M.S. Bojer and H. Ingmer

Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark

weizhewang@sund.ku.dk

Many human pathogens employ communication systems to sense their surroundings and in response either induce or repress virulence gene expression. This type of regulation is being exploited in anti-virulence therapy whereby bacterial pathogens are targeted by compounds that repress virulence factor expression rather than growth. *Staphylococcus aureus* is an opportunistic human pathogen that colonizes many humans but also can cause a variety of serious infections. In *S. aureus*, virulence factor expression is controlled by cell-to-cell communication (quorum sensing, QS) that enables bacterial cells to respond to cell density allowing toxins and other degradation enzymes to be produced at high cell densities. In four allelic variants of the Agr system, Agr I-IV, a matching autoinducing peptide (AIP) is required for virulence induction, and a non-matching AIP results in QS inhibition. Previous research has shown that other *Staphylococci* produce AIPs able to competitively inhibit the *S. aureus* QS system. We report the effects of *Lactiplantibacillus plantarum* CIRM-BIA 1870 on the *staphylococcal* QS in a non-bactericidal manner. Consistent with previous findings that tailless *L. plantarum* AIP undergoes a S → N shift, *L. plantarum* co-culturing with *S. aureus* showed efficient inhibition, where *L. plantarum* supernatants only showed some inhibition in the *S. aureus* quorum sensing system. The use of whole probiotics is a promising avenue for developing anti-virulence therapies against *S. aureus* infections.

P44

COMBINATION OF *BIFIDOBACTERIUM LACTIS* HN019 WITH A MIX OF FIVE HUMAN MILK OLIGOSACCHARIDES INCREASES DEFENSE AGAINST INTESTINAL PATHOGENS

Adrienne Weiss[1], C.A. van Loo-Bouwman[1], W.-L. Hung[2], Y. Yan[2], S. Duan[2], I. Man-Yau Szeto[2] and G. Smit[1]

[1]Yili Innovation Center Europe, the Netherlands; [2]Yili Innovation Center, Inner Mongolia Yili Industrial Group Co., Ltd., China

adrienne.weiss@yili-innovation.com

Human milk comprises a complex mixture of immune-enhancing components. Those include beneficial bacteria and human milk oligosaccharides (HMOs), which have been individually studied for their anti-pathogenic effects. To which extent these effects are increased by combining a probiotic with HMOs is unclear. We aimed at exploring the potential of probiotic *Bifidobacterium animalis* spp. *lactis* HN019 combined with a mix of five HMOs on increasing protection from targeted intestinal infections and strengthening gut barrier integrity. *B. lactis* HN019 and a mix of five HMOs (2'-fucosyllactose, 3-fucosyllactose, lacto-N-tetraose, 3'-siallylactose, 6'-sialyllactose) were tested individually and in combination for their ability to increase barrier integrity of intestinal epithelial cells to protect against disruption induced by enterotoxigenic *Escherichia coli* (ETEC). Secondly, their influence on intestinal pathogen adhesion was analysed with enteropathogenic *Escherichia coli* (EPEC). Thirdly, in the nematode *Caenorhabditis elegans* challenged with *Staphylococcus aureus*, their effect on innate immune pathways and survival was measured *in vivo*. *B. lactis* HN019 combined with the HMO mix increased the barrier integrity of epithelial cells upon ETEC exposure in comparison to the individual components. The adhesion of EPEC to intestinal cells was lowered with the probiotic alone as well as with the HMO mix alone, but more effectively when both were applied together. The tested compounds each increased survival of *C. elegans* and showed synergistic effects when combined. *In vivo*, the combination of *B. lactis* HN019 and HMOs was effective when used as a treatment during the challenge with *S. aureus* as well as when given preventative before the pathogen challenge. In conclusion, the combination of the probiotic *B. lactis* HN019 with a mix of five HMOs might improve the intestinal defense against pathogens through increased barrier integrity, anti-adhesion effects and enhanced innate immunity. This combination might increase intestinal protection when added to infant formula.

P45

ENGINEERING LACTIC ACID BACTERIA AS DELIVERY VEHICLES FOR *CLOSTRIDIOIDES DIFFICILE* ANTITOXIN PROTEINS

Abida Zahirović[1], K. Bozovičar[2], M. Rupnik[3,4], T. Bratkovič[2] and A. Berlec[1,2]

[1]Department of Biotechnology, Jožef Stefan Institute, Slovenia; [2]Faculty of Pharmacy, University of Ljubljana, Slovenia; [3]Department for Microbiological Research, National Laboratory of Health, Environment and Food, Slovenia; [4]Department of Microbiology, University of Maribor, Slovenia

abida.zahirovic@ijs.si

Clostridioides difficile is an opportunistic pathogen often carried asymptomatically in the healthy human gastrointestinal tract. Disruption of the gut microbiota by frequent use of antibiotics can lead to proliferation of *C. difficile* and secretion of toxins. Toxin A (TcdA) and toxin B (TcdB) are major virulence factors of *C. difficile* and inducers of disease symptoms ranging from diarrhoea to pseudomembranous colitis. *C. difficile* infection is treated with antibiotics, but due to the emergence of antibiotic-resistant strains, the recurrence rate has greatly increased (up to 25%). In search of new strategies to combat *C. difficile* infection, we constructed *Lactococcus lactis* as a delivery vector for anti-toxin proteins based on non-immunoglobulin scaffolds. The aim is to develop a new treatment method that combines local neutralization of *C. difficile* toxins with the beneficial effects of orally administered probiotics. Antitoxins delivered directly into the intestinal lumen by probiotic bacteria may have better therapeutic efficacy than parenterally administered antitoxins. Genes for antitoxin proteins (affimers targeting TcdA or TcdB and DARPin targeting TcdB) were fused to signal peptide and cloned into lactococcal plasmids with or without cell wall anchor cAcmA. In addition, antitoxin proteins were expressed in *E. coli*, purified, and used as standards for characterization of engineered *L. lactis*. Successful expression of antitoxin proteins in *L. lactis* was confirmed with immunoblot by detecting bands of appropriate molecular weight in whole-cell lysates. DARPin present in the whole-cell lysate of *L. lactis* was able to bind toxin B from the conditioned growth medium of the *C. difficile* reference strain VPI 1046, as demonstrated by enzyme-linked immunosorbent assay. In order to determine the location of the expressed proteins, cell fractionation of *L. lactis* was performed. Affimers were present in all cellular fractions and in the bacterial culture supernatant, whereas DARPin was found only in the membrane fraction and could not be detected in the culture supernatant. Inefficient secretion may be due to protein arrest during transport across the cell membrane or during passage through the peptidoglycan cell wall. Current experiments are focused on optimizing DARPin structure to achieve its export to the extracellular environment. In addition, a non-GMO variant of the bacterial-based vector system was prepared by coating wild-type *L. lactis* with antitoxin proteins from the whole-cell lysates. The surface display of antitoxin proteins was confirmed by dot-blot immunoassay. The proteins were non-covalently anchored to the surface of wild-type bacteria by a peptidoglycan binding domain cAcmA.

P46

MODIFICATIONS OF THE RAFFINOSE FAMILY OLIGOSACCHARIDES PROFILE IN PEAS BY PLANT BREEDING INFLUENCE THE HUMAN GUT MICROBIOTA STRUCTURE AND FUNCTION

Aryana Zardkoohi[1-3], T. Rayner[2], A. Bell[1], C. Domoney[2] and N. Juge[1]

[1]Gut Microbes and Health, Quadram Institute Bioscience, UK; [2]Department of Biochemistry and Metabolism, John Innes Centre, UK; [3]Medical School, University of East Anglia, UK

aryana.zardkoohi-burgos@quadram.ac.uk

The raffinose family oligosaccharides (RFOs) are a group of galacto-oligosaccharides stored in the seeds of legumes with important biological effects on plant fitness such as resistance to drought or environmental stressors. Here we evaluated the role of RFOs in peas on human health using *Pisum sativum* lines, a commercial cultivar, Cameor, a mutant lacking a major raffinose synthase enzyme (*rfs*) and lacking RFOs, BCFN 1551, obtained through fast-neutron mutagenesis; and a line obtained through targeting induced local lesions in genomes (TILLING) with intermediate depletion of RFOs. LC-MS analyses confirmed a significant reduction in RFOs on both mutant lines compared to the commercial cultivar in both flour and extracts. Using shotgun metagenomics, we showed that supplementation of faecal samples from human donor with individual raffinose and pea flour from Cameor led to increased *Bifidobacterium* population, associated with health-promoting properties. Untargeted metabolomics analysis and LC-MS analysis of short-chain fatty acids of the supernatant revealed an increase in metabolites, such as 12-ketolithocholate in the batch fermentations supplemented with BCFN 1551 fermentation, while no major differences were observed in total SCFA or acetate irrespective of the material used for supplementation, pea flour or whole cell extracts. Gas production was monitored using the ANKOM RF gas production system. The cumulative pressure was highest for the fermentation of RFO-containing pea flour, either from Cameor cultivar or the TILLING mutant. These results showed that the deletion of *rfs* and reduction in RFOs within pea seeds influenced the human gut microbial distribution and metabolic profile after fermentation. Work is on-going to test the effects of peas with variable levels of RFOs on gut barrier function using human intestinal organoids gown on transwells or gut-on-chips.

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SMALL INTESTINE VS. COLON ECOLOGY AND PHYSIOLOGY – WHY IT MATTERS IN PROBIOTIC ADMINISTRATION

Nikoletta Vidra[1], B.A.H. Jensen[2], M. Heyndrickx[3], D. Jonkers[4], A. Mackie[5], S. Millet[3], M. Naghibi[6], S.I. Pærregaard[2], B. Pot[1], D. Saulnier7, C. Sina[8], L.G.W. Sterkman[9], P. Van den Abbeele[10], N.V. Venlet[11], E.G. Zoetendal[12] and A.C. Ouwehand[13]

[1]Yakult Europe BV, the Netherlands; [2]University of Copenhagen, Denmark; [3]Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Belgium; [4]Maastricht University, the Netherlands; [5]University of Leeds, UK; [6]ADM Health & Wellness, UK; [7]Novozymes Berlin GmbH, Germany; [8]University Medical Center of Schleswig-Holstein (UKSH) and University of Lubeck, Germany; [9]Caelus Health, the Netherlands; [10]Cryptobiotix, Belgium; [11]ILSI Europe, Belgium; [12]Wageningen University & Research (WUR), the Netherlands; [13]International Flavors & Fragrances (IFF), Finland

nvidra@yakult.eu

Research on the gut microbiota typically focusses on studying faecal samples, which represent the content at the end of the large intestine. However, the small intestine is responsible for nutrient absorption and has different properties that affect how microbes interact with the host. Understanding the local differences in host-microbe interactions between the small and large intestine is important for manipulating the gut microbiota, such as through probiotics, in a way that is beneficial for the host. A narrative review of these location-specific differences and how they affect the gut microbiota composition and function, is conducted by an ILSI Europe expert group. Expert groups comprise at least 50% scientists from academia and the public sector, and up to 50% scientists from the industry. In conclusion: (i) there is a need to study the microbiome in the upper part of our gut using tools that sample directly in the small intestine, such as capsule systems; (ii) lab models can be used to study how the gut microbiota interact with other organs in the body, but these models need to be improved for better accuracy and translatability; and (iii) various strains of probiotics have been seen to relieve small intestinal health conditions. The expected impact can be summarized as follows: (i) probiotic producers may benefit from investing in research that explores the microbiota in the upper parts of the gut to improve the efficacy of their products; (ii) further research can lead to more targeted probiotic administration and improved host-microbe interactions, which can ultimately result in better health outcomes for consumers; and (iii) to better understand yow probiotics work, we need to expand our knowledge on the role of the gut bacteria beyond the large intestine and study the upper parts of the gut as well.

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MANURE PRO CONDITIONER IMPROVES BEDDING QUALITY AND BACTERIAL COMPOSITION WITH POTENTIAL BENEFICIAL IMPACTS FOR DAIRY COW'S HEALTH

Lysiane Dunière, B. Frayssinet, C. Achard, F. Chaucheyras-Durand, E. Chevaux and J. Plateau-Gonthier

Lallemand SAS, France

lduniere@lallemand.com

Recycled manure solid (RMS) is commonly used as bedding material in cow housing. Due to the high level of organic matter present, this environment can be at risk for pathogens development when spoiled with animal urine and faeces. Mastitis is an important issue in dairy industry, responsible for decreased milk production, increase in veterinary cost and culling rate. *Streptococcus*, *Staphylococcus*, *Klebsiella* and *E. coli* are among bacteria involved in mastitis onset. In indoor housing system, cows spend several hours per day lying, contributing to the transfer of potential mastitis pathogens from the bedding to the udder. The objective of this trial was to study the effect of a bacterial drying bedding agent (ManurePro®, MP) application on bedding and teat skin bacterial communities and milk sanitary quality. MP product (mix of *Bacillus* and LAB strains) was sprayed on the surface of 2 treated pens (110 cows in total) once a week at 1g/m²/week for 1 month and at 0.5g/m²/week for the 2 following months while the 2 control pens (110 cows) remained untreated. Control and MP beddings were sampled at day 1 before application, day 51 and day 87 of the trial. Teat skin samples were collected at the same time points on the same 10 cows/pen. Bacterial population was studied through 16S rDNA sequencing. Milk parameters were recorded regularly during the trial. Dry matter and pH of the bedding were not different between groups over the trial. The application of MP modified bacterial profiles of the beddings and increased diversity. Control bedding samples were significantly associated with potential pathogens, such as *Streptococcus*, *Fusobacterium* and a taxon of the *Enterococcales* order at day 51 and *Staphylococcus* at D87, while no taxa associated to a potential health risk were significantly detected in MP samples. Metabolic pathways prediction identified biogenic amines production as more abundant in control beddings at day 51. Teat skin bacterial diversity was not affected by the treatment, but samples from the control group were characterized by a significant association with *Streptococcus* at D51, *Trueperella* and a taxon of the *Staphylococcaceae* family at day 87. Finally, milk samples from cows housed in MP pens presented significantly lower somatic cell counts than those from the control group, while no impact of treatment was observed on milk total flora and spore content. ManurePro application on RMS bedding had positive effects on bedding and teat skin bacterial populations and improved milk sanitary quality during the experimental period.

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