International Society of Microbiota

TARGETING MICROBIOTA

Towards Clinical Revolution

11TH WORLD CONGRESS

OCT. 14>15, 2024

MALTA

Book of Abstracts

International Society of Microbiota

11th ISM World Congress on

Targeting Microbiota

October 14-15, 2024 – Malta

Cecilia Giron

President of International Society of Microbiota

Marvin Edeas

Founder and Chairman of ISM Scientific Commitee

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International Society of Microbiota

Welcome to the 11th ISM World Congress

It is a great pleasure to welcome you to the 11th ISM Annual Meeting on Targeting Microbiota, which will be held on October 14-15, 2024, at Corinthia Palace Malta.

Targeting Microbiota 2024 aims to bridge the gap between research and clinical practice, emphasizing the gut microbiome as a central determinant of systemic health and a promising target for novel therapeutic strategies. Attendees will engage with the latest scientific insights on microbiome modulation, exploring how these findings can be leveraged to advance the treatment and prevention of chronic diseases, marking a pivotal shift in the future of medicine.

We would like to thank all the scientific committee members and speakers of Targeting Microbiota for their excellent contributions.

We also wish to thank the supporters: **Bac3Gel**, **DNA Genotek**, and **Microbiota and Host**.

We are grateful for ISM media partners: **Bacteriophage.news**, and **VisitMalta Incentives**.

We hope that you will enjoy the Targeting Microbiota 2024 Congress and that your interactions with colleagues from many countries will stimulate a creative exchange of ideas and challenges

All our warmest regards,

Prof. Maria Cecilia Giron

University of Padova, Italy
President of Targeting Microbiota 2024

DOI Information

We are pleased to announce that the Targeting Microbiota 2024 Abstracts Book will be assigned a **DOI (Digital Object Identifier)**, ensuring a permanent and easily accessible online presence for the entire collection of work.

The DOI will be activated **after the congress**, and it will be communicated to all attendees.

How to cite your paper in the Abstracts Book?

To cite a paper presented at the Targeting Microbiota 2024 Congress, include the author's name, the conference date, the paper title (italicized), page number, the conference name, location, and DOI.

Citation example:

Smith, J. (2024, September 19–20). **The role of microbiota in cardiovascular health**. *p. 45*, Targeting Microbiota 2024, Malta. DOI

Practical Information

We would like to take the opportunity to give you some additional information about the meeting arrangements.

The Abstract book contains:
• Speakers' abstracts (the abstracts of the oral presentations follow the order of the program)
• The abstracts of posters on display

Badges

Upon registration you have received your own personal badge. Please wear this badge during the entire meeting including the coffee breaks and lunch.
The conference secretariat will be located in the area in front of the conference hall.

Instructions for participants

Chairpersons: The Chairpersons will be seated at the president's table.

Speakers: Speakers are invited to give their Power Point presentations for downloading on the computer to the technical team outside and not inside the conference hall. As the schedule is rather tight and to allow sufficient time for discussions, we would be very much obliged if the timing requirements were respected.

Poster Contributors: Please ensure that your poster is displayed at the appropriate location, please respect your poster number. Please remember to remove your posters at the end of the conference. The Poster contributors are invited to stand by their poster during the poster sessions.

Speakers Dinner
A dinner is organized on October 14 at Summer Kitchen of Corinthia Palace Hotel.
If you registered for this dinner, please join the group at 20h30 at the restaurant.

Mobile Phones
As a courtesy to the speakers and other delegates, please turn off your mobile phones or to silent whilst in the conference room. Please do not take pictures of the slides without the consent of the presenting author.

Questions
Please state your name and institution or company before asking your question.

Conference Staff
Staff at the conference registration desk will be happy to deal with any queries you may have. If we receive any messages for you, they will be announced at the break in the session and can be collected from the desk.

Personal Belongings
Please keep your valuables and working materials with you at all times. We would advise you to keep your name on the conference notes, as we may not be able to replace these if lost. The ISM and Corinthia Palace can't be held responsible for any loss or damage to your property.

11th ISM World Congress on Targeting Microbiota

October 14-15, 2024
Corinthia Palace, Malta

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Targeting Microbiota

Abstracts of Day 1

October 14, 2024

International Society of Microbiota

PHAGE THERAPY: A NEW ERA OF "OLD" CONCEPT FOR "MICROBIOME" HEALTH

KUTATELADZE, Mzia

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Dr. Mzia Kutateladze's insights into phage therapy will highlight a cutting-edge approach to manipulating the microbiome to combat numerous diseases and improve health outcomes.

She will present a groundbreaking exploration of phage therapy targeting the gut and skin, covering the potential of bacteriophages to revolutionize clinical treatments and improve patient outcomes through precision microbiome modulation.

About Dr. Kutateladze

Dr. Mzia Kutateladze represents the world-renown G. Eliava Institute of Bacteriophages, Microbiology and Virology, headquartered in Tbilisi, Georgia. Currently, she is the Director of Eliava Institute, as well as the President of the Eliava Foundation, a collection of commercial spin-offs. She oversees, coordinates and manages the research directions and programs of the Institute. She is the author or co-author of more than 80 scientific papers. Her scientific background is in microbiology and molecular biology, bacteriophage research and application. Dr. Kutateladze was a manager and a leading scientist of number of scientific research projects. She is serving as a project and papers reviewer for national and international funding agencies and scientific journals.

BACTERIOPHAGES REDESIGNED: TINY KILLERS AND DETECTIVES TO SUPPORT INFECTIOUS DISEASE THERAPY

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While bacteriophages offer great host specificity and killing activity, their therapeutic potential is naturally limited by narrow host-ranges, insufficient antimicrobial activity, lysogeny, and rapid emergence of resistance. However, there are several ways to overcome these limitations and provide smart and effective phage-based antimicrobials [1].

We engineered bacteriophages based on in-vitro DNA assembly and subsequent reactivation of synthetic phage genomes within suitable host cells. For efficient rebooting of viral genomes in Gram-positive bacteria, we use a bacterial L-form based platform, providing cross-genus surrogate hosts for phage amplification. To enhance recombination-based engineering of very large phage genomes unsuitable for synthetic assembly, CRISPR-Cas systems were established in various phage hosts.

Based on a broad selection of phages infecting important pathogens such as *E. coli, Klebsiella*, *Staphylococcus, Enterococcus,* and *Listeria*, it was possible to (i) convert temperate phages to virulent ones, (ii) produce phages carrying a broad variety of additional payload genes for enhanced self- or cross killing activity [2], (iii) broaden phage host ranges by structure-guided design, and (iv) provide a corresponding arsenal of nano-luciferase reporter phages as companion diagnostics for use in clinical phage therapy [3].

Besides using functional bacteriophages, another successful approach is to harness the bacteriolytic function of phages, i.e., the endolysins. Here, we have made significant progress by not only optimizing enzyme activity and in vivo half-life by domain shuffling and fusion to non-phage sequence, but also modification of the enzymes for fine-tuned application in serum and blood, tissue, and intracellular environments [4].

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MYTHBUSTING OUR MICROBIOME

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Public, academic and commercial interest in the human microbiome has increased exponentially over the last two decades. There is now a concerted effort, involving researchers around the world, to manipulate the microbiome for therapeutic purposes. There have been a number of encouraging advances, but much work remains to be carried out before we truly understand the roles the microbiome plays in health and in disease, and how we might reproducibly alter it in beneficial ways.

A key challenge for human microbiome researchers, educators, and those involved in knowledge exchange with the public, is to try and communicate exciting advances, while cutting through the hype that sometimes surrounds this field of research. In my talk, I will cover some illustrative examples that demonstrate the pervasiveness of myths and misconceptions in the human microbiome literature, providing evidence to show the unreliableness of these claims.

A further key message is that it is critical to acknowledge the complex nature of the human microbiome, and convey that nuance is extremely important. Some of what has been reported about the microbiome has not replicated well across studies, and conclusions are often context dependent. Over-simplification, and insufficient critical assessment in the microbiome literature, may therefore push research down unproductive avenues, or undermine public confidence in our results.

Ultimately, it is hoped that these messages will be of use to other researchers in the field, particularly those who may be branching into this area of research for the first time.

Reference

Walker, A.W. & Hoyles, L. (2023). Human microbiome myths and misconceptions. Nature Microbiology 8, 1392-1396.

ADDRESSING RISK FACTORS MAY LEAD TO BROADER USE OF PHAGE THERAPEUTICS

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Introduction: With the collapse of the antibiotic market, there is an undeniable need to develop new therapeutic antibacterials, and this is precisely the reason for the growing interest in bacteriophages. To make progress in the development of therapeutics, it is necessary to consider standardized procedures of development and handling at all levels, including proper interpretation/evaluation of phage/cocktail activity in vitro against bacterial mono- or co-cultures. Here we propose a predictive outline of a procedure for effective and rapid evaluation and selection of candidate phages; and criteria that should be considered when designing and producing phage cocktails.

Material & Methods:

- Evaluation-based selection of phages, considering risk factors such as phage or bacteria inhibition in phage/phage, phage/bacteria and bacteria/bacteria co-proliferation.
- Determine of the mode of action: synergy, proto-cooperation or antagonism.
- Development and upload of ready-to-use phage cocktail formulations.

Results and discussions: The phage bi- and tri-cocktails suppress the growth of phage-resistant mutants and overcome the inhibitory effect in vitro, resulting in synergy or proto-cooperation. Using an evaluation-based approach and developing a large set of ready-to-use phage cocktail formulations is an incredibly efficient way to interactively and rapidly evaluate and select the right candidates and predict their treatment potential.

Supported by Royal Higher Institute for Defense, Brussels, Belgium.

FECAL MICROBIOTA ANALYSIS AS A PREDICTIVE TOOL FOR CHOLESTATIC DISEASES

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Background/Aims: Bile acids play a key role in gut microbiota regulation and maintaining intestinal homeostasis. In cholestatic diseases, where bile secretion is impaired, gut microbiota may be altered. This study aimed to compare fecal samples from cholestatic disease patients and healthy individuals to explore if fecal microbiota could predict cholestatic conditions.

Methods: Fecal samples were analyzed using Next-Generation Sequencing (NGS) from 200 healthy controls (HC) and 50 patients with cholestatic diseases (CS).

Results: Cholestatic patients included those with bile duct stones (36), cholangiocarcinoma (7), pancreatic cancer (5), and ampulla of Vater cancer (2). Alpha diversity showed no significant differences, but beta diversity using UniFrac metrics revealed significant differences (R values: 0.16, 0.136). Synergistota and Fusobacteriota were more abundant in the CS group. LEfSe analysis showed Enterococcus and Escherichia were predominant in CS, while Actinomycetota and Bifidobacterium were more abundant in HC.

Conclusion: Fecal microbiota analysis may help diagnose cholestatic diseases, providing insights into gut microbiota changes in these conditions.

This research was supported by a grant from Healthcheckmate Inc., Daejeon, Korea.

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2. Martinez-Gili L, Pechlivanis A, McDonald JAK et al. Bacterial and metabolic phenotypes associated with inadequate response to ursodeoxycholic acid treatment in primary biliary cholangitis. Gut Microbes 2023; 15(1):2208501.
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GUT MICROBIOME, OBESITY AND DIABETES

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Introduction: Gut microbiota has been implicated in the pathogenesis of various diseases including obesity and diabetes. *Fusimonas intestini*, a commensal species of the family Lachnospiraceae, was highly colonized in diabetes model mice. We therefore investigated the importance of *F. intestini* in the pathogenesis of obesity and diabetes.

Materials & Methods: Prevalence and abundance of *F. intestini* was compared by qPCR between type 2 diabetes patients and healthy controls. The role of *F. intestini* in obesity and insulin intolerance was examined in mice.

Results: We found *F. intestini* was highly represented also in diabetes patients. We also found that *F. intestini* aggravated obesity and insulin intolerance, and that the bacteria produced elaidic acid, a trans-unsaturated fatty acid, in high fat diet-fed mice. Mechanistically, *F. intestini* and elaidic acid impaired intestinal epithelial integrity, by downregulating the expression of genes encoding tight junction components, to induce low grade systemic inflammation and thus promote metabolic endotoxemia. We also found that a bacterium other than *F. intestini* can also produce elaidic acid from oleic acid *in vitro.*

Conclusion: Elaidic acids produced from dietary fatty acids by *F. intestini* induces low grade systemic inflammation and ultimately leads to obesity and insulin intolerance. Reagents to remove free elaidic acid in the intestine could be a useful preventive strategy for obesity and diabetes.

Reference

Takeuchi et al. Cell Metabolism 35:361-375.e9,2023.

GUT MICROBIOME SIGNATURES IN IRRITABLE BOWEL SYNDROME

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Irritable Bowel Syndrome (IBS) is a highly prevalent condition characterized by a complex and multifactorial pathophysiology. Alterations in the gut microbiome have been suggested to play a significant role in IBS; however, the scientific literature, while extensive, is often contradictory. Several bacterial taxa have been linked to IBS, such as members of the *Ruminococcus* genus, but universally accepted microbiome signatures remain elusive. A growing body of research is highlighting microbial metabolites, rather than specific taxa, as more promising biomarkers for IBS. These include histamine, microbial bile salt derivatives, and short-chain fatty acids (SCFAs).

In this presentation, it will be shown that SCFAs, particularly propionate, can aid in the stratification of IBS patients beyond traditional bowel habit classifications. Non-constipated IBS patients with higher fecal SCFA levels appear to respond better to dietary therapeutic interventions. Additionally, these patients exhibit distinct microbiome compositions, with taxa such as *Collinsella aerofaciens* and *Dorea* spp. emerging as both potential targets and predictive markers. These findings suggest that non-constipated IBS patients could benefit more from probiotic treatments, low-FODMAP diet or other microbiome-targeted therapies.

In conclusion, gut microbiome analysis (both microbial taxa and metabolites) may enable a more refined categorization of IBS patients, offering better guidance for personalized treatment approaches.

GUT BACTERIA AND HEART HEALING:
THE HIDDEN PLAYERS IN POST-INFARCTION RESILIENCE

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Dr. Hsieh will cover the following points:

1. Gut microbiota influence heart recovery post-heart attack.

2. Microbes affect immune cells and produce essential fatty acids.

3. Butyrate-producers linked to improved cardiac protection.

4. Evidence from human and animal studies supports this link.

5. Potential for therapeutic interventions to enhance heart healing.

6. Interdisciplinary exploration of microbial metabolites and immune dynamics.

MICROBIOTA-DERIVED EXTRACELLULAR VESICLES: BRIDGING MOTHER AND FETUS

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Background: Reports regarding the presence of bacteria in the fetal environment remain limited and controversial. Recently, extracellular vesicles secreted by the human gut microbiota have emerged as a novel mechanism for host-microbiota interaction. We aimed to investigate the presence of bacterial extracellular vesicles in the fetal environment during healthy pregnancies and determine whether extracellular vesicles derived from the gut microbiota can cross biological barriers to reach the fetus.

Results: Bacterial extracellular vesicles were detectable in the amniotic fluid of healthy pregnant women, exhibiting similarities to extracellular vesicles found in the maternal gut microbiota. In pregnant mice, extracellular vesicles derived from human maternal gut microbiota were found to reach the intra-amniotic space.

Conclusions: Our findings reveal maternal microbiota-derived extracellular vesicles as an interaction mechanism between the maternal microbiota and fetus, potentially playing a pivotal role in priming the prenatal immune system for gut colonization after birth.

DECIPHERING THE ROLE OF ORAL-PLACENTAL AND VAGINAL-PLACENTAL MICROBIOME AXES IN PRETERM BIRTH

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Introduction: Recent studies have challenged the dogma that the placenta is a sterile environment, revealing that it hosts a distinctive microbiome. Despite these findings, significant questions remain regarding the origins of the placental microbiome and its implications on pregnancy and fetal health. Some studies suggest that the placental microbiome may originate from the vaginal tract, while others indicate that bacteria from the oral microbiome can enter the maternal bloodstream and subsequently seed the placenta. Studies that simultaneously analyzed the vaginal, oral, and placental microbiomes within the same cohort are still lacking. In addition, it remains unclear whether the composition of the placental microbiome differs between healthy pregnancies and those with complications such as preterm birth (PTB). PTB continues to be the major contributor to neonatal morbidity and mortality worldwide.

Material & Methods: In our study, we performed 16S rRNA gene sequencing to assess the composition of the oral and placental microbiome in samples collected from 18 women who experienced preterm birth (PTB) and 36 matched controls who delivered at term (TB), all of whom were part of the Molecular Signature in Pregnancy (MSP) study. We used the extensive microbiome data from MSP and our previously published research on vaginal microbiomes to explore the possible origins of the placental microbiome and assess whether its composition varies between healthy and complicated pregnancies.

Results: Our analysis revealed distinct profiles in the oral microbiome of PTB subjects compared to those who delivered at term. Specifically, we observed an increased abundance of Treponema maltophilum, Bacteroides and Prevotella species in the PTB group. Importantly, some of these species showed higher abundances in the PTB group during early pregnancy, suggesting their potential use as biomarkers. When we assessed the placenta microbiome composition, we found that similar to previous findings, Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria were the most dominant phyla. Interestingly, species such as Ureaplasma urealyticum were more abundant in PTB placenta samples.

Conclusion: Our findings indicate a notable difference in the placental microbiome composition in PTB subjects compared to those who delivered at term. Specifically, our data revealed that in PTB cases, the placental microbiome exhibited a closer resemblance to the vaginal microbiome, whereas in term pregnancies, the placental microbiome was more similar to the oral microbiome. This underscores the significant differences in the oral-placental and vaginal placental microbiome axes in term and preterm birth.

This work was supported by funds from Sidra Medicine

USING ROSEOFLAVIN, A NATURAL RIBOFLAVIN ANALOGUE, TO MODIFY THE HUMAN MICROBIOTA

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Introduction: The antivitamin roseoflavin, produced by the soil bacterium *Streptomyces davaonensis* and a few relatives, is a structural analogue and competitive inhibitor of riboflavin, also known as vitamin B2 [1]. We conducted pilot cultivation-based and molecular investigations on the effect of riboflavin and roseoflavin on the human oral microbiota to find out whether oral application of roseoflavin might be a new approach to modify this microbiota for the sake of human health, e.g., to prevent caries.

Material & Methods: For cultivation-based tests [2], microbial isolates from saliva samples of 41 healthy volunteers were obtained and identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. 30 different microbial isolates were investigated regarding signs of riboflavin auxotrophy and susceptibility to roseoflavin using growth experiments. In order to account for yet-uncultivated species, NGS-based analyses were conducted, too. For this, we obtained saliva samples from 15 healthy volunteers, which were pooled and incubated with or without roseoflavin for 24 hours, respectively. After DNA and RNA extraction, 16S rRNA (cDNA) and 16S rRNA gene sequencing were used to investigate shifts in the community composition of the present (DNA-based) or active (cDNA-based) saliva microbiota. Similar studies are ongoing for skin microbiota samples.

Results: As expected, cultivation-based studies suggested that mainly the growth of gram-positive bacteria is affected by riboflavin and roseoflavin. Notably, growth of cariogenic *Streptococcus mutans* was significantly enhanced by riboflavin, and this effect was at least partly reversed by supplementing the growth media with roseoflavin. Recently it was suggested that *S. mutans* might indeed be auxotrophic for riboflavin and hence susceptible to roseoflavin [3]. Data from the molecular studies are currently being evaluated.

Conclusion: We carefully speculate that roseoflavin treatment might be an option to particularly modulate the gram-positive fraction of the microbiota of the human oral cavity, and presumably of additional niches of the human body, for the sake of human health. Future research aims at characterizing the inhibited bacteria and the molecular mechanism(s) of the underlying inhibition processes in more detail as well as the effect of roseoflavin on human cells.

Supported by: Microbiome programme of the Baden-Württemberg foundation, Germany

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CULTURING THE COMPLEXITY OF INTESTINAL MICROBIOTA IN-VITRO IN GUT3GEL

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Introduction: Intestinal microbiota modulates inflammation and degenerative diseases. Culture methods fall short studying these interactions due to challenges maintaining the complex community, limiting research on the connection of microbiota with chronic diseases and, validation of molecules in a representative model. Herein, Gut3Gel (intestinal mucus model) ability to sustain microbiota in vitro was evaluated.

Materials and Methods: Isolated microbiota from 5 donors using European guidelines [1] was inoculated in: Gut3Gel or Brain Heart Infusion Medium (BHI) and incubated (37°C) for 24, 48 and 72h. Next-Generation Sequencing was performed to determine if Gut3Gel could sustain microbiota diversity. Fatty acid concentration was determined through metabolomics. Exopolysaccharides secretion was identified through rheology. Local pH, O2 and RedOx were measured through Unisense.

Results: Bacterial population in Gut3Gel was coherent with the communities reported in adult gut microbiota. The most abundant being Bacteroidetes and Firmicutes, with lower levels of Actinobacteriota. In BHI condition, Proteobacteria increased; while in Gut3Gel, Actinobacteria and Firmicutes boosted, and previously unculturable bacteria increased over time. Mechanical properties of the matrix increased hinting production of EPS. Metabolite analysis revealed lactate production within Gut3Gel group.

Conclusion: Gut3Gel sustains microbiota's complexity in-vitro, serving as a bench-test for research in healthy gut microbiota.

Reference

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FROM MICROBIOMES AND (META)GENOMES TO THE LAB AND BACK - IDENTIFICATION, PRODUCTION AND APPLICATION OF BACTERIOCINS

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Bacteriocins are antimicrobial peptides produced by bacteria to inhibit competitors in their natural environments. Some of these peptides and/or their natural producers are widely used as food preservatives. As a number of bacteriocins have potent activity against bacteria pathogenic for humans and animals they also have other interesting fields of application in animal feed or as microbiome modulators. In face of the global increase in problems with antibiotic resistances of many important human pathogens, bacteriocins may also become interesting alternative therapeutics in some clinical settings.

To allow clinical application and entry of novel bacteriocins into the market, efficacy of production and purity of the products need to be improved. Currently, industrial production of bacteriocins is performed exclusively with natural producer organisms on complex substrates and products are commercialized as semi-purified preparations or crude fermentates.

One possibility is to shift production to recombinant biotechnological production hosts. Due to the intrinsic antimicrobial activity of bacteriocins, this is, however, not a trivial task. Here, we will report on some of our recent efforts identify (novel) bacteriocins in environmental microbiomes and (meta)genomic data. We will highlight approaches to establish recombinant production of several well-characterized bacteriocins using the industrial workhorse organism *Corynebacterium glutamicum*. Moreover, we will discuss problems associated with recombinant production and possible solutions. Finally, we will provide examples for potential application of bacteriocins in different settings.

RESIDENT MICROBIOTA PROMOTE THE ACQUISITION AND PATHOGENESIS OF EBV AND HIV

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Introduction: Germ-free (GF) mice, which have no resident microbiota, are the gold standard for exploring the microbiome's role in health and disease but have limited value to study human-specific pathogens because they do not support their replication. Here, we developed GF mice systemically reconstituted with human immune cells (humanized GF mice) and use them to evaluate resident microbiota's role in the acquisition, replication, and pathogenesis of two human-specific pathogens, Epstein-Barr Virus (EBV) and Human Immunodeficiency Virus (HIV).

Material & Methods: We created GF and conventional (CV) humanized mice. GF and conventional (CV) humanized mice were infected with HIV or EBV and then we measured rates of infection, T cell levels, and in the case of EBV, tumor formation.

Results: Resident microbiota enhance the establishment of EBV infection and tumor formation, increasing the different organs where tumors were found. HIV mucosal acquisition and replication are higher in CV-humanized mice and HIV-RNA levels are higher in plasma and in tissues of CV-humanized mice. Interestingly, the frequency of CCR5+ CD4+ T cells throughout the intestine was higher in CV-humanized mice, indicating that resident microbiota determines HIV target cell levels.

Conclusion: Resident microbiota promote the acquisition and pathogenesis of two clinically relevant human-specific pathogens.

Supported by National Institute of Health grant R01DK131585.

RESIDENT MICROBIOTA REGULATES HIV INFECTION IN THE CENTRAL NERVOUS SYSTEM

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Introduction: The central nervous system (CNS) is an important site of HIV infection. HIV-associated neurological disorders affect up to 50% of people with HIV (PWH). Disturbances in the gut microbiome have also been associated with neurocognitive impairment in PWH. Thus, we hypothesized that resident microbiota may modulate HIV replication in the CNS.

Material & Methods: We used humanized mice, immune deficient mice systemically reconstituted with human immune cells, to evaluate the role of resident microbiota in HIV replication in the CNS. We compared HIV-RNA and HIV-DNA levels, as measured by real-time PCR, in the brain of HIV-infected germ-free (GF) humanized mice and humanized mice colonized with conventional (CV) resident microbiota.

Results: HIV-DNA levels were 6.7 fold higher in the brain of HIV-infected CV humanized mice compared to GF humanized mice (664 vs 98 HIV-DNA copies/100,000 cells respectively, p=0.0295). Similarly, HIV-RNA levels were 8.4 fold higher in the brain of HIV-infected CV humanized mice compared to GF mice (36,219 vs 4,053 HIV-RNA copies/100,000 cells respectively, p=0.0065).

Conclusion: Our data demonstrate that resident microbiota regulate HIV replication in the CNS. In the future, our results will help inform the development of novel HIV treatment approaches aimed at ameliorating neuroHIV infection.

Supported by National Institute of Health grants R01MH131441 and R01DK131585.

THE GUT MICROBIOTA MODULATES ANASTOMOTIC HEALING IN PATIENTS WITH COLORECTAL CANCER UNDERGOING SURGERY

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Anastomotic leak (AL) is a major complication in colorectal surgery and significantly increases morbidity and mortality. Our objective was to investigate the role of gut microbiota in anastomotic healing.

Preoperative fecal samples were collected from patients with colorectal cancer (CRC). Fecal microbiota transplantation (FMT) was performed in mice using samples from patients with and without AL, after which transplanted mice underwent colonic surgery. At day 6 after surgery, anastomotic healing, gut barrier integrity, and gut microbiota composition were analyzed. Bacteria of interest were isolated and assessed in vitro and in vivo.

We found that, compared to mice transplanted with fecal microbiota from donors without AL, mice receiving fecal transplants from donors with AL displayed poorer anastomotic healing, increased gut permeability, and higher levels of colonic pro-inflammatory cytokines, resulting in higher AL rates. We identified a strain of Parabacteroides goldsteinii, which exerted a beneficial anti-inflammatory effect and improved anastomotic healing, and a deleterious Alistipes onderdonkii strain, which promoted inflammation and increased leakage.

These results suggest a causal role of the preoperative gut microbiota in surgical recovery of the colon, paving the way toward microbiota-targeted interventions to improve anastomotic healing and prevent AL.

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THE HOST GENOTYPE ACTIVELY SHAPES ITS MICROBIOME ACROSS GENERATIONS

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The microbiome greatly affects health and wellbeing. Evolutionarily, it is doubtful that a host would rely on chance alone to pass on microbial colonization to its offspring. However, the literature currently offers inconclusive evidence regarding two alternative hypotheses: active microbial shaping by host genetic factors or transmission of a microbial maternal legacy. To untangle the influence of host genetics and maternal inheritance, we collected 2-cell stage embryos from two representative wildtypes, C57BL6/J and BALB/c, and transferred a mixture of both genotype embryos into hybrid recipient mice to be inoculated by an identical microbiome at birth. Observing the offspring for six generations unequivocally emphasizes the impact of host genetic factors over maternal legacy in constant environments, akin to murine laboratory experiments.

Interestingly, maternal legacy solely controlled the microbiome in the first offspring generation. However, current evidence supporting maternal legacy has not extended beyond this initial generation, resolving the aforementioned debate.

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IMPACT OF OMEGA-3-RICH FISH CONSUMPTION ON FAECAL SHORT CHAIN FATTY ACID AND GUT MICROBIOTA PROFILES

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Introduction: Despite there are several studies focused on the effects of carbohydrates on gut microbiota in humans, the impact of dietary fats is still less known1. Then, we explored how changes in the intake of omega-3-rich fish would affect faecal short chain fatty acid (SCFA), and gut microbiota profiles.

Material & Methods: A clinical intervention study was conducted with 18 healthy adults who were asked not to consume any omega-3-rich food during one week and to consume two servings per week of omega-3-rich fish (mackerel) for the next two weeks. Faeces and food intake habit were collected at baseline, no-fish, and at fish-diet points. Faecal SCFA were analysed by GC-MS and bacterial metataxonomy was assessed by V3-V4 16S metagenomics. Univariate, bivariate, and multivariate analyses were performed in R.

Results: There was a shift in the SCFA profile of volunteers two weeks after omega-3-rich fish consumption, which correlated negatively with acetate levels (ρ=-0.43, $p<0.01$). The gut bacterial community changed between regular and non-regular consumers of omega-3-rich fish; Coprococcus genera relative abundance doubled and correlated positively with regular consumption of omega-3-rich fish (ρ=0.60, $p<0.01$).

Conclusion: SCFA and gut microbiota profiles are impacted by omega-3-rich fish intake in the short and long-term, respectively.

Supported by: The European Union under Horizon Europe grant number 101084642. Laura García receives a predoctoral student grant from the Basque Government (IKERTALENT Scholarship Program 2021).

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INTERPLAY BETWEEN HOST GENETICS AND GUT MICROBIOME COMPOSITION IN THE JAPANESE POPULATION

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Introduction: Host genetic variation affects the gut microbiota through a complex interplay involving both environmental and genetic factors. Despite significant research focused on discovering the relationship between bacterial composition and disease onset, the role of host genetics in shaping the microbiota remains poorly understood, especially in the Japanese population.

Material & Methods: We performed Whole Genome Sequencing on Japanese individuals and obtained their gut microbiome compositions using 16S rRNA sequencing and shotgun metagenomic sequencing. We applied a series of analyses, including genome-wide association studies to identify associations between human gut microbiota and host genetic variants. We also carried out phenome-wide association studies on loss of function variants.

Results: We identified 27 significant independent SNP-taxon associations and 33 taxa associated with loss of function variants. Post-GWAS analysis revealed functional links between taxa and different traits and disorders, including rs79675168-A's mapped gene LIMA1 association with Bifidobacterium bifidum and cholesterol homeostasis. Also, post-PheWAS analysis identified functional associations between Blautia and rheumatoid arthritis, and Escherichia and innate immune response.

Conclusion: The findings in this study contributes to a better understanding of the complex role of host genetics in shaping the gut microbiota and its relationship with complex traits and disorders in the Japanese population.

Supported by RIKEN's Junior Research Associate program

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AIR POLLUTION, NASAL MICROBIOTA, AND BRONCHIOLITIS: UNDERSTANDING THEIR INTERPLAY THROUGH A MULTILEVEL APPROACH

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Respiratory syncytial virus (RSV) is a leading cause of lower respiratory infections in infants, contributing to significant global morbidity and mortality. The factors influencing RSV severity are not fully understood, but environmental exposures, particularly to particulate matter (PM10 and PM2.5), are suspected to worsen RSV-related bronchiolitis by affecting inflammatory pathways. Previous research linked PM exposure up to three weeks before hospitalization to increased bronchiolitis severity in infants. The bacterial nasal microbiota (bNM) also modulates immune responses and may influence RSV pathogenesis in response to environmental triggers.

This study examines the effects of PM10 and PM2.5 on bNM composition in RSV bronchiolitis and explores the role of bacterial extracellular vesicles (bEVs) from the nasal microbiota in immune modulation using a zebrafish model. In a case-control study of 110 infants with RSV bronchiolitis and 49 healthy controls, bNM composition differed significantly, with RSV cases showing a higher abundance of Haemophilus influenzae (Hi), which increased with higher PM exposure. Hi-derived bEVs injected into zebrafish embryos triggered a pro-inflammatory response, including neutrophil recruitment and upregulation of inflammatory genes.

These findings suggest that air pollution alters bNM composition, potentially exacerbating bronchiolitis through inflammatory mechanisms, and highlight the role of bEVs in RSV-bronchiolitis pathogenesis.

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A NOVEL IN VITRO APPROACH TO IDENTIFY MICROBIAL AND CELLULAR SIGNATURES OF SHORT BOWEL SYNDROME (SBS)

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Introduction: Short bowel syndrome (SBS) is characterized by reduced bowel length, causing intestinal failure and alteratered microbial ecology1. In half of SBS patients, spontaneous morphological changes (adaptation) in the intestinal mucosa improve nutrient absorption and survival2-3. Yet, the SBS-induced changes and adaptation are poorly characterized. Here, we present an in vitro approach to characterize microbial, metabolic and cellular hallmarks of SBS.

Material & Methods: A Simulator of the Human Intestinal Microbial Ecology (SHIME) model incorporating oral, small intestinal and colon microbiota from a healthy donor is developed and used to simulate SBS(Type II)-resection. Microbial compositon and metabolic activity in in vitro SBS and non-SBS SHIME are quantified by 16S rRNA-sequencing and gas-/ion-chromatography. Coculture model of entero-/colono-cytes, goblet- and enteroendocrine-like cell lines are employed to assess alterations induced by in vitro microbiota (SBS and non-SBS) on barrier permeability, integrity, mucus and cytokines secretion.

Results: Microbial composition, organic acid profiles and epithelial functions are altered in in vitro SBS condition. In particular, changes in lactate, butyrate production and bacterial genera modulation align with the literature on SBS patients.

Conclusion: By combining SHIME and cellular models we provide a deeper understanding on alterations of both microbial ecology and host-bacteria interaction in SBS.

Supported by EU Marie Sklodowska-Curie Individual fellowship

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EXPLORING CONNECTIONS BETWEEN THE AUTONOMIC NERVOUS SYSTEM, MICROBIOTA-GUT-BRAIN AXIS AND RESPIRATORY SYSTEM: A SCOPING REVIEW

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Introduction: Much has been written about the relationship between the gut and Autonomic Nervous System (ANS)1, as well as the connection between the gut and lungs[2]. Research has also shown the connection between the lungs and ANS3. Given this, I hypothesize that the vagus nerve, by innervating several organs involved in these systems and exercising its immunomodulatory role, is key in the interconnection of the gut and lung microbiota and ANS.

Material & Methods: A retrospective Scoping Review of scientific papers published between 2014-2024.

Results: No studies were found that consider the role of the vagus nerve in the connection between gut and lung microbiota. However, some papers show an efferent pathway given by the hypothalamic-pituitary-adrenal axis and ANS, which, when injured, generates inflammation, altering the intestinal barrier, generating dysfunction in its microbiota[3]. In an afferent sense, inflammation of the intestinal tract and dysbiosis generates dysfunction in the ANS, which can generate pathologies, in the digestive, nervous, and respiratory systems[3].

Conclusion: While understudied, the vagus nerve may present an intersection point between the ANS and gut and lung microbiota. If true, such insight could lead to enhanced clinical approaches in the prevention and treatment of neurological, respiratory and gastrointestinal conditions.

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11th ISM World Congress on

Targeting Microbiota

Abstracts of Day 2

October 15, 2024

THE MYSTERY OF THE MICROBIOME GENOME: THE POTENTIAL OF 2 TO 20 MILLION MICROBIAL GENES TO TRANSFORM OUR HEALTH

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The human microbiome harbors an estimated 2 to 20 million microbial genes, representing an extensive genetic reservoir that has the potential to significantly impact human health. This presentation will explore the vast genetic diversity within the microbiome and its implications for our understanding of human biology.

I will discuss how these microbial genes may interact with the human genome, potentially allowing us to "outsource" or delegate various biological functions to the microbiome. This outsourcing could pave the way for novel approaches in disease prevention, treatment, and even extending human lifespan. By elucidating the interactions between microbial and human genes, this research aims to identify new strategies to modulate these relationships and enhance health outcomes.

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DRUG EFFECTS OF GUT MICROBIOTA IN HUMANS: CLINICAL CONSEQUENCES

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The comparative and functional meta-omics approaches have made it possible to get a molecular snapshot of microbial function at a certain time and place. It became evident that the activity and composition of the microbiota is affected by genetic background, age, diet, and health status of the host. However, together with disease, drugs can profoundly affect gut microbiota, exerting in that way undesirable and/or desirable effect on patients. Besides antimicrobial compounds, whose action on gut microecology is expected and well-known, many different drugs or drug classes can influence the composition of the gut microenvironment. A seminal study evaluated 1.000 marketed drugs against 40 representative gut bacterial strains, and found that 24% of the drugs with human targets, including members of all therapeutic classes, inhibited the growth of at least one strain in vitro. The anticommensal activity was displayed at concentrations lower than those achieved in the small and large bowel [1]. A systematic review of non-antibiotic drugs reported a long list of drug-induced dysbiosis, including proton pump inhibitors (PPIs), non-steroidal anti-inflammatory drugs (NSAIDs), opioids, statins, antipsychotics and metformin [2].

In this presentation, the mechanisms by which some medicines (namely PPIs, NSAIDs, opioids, systemic and topical antibiotics) affect gut microbiota and the clinical consequences of drug-induced dysbiosis will be discussed.

In addition, the bidirectional interaction between drugs and microbiota will be outlined. Indeed, gut microbiota is one the potential causes of individual variability in drug response, one major cause of adverse drug reactions resulting in a substantial health and economic burden [3]. Thanks to wide array of bacterial enzymes, the metabolic activities of the microbiome (as well as its metabolites) can also influence drug pharmacokinetics and pharmacodynamics. This field has given rise to pharmacomicrobiomics, a fascinating new discipline that represents a path forward the precision medicine.

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TARGETING THE MICROBIOTA BY MEDICAL PROFESSIONALS: WHERE ARE WE NOW?

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Introduction: The important role that the gut microbiota plays in health and disease of the host has clearly been established over the last couple of decades. Dysbiosis of the gut microbiota composition has been shown in many different diseases. However, all of this insight has led to limited successful applications in real-life. This is partly due to the gut microbiota composition showing large inter-individual differences that are primarily defined by host genetics, diet, lifestyle and use of medication. Even during the day, large fluctuations seem to occur. On a functional level however, the similarities between individuals are thought to be much higher, due to large functional overlaps between different microbial taxa.

Material & Methods: This presentation will give a high-level overview of the state-of-the-art of gut microbiota research and tools to modulate it. Subsequent presentations in the session will be more granular with respect to (potential) applications.

Results: Only for a few taxa we know the correlations between their presence or absence and certain diseases or disorders. And even then, there is not always a causal connection: the difference in abundance may be due to the disease, rather than that the difference causing the disease. Therefore, the chicken-and-the-egg dilemma still holds very much for targeting the microbiota. Nevertheless, numerous start-up companies are using the (fecal) microbiota composition as a marker to optimize your health status. Specifically medical professionals in academic hospitals have adopted the conviction that the microbiota is extremely important in health and disease. This has led to a multitude of different studies, but rarely to true (clinical) applications, perhaps with the exception of fecal microbiota transplantation for *C. difficile* infection, which is extremely effective, with an even better efficacy than many a drug. However, many healthcare professionals do not know which probiotic strain to choose for a particular patient (next talk in the session). Other talks in the session address i) the microbiota-gut-brain axis in neurodevelopment in early life and ii) depression, and iii) targeting the microbiota using precision nutrition based on microbiota profiling in obesity

Conclusion: This introduction and the subsequent talks in the session are a prelude to the discussion on how to target the microbiota in the program-item '*Discussion: Probiotic & Prebiotic Prescribing Practices: How to Empower Medical Actors to Improve Patients' Health*?'

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PROBIOTICS & HEALTH: HOW TO SELECT THE RIGHT STRAIN?

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The use of probiotics in the food and pharma industry has been blooming for the past decades. As feedback from healthy consumers is rather enthusiastic, a lot of effort is currently directed in elucidating the mechanisms of interaction between beneficial microbes and barrier and immune function of the host.

In most cases, the effect of the administered probiotic is evident when the microbes are still alive at the time they reach the small and large intestine, suggesting that it is dependent on the metabolic activity of the bacteria. Subsequently per definition we call it a probiotic. However, on some occasions it has been shown that even the culture supernatant of these microbes mediates effects or a combination with so called prebiotics. Even dead microbes have been show to confer positive results to the host.

In a clinical setting, a practitioner may conceptually believe that a patient would benefit from a probiotic. The problem is which one(s). Often the advice is a generic “take a good probiotic”; a suggestion bordering on magical thinking. For those that wish to avoid this wishful thinking several paths are possible to the selection of the right strain. Those paths will be presented within the talk.

Gut3Gel: INNOVATIVE IN VITRO PLATFORM FOR EVALUATING MICROBIOME-MODULATING MOLECULES

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Introduction: Modulating microbiota can prevent degenerative diseases and mental disorders. *In vitro* technologies that efficiently replicate the complexity of microbiota are required to validate the effect of microbiome-modulating drugs in a high throughput manner. Gut3Gel is a standalone solution to culture intestinal microbiota *in vitro*. Its power as a test bench to evaluate microbiome-modulating drugs was assessed with clinically tested prebiotics.

Materials and Methods: Microbiota isolates were inoculated in Gut3Gel and incubated for 24 hours. Afterwards, microbiota in Gut3Gel were supplemented with microbiome-modulating drugs and further incubated for 24 and 48h. Growth profiles of these communities were assessed through DNA quantification, 16S rRNA NGS, and metabolic activity.

Results: Nutraceuticals had a different effect depending on microbiome composition between donors. Microbiota in Gut3Gel supplemented with inulin resulted in higher Actinobacteria and inhibited Rikenellaceae and Oscillospiraceae while when supplemented with Nutriose®, Lachnospiraceae and Parabacteroides increased in two donors, similarly to what was observed in clinical studies.

Conclusion: Initial microbiota composition promotes a variable effect of nutraceuticals. Gut3Gel closely matches observations from clinical studies, indicating that Gut3Gel serves as an effective *in vitro* platform to understand the impact of nutraceuticals on microbiota behaviour. More studies will be repeated with a higher number of donors and nutraceuticals.

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INVESTIGATING WHETHER POSTBIOTIC-RICH SOURDOUGH BREAD HAS THE POTENTIAL TO CONFER SIMILAR HEALTH BENEFITS TO CONSUMERS WITH DIFFERENT GUT MICROBIOTA PROFILES

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Sourdough bread is a type of heat-treated functional fermented food that uses specific microbes to produce postbiotics associated with potential health benefits. This study used a long-term in-vitro gut model to assess the extent that consuming postbiotic-rich sourdough bread impacts key factors of human health following digestion and gut fermentation.

The microbial strain used in this study was highlighted in preceding studies to have the greatest overall potential to confer a range of health benefits to a consumer. This microbial strain was used to create sourdough bread with a distinct postbiotic profile and, following simulated digestion, the digested sourdough bread was used to supplement the nutritional feed for a long-term in-vitro gut model (M-SHIME) to simulate gut fermentation using microbiota provided by a range of faecal donors. At key endpoints factors that influence human health were assessed including organic acid production, antioxidant capacity, gut barrier strength, and both metabolomic and metagenomic profiles.

The preliminary assessment confirms that while the sourdough bread produced consistently greater health benefits compared to the non-sourdough reference bread, there was noticeable differences in several potential health benefits due to gut microbiota variability between donors. (Additional analyses will be conducted prior to conference in October).

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GUT MICROBIOTA IN EARLY LIFE: IMPACT ON NEURODEVELOPMENTAL OUTCOMES

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Introduction: The gut microbiota is now recognized as a key regulator of brain development and behaviour, and a potential susceptibility factor in neurodevelopmental and psychiatric disorders such as autism spectrum disorder (ASD). Although the exact role of gut microbiota in ASD is still being clarified, identifying microbiota-based therapeutic strategies is crucial for prevention and management.

Materials & Methods: We conducted a longitudinal study examining the fecal microbiota and metabolome in infants with and without a family history of ASD over the first 3 years of life, a critical period when the gut microbiota and brain are both undergoing rapid development. Developmental assessments included the Mullen Scales of Early Learning (MSEL) at 5 and 36 months and the ADOS-2 at 36 months. Preclinical studies assessed the impact of multispecies probiotic supplementation from gestational day 6 to birth on anxiety-like behavior and gene expression related to gut barrier and blood-brain barrier (BBB) integrity, as well as gut microbiota composition in juvenile and adult offspring.

Results: At 5 months, infants at elevated likelihood (EL) of ASD had altered gut microbiota, including reduced *Bifidobacterium* and increased *Clostridium* and *Klebsiella* species, compared to low-likelihood (LL) infants. LL infants excreted more fecal γ-aminobutyric acid (GABA), which declined with age, while GABA levels in the EL group remained low. Positive correlations were found between GABA and *Bifidobacterium species*, and negative correlations with *Clostridium species*. Behavioral assessments showed no significant differences at 5 months, but by 3 years, EL infants had lower MSEL and ADOS-2 scores. Preclinical studies indicated that prenatal multispecies probiotic supplementation improved emotional behavior, gut microbiota composition, and gene expression, with males showing greater sensitivity to the intervention.

Conclusions: Our findings suggest that gut microbiota may influence behavioral outcomes in early life, with implications for using prenatal probiotic interventions to support neurodevelopment. These results highlight the potential for precision microbial therapies to enhance healthy neurodevelopment and behavior.

Supported by: Olle Engkvist Foundation, Swedish Medical Council, and Swedish Brain Foundation (Hjärnfonden).

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MICROBIOTA ALTERATIONS IN PROLINE METABOLISM IMPACT DEPRESSION

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Introduction: The microbiota-gut-brain axis has emerged as a novel target in depression, a disorder with low treatment efficacy. However, the field was dominated until recently by underpowered studies focusing on major depression not addressing microbiome functionality, compositional nature, or confounding factors.

Material & Methods: We applied a multi-omics approach combining pre-clinical models with three human cohorts including patients with mild depression.

Results: Microbial functions and metabolites, particularly those converging onto glutamate/GABA metabolism and proline, have been linked to depression. High dietary proline intake was identified as the strongest dietary factor influencing depression. Disruptions in whole-brain dynamics, specifically within rich club networks, were associated with both depression and circulating proline levels. In mice, proline supplementation exacerbated depression and increased microbial translocation. Additionally, human microbiota transplantation induced emotional impairments in mice and altered prefrontal cortex genes related to GABA, proline, and the extracellular matrix. In Drosophila, RNAi-mediated knockdown of proline and GABA transporters, along with mono-association with Lactobacillus plantarum (a high GABA producer), provided protection against depression-like states.

Conclusion: Targeting the microbiome and dietary proline may open new windows and add new complementary strategies in the treatment and/or prevention of depressive traits.

Supported by: This work was partially supported by Instituto de Salud Carlos III (Madrid, Spain) through the research grants PI15/01934, PI18/01022, and PI21/01361.

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DOES THE MICROBIOME CONTROL CRAVINGS AND ADDICTIVE BEHAVIOR OF THEIR HOST: A NEW PROSPECTIVE AND HOPE FOR TREATING ADDICTION

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Addictions to opioids, pain relievers, alcohol, and tobacco represent an extremely challenging and persistent problem. The recidivism rate following in-patient or outpatient treatment programs can be as high as 90% for most of these addictions.

This presentation will examine the startling new findings of how gut dysbiosis and intestinal permeability drive addictive behavior, making the host seek out compounds that benefit these dysbiotic microbes, usually by pain and/or reward pathways.

Thus, treatment regimens need to be restructured towards changing the gut terrain via targeted pro, pre, and postbiotics; truly a paradigm shift in rehabilitation philosophies.

GENE-ENVIRONMENT INTERACTIONS RELEVANT TO AUTISM AND IMMUNE IMPAIRMENT CAUSE GUT MICROBIAL DYSBIOSIS IN A MOUSE MODEL OF COLITIS

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Introduction: Autistic individuals often experience inflammatory disorders and gastrointestinal issues1. We investigated impacts of acute inflammatory insult (dextran-sodium-sulfate (DSS)-induced colitis2) on the gut microbiome in Nlgn3R451C mice expressing an autism-associated variant in Neuroligin-33, and IFNAR1-/- mice lacking the interferon-alpha/beta-receptor-subunit-1.

Material & Methods: 3%-DSS treatment for 7-days generated ulcerative-colitis symptoms in co-housed 8-week-old Nlgn3R451C, IFNAR1-/-, Nlgn3R451CxIFNAR1-/-, and wild-type mice. Faecal bacterial 16S rRNA sequences were analysed to determine microbial diversity, richness, evenness and relative abundance.

Results: DSS treatment negatively impacted microbial alpha-diversity in wild-type mice (P=0.017), particularly on Day 8 compared to SHAM-treated wild-types (P=0.03). Microbial diversity was altered in DSS-treated Nlgn3R451CxIFNAR1-/- (P=0.013) and IFNAR1-/- mice (P=0.02). Interestingly, DSS-treated Nlgn3R451C alpha-diversity was unchanged during the experiment. Nlgn3R451Cmice exhibited treatment-responsive changes in microbial composition over time compared to SHAM controls (R2: 0.146754, P=0.015). In all mouse models the genus Lachnospiraceae Nk4A136 group dominated, however, Romboutsia and Akkermansia increased with DSS treatment, especially on Day 8. Akkermansia was abundant in Nlgn3R451C and IFNAR1-/- mice.

Conclusion: Gut microbial alpha diversity was unchanged in Nlgn3R451C mice after DSS treatment. Akkermansia relative abundance increased with DSS treatment in Nlgn3R451C mice suggesting opportunistic and potentially protective growth of Akkermansia in response to DSS-induced colitis in Nlgn3R451C mice.

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IN SILICO ASSESSMENT OF DIET'S EFFECT ON GUT MICROBIOTA INTERACTIONS WITH AUTISM SPECTRUM DISORDER SYMPTOMS USING MACHINE LEARNING DERIVED PREDICTORS

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Introduction: Previous research has linked the gut microbiota composition (GMC) to autism spectrum disorder (ASD), but findings on specific bacteria have been inconsistent. This study builds on prior work by using in silico simulation (ISS) with machine learning (ML) predictors to assess microbial interactions and their influence on ASD symptoms.

Material & Methods: Candidate bacteria for the simulation were selected based on the AGORA database and identified by ML models in feces samples from children aged 2-11. The study considered two diets: Western-Diet (WD) and High-Fiber Diet (HFD). Bacterial interactions commensalism, mutualism, and parasitism were evaluated through ISS, replicating microbiota conditions under varying oxygen levels.

Results: The simulation revealed that under WD with oxygen, commensalism and parasitism dominated, while neutralism increased in the absence of oxygen. In HFD with oxygen, amensalism was more prominent, remaining significant without oxygen, alongside increased neutrality. Oxygen was crucial in shaping these interactions, with WD favoring commensalism and HFD showing more amensalism and neutrality.

Conclusion: The importance of dietary choices in influencing GMC and the potential benefits of multi-objective optimization techniques in microbiota research has been confirmed by the selected ML technique. The identification of the key predictors can be used to amminorate ASD symptoms.

Supported by ITESM Computer Sciences Doctoral Program, CONAHCyT doctoral stipend, and project ID IKXT025-23UI60001

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MICROBIAL PHENOTYPES IN OBESITY: IMPLICATIONS FOR PRECISION NUTRITION

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Globally, the prevalence of obesity and its associated conditions such as type 2 diabetes mellitus (T2D), cardiometabolic diseases, and impaired mental health has significantly increased since the 1980s. There is an imperative need for precision lifestyle programs aimed at augmenting the efficacy of dietary interventions. Variations in insulin resistance (IR) across diverse tissues, including adipose tissue, muscle, and liver, exert a pivotal influence on cardiometabolic risk, modulating the body's nutrient processing in response to dietary interventions. Even within cohorts exhibiting overweight and normal glucose tolerance, discernible phenotypes of liver or muscle IR can be delineated, characterized by distinct microbial, metabolomic, lipidomic, and adipose tissue transcriptome profiles.

Our recent findings underscore that tailoring the macronutrient composition of diets, while adhering to healthy nutrition guidelines, based on an individual's tissue-specific insulin-resistant phenotype, can yield noteworthy enhancements in cardiometabolic health among individuals with overweight or obesity. Obesity and T2D correlate with microbial dysbiosis, with initial microbial composition emerging as a potential determinant in lifestyle-induced cardiometabolic health outcomes. These insights into tissue metabolism will be deliberated within the framework of devising targeted lifestyle strategies to ameliorate cardiometabolic health in individuals with obesity.

THE SMALL INTESTINAL MICROBIOTA: A HIDDEN PLAYER IN HUMAN HEALTH AND DISEASE

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Introduction: Worldwide, almost one out of four children suffer of stunted growth, with most of the children affected living in Sub-Saharan Africa and South-East Asia, leading to severe long-term consequences. Environmental enteric dysfunction (EED) is an inflammatory disease postulated to contribute to stunted child growth and to be associated with intestinal dysbiosis and nutrient malabsorption. Here, we aimed to assess for changes in the proximal and distal intestinal microbiota in the context of stunting and EED and to test for a causal role of these bacterial isolates in the underlying pathophysiology.

Material & Methods: 920 children aged 2-5 years were recruited in two study sites, Bangui, CAR and Antananarivo, Madagascar from 2016-2018. DNA of the gastric, duodenal and fecal samples was subjected to DNA extraction and analysed using amplicon sequencing directed against the 16S & 18S rRNA gene and ITS1 region. Small intestinal bacterial overgrowth (SIBO) was assessed by plating. Biomarkers for inflammation were measured in the feces and blood alongside markers of nutritional status including anthropometry and hemoglobin levels. Sequencing was analysed on R using the phyloseq pipeline. Cell culture assays using polarized mICcl2 cells and mice experiments in C57BL/6 mice were performed with clinical bacterial isolates.

Results: We find that the pro- and eukaryotic microbiome differs along the gastrointestinal tract and is strongly influenced by country of origin. In the feces, we find a decrease of butyrate-producing bacteria as well as higher levels of *Giberella intricans* and lower levels of *Blastocystis.* In the small intestine, we find high prevalence (80%) of small intestinal (oral) bacterial overgrowth (SIOBO) associated with increased inflammation and replacement of classical small intestinal strains. Last, we experimentally show that SIOBO strains increase permeability and directly decrease lipid absorption in both cultured enterocytes and mice.

Conclusion: Our work shows that the intestinal microbiota is changed in the context of stunted child growth and that SIOBO plays a direct role in the pathophysiology underlying stunting and EED. The small intestine might be an interesting target for microbiota-directed interventions allowing all children a healthy growth.

Supported by the Total Foundation, Institut Pasteur, the Swiss National Science Foundation, the Nutricia Research Foundation, the Petram Foundation as well as the Odyssey Re Foundation.

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PROSPECTS FOR LEVERAGING THE MICROBIOTA AS MEDICINE FOR HYPERTENSION

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Dr. Joe will cover the following points:

- Current evidence for microbiota as context-dependent causal factors for hypertension.
- Proof-of-concept for engineering microbiota as medicine for hypertension.
- Microbiota-host interactions in the regulation of blood pressure.

MALE FERTILITY AND GUT MICROBIOTA: CURRENT KNOWLEDGE AND FUTURE DIRECTIONS

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Human infertility is rising worldwide. The percentage of couples that struggle with infertility increased from 8% in the 1980s to almost 16% today, and male infertility accounts for half of the cases of infertile couples. Additionally, the causes of male infertility are unknown in 35% of cases. The detrimental effects of oxidative stress on male fertility are well known. Oxidative stress decreases sperm motility and capacitation and promotes oxidation of the paternal genome. The oxidative stress induced by the absence of peroxiredoxin 6 (PRDX6) promotes infertility in humans and mice. An association of microbiome dysbiosis and male infertility has been reported recently, suggesting the existence of a gut testis axis. However, the research on this subject is primarily descriptive and incomplete, as the mechanisms behind this association have not been elucidated. Only a few parameters were studied; low sperm production and decreased sperm motility in infertile men and male mice were associated with gut microbiota dysbiosis. An increased abundance of Bacteroidetes is associated with male infertility. We found that lipid peroxidation and DNA oxidation were increased, and pup numbers, sperm motility and capacitation were reduced in *Prdx6*$^{-/-}$ males compared to wild-type (WT) controls. Bacteroidetes, Proteobacteria, Verrucomicrobia, Cyanobacteria and Fusobacteria were increased, and Firmicutes were reduced in *Prdx6*$^{-/-}$ compared to WT. Butyrate, propionate and acetate support sperm capacitation. A significant decrease in propionic acid and butyric acid (normally support capacitation) was found in *Prdx6*$^{-/-}$ compared to WT. In humans, we observed that carboxymethyl-lysine (CML) and deoxycholic acid (DCA), metabolites produced by gut microbiota in obese patients, impair human sperm capacitation without altering sperm motility. The gut microbiota dysbiosis and alteration of short-chain fatty acid production in *Prdx6*$^{-/-}$ indicate an indirect mechanism, providing a new layer of complexity to understanding the pathophysiology of male infertility. The modulation of the gut microbiota using antioxidants could be a novel therapeutic strategy to tackle male infertility.

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RADIO-CHEMOTHERAPY TREATMENT EXACERBATES INTESTINAL DYSBIOSIS IN CERVICAL CANCER PATIENTS FROM WESTERN MEXICO. PIONEERING STUDY CHARACTERIZING MICROBIOTA AND NK CELL EXHAUSTION

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Introduction: Intestinal Microbiota (IM) influences NK Cell (NKC) regulation; however, its role in Cervical Cancer (CC) NKC exhaustion remains unclear. This study characterizes IM of CC patients undergoing radio-chemotherapy and its association with peripheral putatively exhausted NKCs.

Material & Methods: Cross-sectional analytical study with 19 CC patients pre-treatment (pre-tx), 9 patients post-treatment (post-tx) and 25 healthy women (HD). We analyzed stool samples with 16S NGS and peripheral blood by flow cytometry for NKC exhaustion.

Results: CC-pre-tx patients IM displayed expansion of Prevotella and significant reduction in α-diversity, which was accentuated post-tx, with further expansion of Escherichia-Shigella and Phascolarctobacterium, alongside decreased short-chain fatty-acid-producing bacteria. β-diversity analyses displayed significant differences between IM of HD and CC-pre-tx, and greater post-tx. Prediction of bacterial metabolic pathways indicated a pro-inflammatory profile driven by LPS in CC groups. Immunophenotype analysis revealed enhanced expression of PD-1, LAG-3 and BTLA on CD56dimNKCs post-tx. Putative exhausted phenotype, defined by persistent expression of multiple inhibitory receptors, was confirmed with co-expression of PD-1+BTLA+ and PD-1+TIGIT+ on CD56dimNKCs in CC post-tx.

Conclusion: Radio-chemotherapy exacerbates intestinal dysbiosis driven by potentially inflammatory bacteria, which may influence NKC exhaustion. Pre- and during-treatment IM interventions could offer a novel strategy to enhance anti-tumor responses in CC.

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OPTIMIZING GUT-ON-A-CHIP TECHNOLOGY WITH PROBIOTICS TO DECREASE THE GAP BETWEEN PRECLINICAL AND CLINICAL DATA

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Introduction: Cell-based in vitro assays have long been used to screen for novel probiotic strains and elucidate the specific mode of action. However, the gap between clinical and preclinical data is evident. To minimize this and enhance data translatability, we have optimized a gut-on-a-chip assay in combination with live bacteria.

Materials and Methods: Using commercially available 3-channel chips [1] (Mimetas B.V.), we developed a human gut model. A 3D tubule with Caco-2 and HT-29-MTX cells matured over 4 days with continuous medium flow. Thereafter, strains of Lactobacillus or Bifidobacteria were added to the luminal compartment. Time-lapse TEER measurements were taken every 16 minutes, and after 24 hours, medium from both luminal and basolateral compartments was analyzed. Additionally, an inflammatory cytokine challenge [2] and probiotics were added in combination to study potential beneficial effects on TEER and cytokine release.

Results: Dose-dependent increase in TEER was observed for the probiotic strains. Interleukin-8 release increased with challenge treatment, and a TEER decrease was noted, which was rescued by probiotic addition.

Conclusion: Gut-on-a-chip technology with probiotics shows great potential to make preclinical results more translatable to man. We are now working on integrating immune cells, neuronal cells, and biopsy-derived organoids [3] for even more physiologically relevant models.

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UNCOVERING THE POTENTIAL OF NUTRITIONAL INTERVENTIONS TO REBUILD GUT HEALTH POST-ANTIBIOTICS

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Introduction: Antibiotics disrupt human gut microbiome composition and functions by affecting beneficial bacteria, reducing colonization resistance to pathogens, and promoting antibiotic resistance and the emergence of antibiotic-resistant microbes, causing stable dysbiosis (1,2). This may negatively interact with the host and impact human health leading to chronic disorders (3). Nutritional strategies have been suggested to restore the microbiome and improve its function (4).

Aim: Investigate the impact of nutrition interventions on gut microbiome restoration and host metabolites.

Methods: Forty-three participants (mean age of 40.2±10 and mean BMI of 25.4±4) who have received at least one course of antibiotics within the last 90 days were randomly assigned to one of four different nutritional interventions containing probiotics + prebiotics (PP n=10), PP + essential oils (n=10), PP + micronutrients (n=12) or PP + polyphenol (n=11) for 8 weeks. Biospecimens were collected at baseline and 8 weeks for multi-omics analysis including metagenomics, metabolomics, gut permeability, and cytokine analysis.

Results: PP significantly ($p<0.05$) increased gut microbiome richness at 8 weeks. Metabolite analysis revealed significant ($p<0.05$) changes in saliva, urine, and blood metabolites, notably in the PP + micronutrients group.

Conclusion: Prebiotics and probiotics can effectively help the restoration of the gut microbiome and may improve overall health.

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IN VIVO SYNERGISTIC EFFECTS OF LACTICASEIBACILLUS PARACASEI 11W AND GALACTOOLIGOSACCHARIDE ON PSYCHOBIOTIC AND ANTIDIABETIC EFFICACY IN FEMALE MICE

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Introduction: Synbiotics (probiotics + prebiotics) enhance beneficial microbes, regulate immunity, reduce inflammation, mitigate diabetes, and improve cognition. This research evaluates Lacticaseibacillus paracasei 11w (11w) with 4% galactooligosaccharides (GOS) in high-fat diet (HFD) female mice.

Material & Methods: In a 6-month study, 35 female C57BL/6J mice were divided into control, HFD, HFD + 11w, and HFD + 11w + 4% GOS groups. Weekly body weight, glucose levels, 4-month behavioural evaluations, glucose tolerance tests (GTT), tissue sampling, colon histology, and cytokine ELISA were conducted.

Results: After a 6-month dietary intervention, HFD + 11w ($p<0.05$) and HFD + 11w + GOS ($p<0.01$) improved glucose metabolism in the GTT at 120 minutes when compared to HFD. The HFD + 11w + GOS group showed reduced depression-like behaviour ($p<0.01$), while HFD + 11w improved learning memory ($p<0.001$). Histology revealed increased goblet cell count and villi length in HFD + 11w and HFD + 11w + GOS. ELISA showed better insulin levels in HFD + 11w + GOS ($p=0.07$) and reduced proinflammatory cytokines TNFα ($p<0.01$) and IL-6 ($p<0.001$) in HFD + 11w.

Conclusion: The investigation highlights 11w and 11w + GOS as promising psychobiotic and antidiabetic agents, demonstrating improved glucose metabolism and diabetes-related mental health outcomes.

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Ashwinipriyadarshini Megur, Eric Banan-Mwine Daliri, Toma Balnionyte˙, Jonita Stankevicˇiu̇te˙, Egle˙ Lastauskiene˙, and Aurelijus Burokas 2023. "In Vitro Screening and Characterization of Lactic Acid Bacteria from Lithuanian Fermented Food with Potential Probiotic Properties." Frontiers in Microbiology 2023.

THE KNOWN UNKNOWNS - THE MYSTERY OF EXTRACELLULAR VESICLES OF BIFIDOBACTERIUM IN ALLERGY DEVELOPMENT

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There is a great concern about allergies which prevalence is rising dramatically worldwide. It is necessary to create a solution that will be able to treat/prevent allergies easily and safely. Scientific evidence indicates that bacteriotherapy, especially by probiotic strains can give an advantage in fighting the allergy. A relatively new research direction is using extracellular vesicles (EVs) produced by probiotic bacteria. Numerous in vivo and in vitro assays reported beneficial immunomodulatory effects exerted by EVs derived from probiotics. Several authors consider administering these EVs as safe and efficient to provide the beneficial effects exerted by probiotics. EVs possess the capacity to spread and directly migrate to other tissues and/or interact with different cells of the immune system of the host. At the same time, it's difficult for the whole probiotic cells.

My project deals with completely unfathomed areas, since the topic of EVs of Bifidobacterium, is just beginning to be explored. I'll present our recent studies about the physicochemical and immunomodulatory properties of EVs from Bifidobacterium strains and discuss thier potential role in the allergy prevention.

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MICROBIAL AND METABOLIC SHAPE OF REMISSION AND EXACERBATION IN ULCERATIVE COLITIS PATIENTS

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Introduction: The course of ulcerative colitis (UC) involves remissions and exacerbations but is difficult to predict. Are periods of exacerbation and remission associated with specific disturbances in the composition of the intestinal microbiota and its metabolome? Our goal was to answer this question and to identify bacterial taxa and metabolites that are necessary to maintain the remission.

Material & Methods: We enrolled 65 individuals, including 20 UC patients in remission and 15 in exacerbation as well as 30 controls. Metagenomic profiling of the gut microbial composition was performed based on 16S rRNA sequencing. Stool metabolic profiles were studied by chromatography combined with mass spectrometry.

Results: We revealed significant differences in the gut bacterial and metabolic composition between remission and with active UC, as well as high similarity between remission and controls. Remission was associated with a higher F/B ratio and greater abundance of Akkermansia, Family XIII AD3011 group, Lachnoclostridium, Subdoligranulum, Coprostanoligenes, and Agathobacter, as well as with elevated levels of the metabolic markers – acetic, propionic and nicotinic acid and tocopherol gamma.

Conclusion: We identified specific bacterial taxa, metabolites, and their correlations, which are crucial in the remission of Polish UC patients, promising for development of new therapeutic strategies for UC.

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WHY SETTLE FOR ONE? COMBINE 16S AND MCRA SEQUENCING

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Introduction: Methanogenic archaea have been increasingly investigated concerning their impact on the environment, role in human health, and technological applications, including the industrial production of biofuel methane as a part of a sustainable energy economy. A reliable tool to asses their diversity in a given sample is thus in great demand.

Material & Methods: We compared the approach of 16S rRNA gene sequencing with novel mcrA (methyl coenzyme M reductase) gene sequencing using dual-index barcoding with Illumina MiniSeq platform (2×150 bp) and current bioinformatic pipelines (DADA2). For the mcrA gene taxonomical assignment, a comprehensive mcrA sequence database was curated.

Results: Our results show mcrA sequencing can offer significant advantages regarding detection and taxonomical assignments, especially true in highly diverse samples. However, it falls short of 16S rRNA sequencing in providing reliable information about the abundances of detected species.

Conclusion: Based on the results we recommend supplementing any 16S rRNA sequencing of methanogenic archaea with our mcrA sequencing approach as well to gain deeper and reliable taxonomical resolution without sacrificing abundance information. Combining both libraries in a single sequencing run offers a cost-effective option for analysing populations of methanogenic archaea.

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MODELING MICROBIOMES AS (APPROXIMATELY) THE SUM OF THEIR PARTS

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Introduction: Clustering of microbiome compositions can help to summarise broad structure in complex populations [2]. However discrete clustering can introduce artificial boundaries between assemblages, and assigning a single label to a sample can obscure an underlying mixed nature. We developed the software 'cvaNMF' to identify a small number of "Signatures", commonly co-occurring features, allowing a description of microbiomes as a gradient of these signatures.

Material & Methods: Our python package 'cvanmf' and accompanying nextflow pipeline facilitate use of Non-negative Matrix Factorisation (NMF) on large biological datasets. Gut microbiome signatures were generated from two collections of public data processed with different metagenomic tools.

Results: The healthy gut microbiome can be well described as a mixture of five "Enterosignatures" [1], which can be consistently identified from multiple separate datasets. Additionally signatures and measures of model fit show association to diseases such as Crohn's disease. We have also applied our software to data outside the gut microbiome to identify biologically meaningful signatures.

Conclusion: Coarse grained "Signature" models provide an intuitive description of bacterial guilds in microbiomes, and can identify broad disease associated patterns in microbiome composition. These signatures can be recovered from global samples including non-westernised gut microbiomes.

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ASSESSMENT OF SKINOSAL, A SYNBIOTIC SUPPLEMENT, IN REDUCING ALLERGY SYMPTOMS IN RAGWEED-SENSITIZED MICE

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Allergies are increasingly prevalent in developed countries, yet existing treatments primarily manage symptoms. Skinosal, a novel synbiotic formulation aims to prevent cutaneous and respiratory allergies. This study evaluated Skinosal's effects on allergic responses in a murine model of ragweed pollen allergy.
Balb/c mice were sensitized to ragweed pollen over three weeks and divided into four groups: control (PBS), low and high-dose Skinosal, and negative control. After sensitization, mice were challenged with ragweed pollen, and allergic reactions were assessed through clinical scoring, IgE, and cytokine profiling. Data were analyzed using ANOVA and non-parametric tests.

Results showed that control groups had significant allergic symptoms after the challenge. The high-dose Skinosal group had a delayed onset and reduced severity of symptoms compared to the PBS control group. Although IgE levels increased in all challenged groups, the high-dose Skinosal group tended to have lower IgE levels, indicating a potential mitigating effect. Cytokine analysis revealed significant upregulation of IL-5 and TNF-α, and downregulation of IL-1β in males in the positive control group. Skinosal-treated groups showed a trend towards modulating these cytokine responses.

Skinosal show promise for allergy prevention by delaying reactions, reducing symptom severity, and modulating immune responses. Further studies are needed to confirm efficacy.

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11th ISM World Congress on

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Abstracts of Poster Presentations

Alphabetical Order

ROLE OF ORAL MICROBIOME IN SALIVARY GLAND HYPOFUNCTION

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Introduction: Dry mouth may be associated with salivary gland hypofunction (SGH) due to conditions such as sicca syndrome, and Sjögren's disease, impacting patient's quality of life. Our objective was to determine salivary microbiome profiles of patients with sicca and Sjögren's disease (SGH group) compared to healthy controls (HC group).

Material & Methods: A cohort of SGH patients (sicca: N=10; Sjögren's: N=19) and HC subjects (N=38) was recruited. Saliva samples were collected and sequenced using 16S rRNA gene sequencing for determination of relative abundance of bacterial taxa. Alpha and betadiversities were determined. LEfSe analysis and Mann-Whitney U-tests were used to identify differential species. CombiROC analysis was completed for species with AUC>0.75.

Results: Alpha- and beta-diversities of SGH and HC groups differed (p=0.001 and p=0.0001, respectively). LEfSe analysis identified ten species characterizing SH group and two species characterizing HC group. Multi-species signatures were identified distinguishing SH group from HC group using CombiROC analysis. Most significant 'combo' signature consisted of Prevotella fusca, Prevotella. Melaninogenica, Rothia Dentocariosa, Veillonella atypica, and Veillonella dispar (AUC=0.939, Sensitivity=0.947, and Specificity=0.828).

Conclusion: There were significant differences in oral microbiome profiles of SGH versus HC groups. The differential species identified, possibly constitute a multi-marker signature.

Supported by Atrium Health Research Fund.

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EXPERT OPINION ON THE USAGE OF PROBIOTICS IN CHRONIC KIDNEY DISEASE MANAGEMENT: PERSPECTIVES OF INDIAN NEPHROLOGY EXPERTS

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Introduction: CKD patients experience gut microbiota imbalances, intestinal barrier disruption, and increased inflammation. Probiotics may improve gut health and reduce inflammation, though research is limited. To explore the role of a multispecies probiotic formulation in CKD care, expert discussions were held with Indian nephrologists.

Material & Methods: 10 digital expert group discussions were held with 60 Indian nephrologists, supplemented by a comprehensive literature review from the PubMed database. Advisory board meetings used a qualitative question and answer format to encourage interactive dialogue. Key expert opinions were formed based on the majority consensus from both the discussions and literature review.

Results: Multispecies probiotics are recognized as supportive therapy in CKD management, with potential to delay kidney function deterioration. Benefits include improved gut barrier integrity, antimicrobial effects, reduced inflammation, and enhanced immunity. Clinical improvements were seen in patients with high urea levels, obesity, rising creatinine, or uremic symptoms. Probiotics, prescribed at 45 million units daily for 1-3 months, reduced BUN and creatinine within 3 to 4 weeks.Non-dialysis patients reported increased appetite, weight gain, and stabilized GFR.

Conclusion: Probiotics may relieve symptoms, reduce inflammation, and slow CKD progression, but larger studies are needed to confirm their benefits.

CORRELATION OF MICROBIOME SEQUENCING DATA AND QUANTIFIED SHORT-CHAIN FATTY ACIDS BY LC-MS/MS IN IBD AND NON-IBD STOOL SAMPLES

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Introduction: Short-chain fatty acids (SCFAs), primarily acetic acid (AA), propionic acid (PA), and butyric acid (BA), are key metabolites produced by bacterial fermentation of dietary fiber and play a crucial role in various metabolic pathways such as energy balance and anti-inflammatory processes. A deviation from a molecular ratio of 60:20:20 (AA:PA:BA) may indicate bacterial dysbiosis. While most SCFAs have positive effects, the impact of branched SCFAs like isobutyric acid and isovaleric acid is less understood. To evaluate the diagnostic potential of a broader SCFA panel, a novel LC-MS/MS method was developed to quantify 11 SCFAs in human fecal samples.

Materials & Methods: A microbiome sequencing study was conducted focusing on the role of SCFAs in patients with inflammatory bowel disease (IBD) and their correlation with microbiome composition. Stool samples of 19 patients (8 male, 11 female), including four IBD patients, were collected and analyzed for their microbiome. SCFA profiles were determined from the same stool samples using UPLC-ESI-MS/MS.

Conclusion: The LC-MS/MS method allowed sensitive and robust SCFA measurement, revealing altered AA:PA:BA ratios in IBD patients, which correlated with significant dysbiosis in IBD patients compared to healthy individuals. Larger studies are needed to confirm the diagnostic potential of these findings.

The presenter is an employee of Immundiagnostik.

STAPHYLOCOCCUS SPP. SKIN COLONIZATION IN ANTI-IL-4/IL-13 AND ANTI-JAK-TREATED ATOPIC DERMATITIS PATIENTS: PROPOSAL OF A STANDARDIZED PROTOCOL FOR RESEARCH AND CLINICAL APPLICATIONS

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Atopic dermatitis (AD) is a chronic inflammatory autoimmune skin disease, characterized by intense pruritus, eczematous lesions, microbiota dysbiosis and skin barrier dysfunction, affecting million people worldwide. These alterations facilitate microbial overcolonization and virulence, mainly by the opportunistic pathogen *Staphylococcus aureus* (SA), contributing to the disease severity. Novel biologic systemic treatments, wherein the anti-IL-4/IL-13 Dupilumab antibody and the JAK inhibitor Upadacitinib, changed the therapeutic scenario3. Since *Staphylococcus spp.* role in AD is still poorly understood, it must be clarified if the increased levels precede symptoms, contributing to the inflammation onset or are a consequence. Therefore, this pilot study proposes a standardized procedure to monitor *Staphylococcus spp*., mainly SA skin colonization during treatment, to likely clarify the potential interactions with the drug mechanisms of action, predict therapy response, and create interactive databases. Swabs from lesional and non-lesional skin, collected with validated protocols, are both bio-banked and let them growth onto selective/differential media for *Staphylococcus spp* cultural assessment and their DNA isolated for 16SrRNA sequencing for bacterial diversity identification and comparison. The findings obtained with the proposed protocol could lay the basis for its possible integration in the clinical practice, for a more accurate diagnosis and a better personalized management of the disease.

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OVERACTIVE BLADDER IN POSTMENOPAUSAL WOMEN IS ASSOCIATED WITH VARIATIONS IN GUT MICROBIOTA SIGNATURES – A CASE CONTROL STUDY

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Introduction: Overactive bladder (OAB) is a common condition in women characterized by urgency and frequency of urination. The pathophysiology is often unknown, however, studies have suggested that the gut microbiota is involved in bladder health and that specific bacteria may be associated with OAB. Here we aim to investigate if women with OAB have a distinct gut microbiota profile compared to matched women without OAB.

Materials and Methods: Fecal samples were collected from postmenopausal women with (n=43) or without (n=51) OAB. 16S rRNA gene sequencing of the V4 region was performed and results were evaluated by alpha- and beta-diversity measures as well as differential abundance using ANCOM-BC.

Conclusion: We observed several genera that were enriched in postmenopausal women with OAB compared to women without OAB. This included the eligens group of the Eubacterium genus, the genera CAG-56 and UCG-001 of the Lachnospiracaea family, as well as a member of the Victivallaceae family. The observation that postmenopausal women with OAB possessed a gut microbiota that differed from women without OAB, supports the role of a possible gut-bladder-brain axis as a cause of OAB. This may be important for future targeted therapies for OAB.

ALTERATION OF GUR MICROBIOTA COMPOSITION AND DIVERSITY IN ACUTE AND/OR CHRONIC GRAFT-VERSUS-HOST DISEASE FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLATATION

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Introduction: Our research study the gut microbiome in patients having Graft-versus-Host Disease (GvHD) after Allogenetic Hematopoietic Cell Transplantation (allo-HSCT).

Material & Methods: 20 allo-HSCT patients were included, having at least Grade-II acute GvHD and/or moderate or severe Chronic GvHD. Fecal specimens collected at 5-time stamps in 6 months range, with date 0 the allo-HSCT. Next-Generation-Sequencing performed using Ion PGM System (Thermo Fischer Scientific) to sequencing the 16S rRNA gene. Statistical analysis was performed by the programs EZBioCloud, Rhea, LEfSe, and by PERMANOVA test.

Results: Compared with timepoint-1 (Date 0), at timepoint-4 (Date +90) there was significant decrease in the abundance of Proteobacteria phylum (14.22%T1, 4.07%T4, p=0.01) and Enterobacteriaceae family (13.3%T1, <0.05%T4, p<0.05), and increase in Enterococcus species (0.1%T1, 12.8%T4, p<0.05) in acute GvHD patients. A reduction in the abundance of Eurobactereaceae family (1.32%T1, 0.53%T4, p<0.05) and Roseruria genus (3.97%T1, 0.09%T4, p<0.05) was noticed in chronic GvHD patients. Alpha and beta diversity analyses did not reveal a difference in the abundance of bacteria at both groups between T1-T4.

Conclusion: To associate specific diversions in gut microbiome in patients with GvHD after allo-HSCT, further studies should be carried out and increase the addition of clinical parameters in the microbiome analyses.

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GUT MICROBIOTA ALTERATION CONTRIBUTES TO CARDIAC DYSFUNCTION INDUCED BY PARTIAL GENETIC DELETION OF AKAP1 DURING AGING

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Introduction: Partial Akap1 deletion (Akap1+/-) predisposes mice to heart failure (HF) by impairing mitochondrial function and promoting cardiomyocyte apoptosis (1). Here, we used Akap1+/- mice to investigate the interplay between genetic predisposition, gut microbiota (GM), and age-related cardiovascular decline. Specifically, we examined the GM changes during aging in Akap1+/- mice and explored the mechanistic link between abnormal microbial profiles and disease progression through fecal microbiota transplantation (FMT).

Material & Methods: Young and old Akap1+/+ (wt) and Akap1+/- mice were assessed for cardiac function, systemic inflammation, gut barrier integrity, and microbiota composition. FMT was performed from old Akap1+/− to young and old wt mice, and the effects on gut and heart functionality were evaluated.

Results: Old Akap1+/- mice exhibited reduced cardiac function, increased intestinal permeability, and distinct microbiota profiles compared to wt mice. FMT from Akap1+/- to wt mice worsened heart function and increased gut permeability and systemic inflammation, suggesting that Akap1+/--associated microbiota may compromise gut barrier integrity and drive inflammation.

Conclusion: Akap1 plays a critical role in maintaining gut homeostasis and cardiac function during aging. These findings underscore the importance of gut microbiota in influencing heart function, offering insights for potential therapeutic interventions to promote cardiovascular health and longevity.

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STRAIN-SPECIFIC INTERACTIONS OF RUMINOCOCCUS GNAVUS WITH HOST GUT

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Introduction: [Ruminococcus] gnavus, a Gram-positive anaerobic bacterium, is a prevalent member of the human gut microbiota. It has been associated with different diseases but causality remains to be determined. Sequencing data showed that R. gnavus strains are highly divergent and divided into clades, underscoring the need to understand how genomic variability impacts phenotypic traits. We previously showed that the ability of R. gnavus to utilise mucin glycans is strain-dependent. Here, we aim to determine the mechanisms underpinning strain-specific adapatation of R. gnavus in the gut.

Material & Methods: We colonised germ-free mice with R. gnavus: the mucin glycan-foraging strain ATCC 29149 and/or the non-mucin glycan-foraging strain E1. Bacterial colonisation and transcription as well as mucin glycosylation, bile acids and gut metabolites were determined by qPCR, FISH, RNASeq and MS.

Results: Both R. gnavus strains could colonise and co-exist in the gut. Differences in sialylation of the mucin glycans was observed, which correlated with ATCC 29149 unique sialic acid metabolism pathway. Differences in bacterial gene transcription were associated with variation in metabolites observed in the gut.

Conclusion: Several strains of R. gnavus can co-exist and differentially interact within the gut, producing metabolites with different impact on host health.

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EXPLORING THE SMALL INTESTINAL MICROBIOTA: THE POTENTIAL OF SALIVA AND FECES IN A GASTROINTESTINAL IN VITRO MODEL

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To date, the small intestinal microbiota remains mainly unexplored, despite its crucial role in human health and immunity, largely due to sampling challenges and ethical restrictions.

To address these limitations and lack of knowledge, this research attempted to establish the small intestinal microbiota in the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) starting from human saliva and fecal microbiota. A saliva community was established in a mouth reactor and was transferred each meal throughout the in vitro model consisting of separate mouth, stomach, proximal small intestine, terminal ileum and colon compartments. Besides, a proximal colon community, derived from feces, was inoculated in the terminal ileum. The microbial community composition, load and metabolic activity were regularly monitored.

The results show a microbial transfer throughout the in vitro model from mouth to colon, while maintaining unique microbial and metabolic profiles in each gastrointestinal compartment in correspondence with in vivo data. The small intestinal community represents key in vivo taxa (*Streptococcus, Veillonella, Haemophilus, Fusobacterium, Bacteroides, Escherichia/Shigella*) originating from the antegrade or retrograde colonization routes.

In conclusion, through establishing small intestinal communities in vitro we can expand our knowledge on small intestinal microbiome and facilitate novel therapeutic approaches to improve human intestinal health.

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EXPLORING THE MYCOBIOME: INSIGHTS INTO FUNGAL COMMUNITY DIFFERENCES BETWEEN AUTISM SPECTRUM DISORDER AND HEALTHY INDIVIDUALS

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The human microbiome plays a critical role in health and disease, with recent attention focusing on the mycobiome's potential involvement in neurological and developmental disorders1,2. Autism Spectrum Disorder (ASD), a complex neurodevelopmental condition, has been increasingly linked to gut microbiome alterations, though the role of the mycobiome remains underexplored. This study investigates differences in fungal community composition between children with ASD and neurotypical (NT) controls.

Fecal samples were collected from 12 male participants (ages 5-10), including 4 individuals diagnosed with ASD who reported gastrointestinal problems (ASD.G) and 8 NT controls. DNA extraction and high-throughput sequencing were conducted to assess fungal community composition.

Across multiple taxonomic levels, NT children consistently showed greater fungal diversity. In contrast, the ASD.G group showed lower diversity, suggesting a less complex and possibly more imbalanced mycobiome. It was dominated by Saccharomyces and Candida, genera potentially linked to gastrointestinal issues in autism. NT children had a more diverse mycobiome, with higher abundances of Rhodotorula, Schizophyllum, and other genera, which may indicate a healthier fungal profile.

These findings highlight potential fungal biomarkers and therapeutic targets for managing GIT problems in autistic populations. Identifying specific fungal imbalances could lead to targeted probiotic or antifungal therapies to alleviate GIT symptoms.

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IMPACT OF GALLIC ACID ON CARDIOVASCULAR GUT DYSBIOSIS

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Introduction: Cardiovascular (CV) dysbiosis, characterized by gut microbiota composition and functionality alterations, has emerged as a significant contributor to CV diseases [1]. Gallic acid (GA) is a polyphenolic compound abundant in various plants with antioxidant, anti-inflammatory, and cardioprotective effects, making it an attractive candidate for mitigating CV dysbiosis and its associated complications. The study aimed to investigate the potential of GA as a therapeutic agent for combating CV dysbiosis.

Material & Methods: In vitro tests were conducted with CV dysbiosis of hypertension and dyslipidemia [2], while microbiological and qPCR methods analyzed the GA modulation effect, then results correlated with the metabolic profile.

Results: In GA-supplemented conditions, the total number of bacteria increased up to 2 log/mL, as well as the beneficial Lactobacillus spp., and Firmicutes population; while the opportunistic pathogen E. coli contributing to CV pathogenesis decreased by 0.5 log/mL. Targeting the but/buk genes and metabolic profile analysis revealed that butyrate-producer bacteria significantly increased.

Conclusion: Dietary supplementation with GA or GA-rich foods may represent a safe and effective approach for restoring gut microbial homeostasis and preserving cardiovascular health. However, more research is needed to translate these findings into clinical practice for the prevention and management of CV diseases.

This research was funded by the UASVM-Bucharest, project #847/2023-VitisBIOTIC.

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ANALYSIS OF NASOPHARYNGEAL MICROBIOME IN PATIENTS WITH VARYING COVID-19 INFECTION SEVERITY

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Introduction: The aim of this study was to discover specific features of the nasopharyngeal microbiome that could be useful as biomarkers of the severity of COVID-19 infection.

Materials and Methods: 43 nasopharyngeal swabs from patients with COVID-19 infection was collected, 16 of which were managed as outpatients, 27 were hospitalized, while 2 of them in ICU. Extraction of genetic material and RT-PCR were performed using the MagMAX™ Viral/Pathogen Kit (Thermo Fischer Scientific) and TaqPath™ 1- Step RT-qPCR Master Mix diagnostic kit (Applied Biosystems) respectively. Next generation sequencing was performed by amplifying 16S rRNA gene using the Ion PGM System (Thermo Fischer Scientific). Statistical analysis was performed by the programs EZBioCloud, Rhea, LEfSe, and by PERMANOVA test.

Results: 7 bacterial phyla were detected, with Firmicutes (41.2%), Proteobacteria (28.4%) and Actinobacteria (21.5%) being the most abundant ones. 61 families were detected (Streptococcaceae and Staphylococcaceae were the most predominant). 132 genera were detected, with Streptococcus being the predominant genus in outpatients and Staphylococcus in hospitalized patients. Alpha diversity analysis did not reveal a difference, but beta diversity analysis established distinct microbial communities between inpatients and outpatients.

Conclusion: Important information is provided on the association between the nasopharyngeal microbiome and SARS-CoV-2 infection.

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GUT MICROBIOTA INTERACTIONS AND BIOAVAILABILITY OF NATURAL COMPOUNDS FOUND IN LAVENDER FLOWERS

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Lavandula angustifolia Mill., commonly known as true or English lavender, is a valuable medicinal plant, with its flowers (Lavandulae flos) being a key component [2]. While its essential oil is rich in monoterpenoids, lavender flowers are frequently used as self-prepared infusions to alleviate mild anxiety symptoms. These infusions, however, contain only small amounts of essential oil and are rich in polar natural compounds, which may contribute to their beneficial effects in vivo [1]. Previous research has shown that plant materials can influence gut microbiota (GM) biodiversity, and the GM, in turn, can modify natural products in plant extracts, leading to the formation of novel bioactive metabolites [3].

This study aims to provide scientific evidence on the interaction between lavender infusion (LOI) and GM in the context of treating and preventing anxiety disorders in humans. Using UHPLC-DAD-MS analysis, 43 compounds were identified in LOI, including flavonoid derivatives, caffeic acid derivatives, propanoic acid derivatives, ferulic acid derivatives, glucosyl hydroxycinnamic acid, and methoxycinnamic acid derivatives. The chemical composition following incubation with GM was also established using UHPLC-DAD-MS.

Up to know 9 potential metabolites were detected in analysed mixtures.

The research was funded by National Science Centre, Poland grant no. 2023/50/E/NZ7/00155.

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INFLAMMATORY BOWEL DISEASE IN PAEDIATRIC AND ADULT PATIENTS: THE MICROBIOLOGY LABORATORY AS A SUPPORT IN ITS APPROACH AND PRECISION MANAGEMENT

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Introduction: The formation of biofilms in patients with Inflammatory Bowel Disease (IBD) is being of interest, as they can alter the host's immune response and increase susceptibility to infections, such as those associated with Clostridioides difficile. Our objective was to analyze the correlation between the 3D-structure of the faecal microbiota biofilm and IBD.

Material & Methods: Prospective 7-month study in which adult and paediatric patients with IBD had stool samples taken for analysis of the presence of C. difficile and analysis of the area of occupation of a 7-day maturity biofilm of the faecal microbiota by CLSM.

Results: We included 40 samples from adults (n=29) and children (n=11) having a median (IQR) age of 43 (31-58.5) and 13 (7-15) years, respectively. There were 27 (67.5%) and 13 (32.5%) patients with CD and UC, respectively. Only 2 patients had C. difficile and 19% had diarrhea. We found no association between either the percentage of biofilm occupancy area, taking into account the type of underlying disease, whether patients had diarrhea or not, and between adults and children.

Conclusion: No significant associations were detected among IBD patients according to biofilm. We need to further investigate our findings by comparing them to healthy individuals.

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GUT MICROBIOTA PROFILES OF LABORATORY MICE REWILDED IN SEMI-NATURAL AGRICULTURAL SETTINGS

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Introduction: Laboratory mice housed in specific pathogen-free (SPF) environments exhibit reduced microbial diversity and an immature immune system. This study explored the effects of "rewilding" laboratory mice in semi-natural, non-SPF environments. We hypothesized that rewilding would increase gut microbial diversity, aligning it with that of wild mice, and promote immune system maturation.

Materials and Methods: Adult C57BL/6 mice were housed for 12 weeks in a non-SPF indoor enclosure containing natural farm elements such as soil, grass, hay, and insects. Controls remained in SPF conditions. Pet-shop and wild Mus musculus mice were included for comparison. Fecal samples underwent Shotgun Metagenomic Sequencing to assess microbial composition, and immune cell populations were analyzed via flow cytometry.

Results: Rewilded mice showed notable shifts in gut microbial diversity, including specific taxa like Acutalibacter muris. However, their microbiota profiles remained distinct from those of pet-shop and wild mice. Enhanced immune activation was observed in rewilded mice, with increased lymphocyte, neutrophil, and dendritic cell activity, resembling that of wild mice.

Conclusion: Contrary to our hypothesis, the immune profiles, rather than the gut microbiota, of rewilded mice more closely resembled those of wild mice. This approach may improve mouse models for human disease research, particularly regarding microbiome-immune interactions.

THE GREAT IMPORTANCE OF GUT MICROBIOTA IN THE TREATMENT OF A RARE GENETIC DISEASE: A CASE REPORT

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Introduction: Menke-Hennekam syndrome is a rare genetic syndrome, with less than 30 cases reported worldwide. Its charachteristics include intellectual disability, a particular phenotype, congenital malformations, upper airway infections and feeding problems. Allergies are not frequently reported, but may have a link with the alterations in gut microbiota.

Material & Methods: We present the case of a 5-year-old boy with Menke-Hennekam syndrome and chronic urticaria. Diagnosed at 6 months via whole exome sequencing, he was admitted to our clinic several times for respiratory and gastrointestinal tract infections. At 3 years old, he developed multiple allergic reactions, including chronic urticaria, abdominal pain and diarrhea.

Results: In addition to allergy testing, an intestinal microbiome analysis revealed significant dysbiosis, including low biodiversity, a reduced number of butyrate-producing bacteria, and an overgrowth of Clostridia and Candida species. A treatment plan with prebiotics and probiotics was implemented to restore gut microbiota homeostasis, leading to the complete remission of the cutaneous and digestive symptoms.

Conclusion: While Menke-Hennekam syndrome is incurable, identifying and treating underlying issues can significantly improve the patient's quality of life. The gut microbiome is pivotal in managing skin allergies and abdominal discomfort, and targeted treatments can effectively restore balance and alleviate symptoms.

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STAPHYLOCOCCUS AUREUS α-HEMOLYSIN INTERACTION WITH TH17 CELLS – DEEP DIVE INTO SHAPING OF THE IMMUNE RESPONSE

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Introduction: Skin microbiome perturbations have been associated with several immune-mediated diseases. Staphylococcus aureus is one of the common inhabitant of the skin and its enrichment was detected, e.g. in psoriasis and was associated with activation of Th17 response [1]. To better understand the complex nature of interactions between Staphylococcus aureus and the host immune system, we aimed to investigate how its toxin, α-hemolysin, affects differentiation and the transcriptome of Th17 cells.

Material & Methods: The CD4+ were isolated from buffy coats sourced from healthy, anonymous donors and were induced to differentiate into Th17 cells in the presence of α-hemolysin. After 5 days cells were analysed using RNA sequencing and whole-genome bisulfite sequencing, real-time PCR, Western blot and ELISA techniques.

Results: RNA sequencing revealed significant changes in the expression of 1626 genes. Among DEGs, we found genes known to be part of the regulatory network for Th17 cells and genes involved in epigentic regulation. We observed that α-hemolysin increase the acetylation of histones H3 and H4 and significantly change genome methylation.

Conclusion: Our study revealed that a-hemolysin induces changes in histone marks and global methylation, shaping the transcriptome, epigenome, and phenotype of Th17 lymphocytes and attenuating a Th17 response [2].

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EXPLORING THE IMPACT OF RHAMNOGALACTURONAN I ON THE MICROBIOME OF HEALTHY INDIVIDUALS: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED INTERVENTION TRIAL

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Rhamnogalacturonan-I (RG-I), a pectic polysaccharide, has demonstrated prebiotic properties in in vitro and ex vivo models, and in a human intervention study [1]. This study investigated the effect of RG-I supplementation, compared to placebo (maltodextrin), on gut health in healthy adults.

Participants of both sexes (18-70 years old), were pre-screened over two weeks for their average fiber intake (adapted Food Frequency Questionnaire) and their fecal bifidobacteria counts (qPCR) and characterized as high/low fiber intakers and high/low bifidobacteria abundant. Using these clusters, a computational algorithm assigned the participants in a randomized, double-blind manner to one of the intervention groups, i.e. 500 mg daily consumption of RG-I (N=26) or placebo (N=28) in capsule form for 4 weeks. During supplementation subjects' food's tolerance markers and health-related parameters were assessed. Differences between time intervals compared to baseline were analyzed via a GEE procedure.

The analysis revealed that both groups were comparable at baseline e.g. macronutrient intake, bifidobacteria numbers; irrespective of the treatment modality, with no recorded side effects between placebo and RG-I. Also, no differences were recorded in physical activity and overall perception of health.

These findings confirm that RG-I is a safe, well-tolerated dietary supplement with potential for inclusion in functional foods.

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ASSESSMENT OF THE ADHESION CAPACITY OF NOVEL BIFIDOBACTERIA ISOLATES TO CANCER INTESTINAL EPITHELIAL CELL LINES

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Introduction: Bifidobacteria are one of the most important types of microorganisms found in our digestive system. They accompany humans from birth and perform many essential functions in the development of the gut microbiota, the immune system, and later also in regulating the populations of harmful microorganisms. In this study, newly isolated strains of bifidobacteria and a commercial probiotic strain were compared of their adhesion ability to human intestinal epithelial cancer cells in the context of potential anticancer properties.

Material & Methods: The research material consisted of bacterial isolates taken from stool samples of children and infants up to 3 years old, who were fed with breast milk or formula milk. Methods included determining the ability to adhere to mucin and analyzing the ability of bifidobacterial strains to attach to the surface of Caco-2 and HT-29 epithelial cell lines.

Results: The studied isolates exhibited varying degrees of adhesion to the cells of both cell lines and to mucin, with the best adhesive properties shown by isolate 12.8. (B.breve). The comertial probiotic strain B. longum subsp. infantis 35624® was characterized by weak adhesive capabilities.

Conclusion: It is necessary to conduct further in-depth analyses of the adhesion mechanisms of the isolate 12.8.

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KERATIN-TRIMETHYLAMINE-N-OXIDE WOUND DRESSING ACCELERATES WOUND HEALING IN DIABETIC RATS

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Introduction: Impaired wound healing is a major medical problem, especially in diabetic patients. Researchers all over the world are looking for new therapeutic options.

Material and Methods: Keratin dressing was obtained according method proposed by Konop et al.1 and enriched with 0.1% trimethylamine-N-oxide (0.1%TMAO) and then tested in vitro and in vivo on full-thickness skin wounds in diabetic rats.

Results: Physicochemical examination showed that TMAO was slowly released from the dressing for 5 consecutive days. In vitro examination showed that FKDP+0.1%TMAO dressing is non-toxic, and increased keratinocyte viability. In vivo experiments on diabetic rats exhibit that FKDP+0.1%TMAO is tissue biocompatible and accelerates wound healing on days 4, 7, 14 and 21 post-injury ($p<0.05$). Histopathological examination showed that in treated wounds predominant M2 macrophages, compared to control wounds were neutrophiles and M1 macrophages predominant. Moreover, in vitro studies showed that higher mRNA expression for KRT16 and KRT17 was observed in cells treated with examined dressing. These findings correlate with in vivo examination, where we found stronger immunolabeling for KRT16 and KRT17 in FKDP+0.1%TMAO treated wounds.

Conclusion: The results suggest that the FKDP+0.1%TMAO dressing is safe, non-toxic and accelerates wound healing possibly by activation of cytokeratin 17.

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EFFECT OF CURLI PROTEIN ON MOTOR DYSFUNCTION

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Introduction: Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder. Early diagnosis before motor symptoms still a challenge. The prodromal phase of PD often includes gastrointestinal disturbances and microbial dysbiosis (1). Curli-producing gut bacteria hypothesized to contribute to disease progression by interacting with the enteric nervous system and spreading to the central nervous system (CNS) via the vagus nerve (2,3).

Material & Methods: We examined the effects of curli-producing bacteria on motor functions in a wild-type mouse model. After depleting the mice's microbiome with antibiotics, we administered curli-producing (E. coli Nissle, MDS65) or curli knock-out (MDS66) bacterial strains via gastric gavage. The control group's microbiome was restored, and weekly maintenance treatments followed.

Results: Motor function tests revealed no significant differences between the treated and control groups in either behavior or motor-related brain regions. However, immunostaining and qPCR revealed overproduction of phosphorylated alpha-synuclein in the enteric nervous system, specifically in the ileum, colon, and mesentery.

Conclusion: The treatment duration may have been too short to detect alpha-synuclein overproduction in the brain. Additionally, it is possible that curli protein alone is insufficient to trigger Parkinson's disease, and that the contribution of other factors may need to the prion-like spread of alpha-synuclein.

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POTENTLAL UTILISATION OF THE FAECAL MICROBIOTA FROM A PATIENT WITH DIAGNOSED ULCERATIVE COLITIS (UC) IN UC INDUCTION UNDER IN VIVO CONDITIONS IN PSEUDO GERM-FREE ANIMAL MODEL

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Introduction: The aim of our study was to investigate potential use of combined induction of ulcerative colitis in pseudo germ-free (PGF) animal model involving administration of dextransodium sulphate (DSS) and faecal microbiota transplant (FMT) obtained from a patient with UC.
Material & Methods: The study was conducted on 96 PGF female mice of BALB/c line. In the DSS group, acute UC was induced by exposure to DSS, FMT from the patient with UC was administered to UC group, and FMT from UC patient together with DSS were administered to animals from DSS-UC group.

Results: The observed pathology of histological architecture of tunica mucosa and tela submucosa of the colon, manifested as degeneration of epithelium, loss of mucin, infiltration of neutrophils in lamina propria and submucosa and cryptitis in the intestine, corresponded to histological picture of induced acute UC in both models (DSS, DSS-UC). Dysbiotic composition of faecal microbiota in our patients with UC showed similarity in trends of increases and decreases in taxonomic representatives of the caecal microbiota associated with UC.

Conclusion: We confirmed that the animal model with combined induction of UC appears optimal with respect to its similarity with histopathology of intestinal mucosa with UC and dysbiotic microbiota in patients with UC.

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IDENTIFING THE MICROBIOME SIGNATURES THAT DISTINGUISH HEALTHY FROM DISEASED GUTS

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Introduction: The microbiome is associated with various human diseases. Healthy individuals typically have diverse gut microbiota (eubiosis), whereas unhealthy guts generally lack microbial diversity (dysbiosis). We sought to profile healthy and diseased guts to identify microbiome signatures and develop a panel of key indicator organisms using OpenArrayTM, a high-throughput real-time PCR platform.

Material & Methods: We profiled 96 stool samples from diseased individuals and normal controls by real-time PCR and NGS. We used a pan-bacterial assay to normalize for sample input and developed a preamplification protocol to increase less abundant microbial detection. Data were sorted by abundance levels for PCR vs. NGS.

Results: Healthy controls consist predominantly of "good bacteria" at abundant levels such Faecalibacterium prausnitzii and other small chain fatty acid producers, whereas diseased samples often lack "good bacteria" or have much reduced levels. We developed the panel that gives distinct signatures of normal and diseased samples. The panel is validated by NGS and high concordance is observed between qPCR and NGS.

Conclusion: We identified a microbial panel that can distinguish healthy and diseased guts. This microbiome panel features the most value-added targets on a high-throughput OpenArray real-time platform, and it can provide useful tools for microbiome research.

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DETERMINATION OF A POSSIBLE SYNERGISTIC ANTIMICROBIAL ACTIVITY OF ACID-WHEY DERIVED LACTOFERRIN AND NISIN

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Introduction Acid whey, a byproduct of dairy industry, is rich in valuable nutrients like lactoferrin1 and serves as medium for cultivation of lactic acid bacteria2. The aims were to produce nisin in acid whey-based media and to investigate possible synergistic antimicrobial interactions between nisin and lactoferrin derived from acid whey.

Material & Methods: Nisin was produced in permeate of ultrafiltered acid whey supplemented with yeast extract and minerals using three nisin-producing Lactococcus strains and underwent purification by salt precipitation and chromatography. Purified lactoferrin from acid whey was provided by Arhel Ltd [1]. To assess potential synergistic antimicrobial interaction of nisin and lactoferrin against 19 indicator bacteria, spot tests and microdilution tests were conducted.

Results: The highest production of nisin was produced by Lactococcus lactis IM145 in ultrafiltered acid whey supplemented with yeast extract. Nisin (90% pure) showed two-fold increase in antimicrobial activity against Latilactobacillus sakei when combined with lactoferrin, while no synergism was observed against other strains.

Conclusion: A weak synergistic antimicrobial effect observed between nisin and lactoferrin, which warrants further investigation in subsequent studies. These results are important to provide new scientific basis for pre-clinical and clinical studies with nisin and lactoferrin and for the development of biotherapeutic products.

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MAPPING MICROBIAL COMMUNITIES: INSIGHTS FROM MULTI-SITE SAMPLING DURING COLON SURGERY

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Introduction: Perioperative interventions can potentially induce considerable changes in microbiota composition. It is therefore crucial to examine how the sampling site affects the microbiota before and during surgery, given the established link between the intestinal microbiota and postoperative complications and long-term surgical outcomes (1).

Material & Methods: The microbial composition in rectal swabs was analyzed together with the swabs and biopsies obtained during the surgical process by 16S rRNA gene sequencing of the V1-V2 region (2) to reduce off-target amplification in biopsy samples with the Illumina MiniSeq platform and the bioinformatic pipelines (DADA2).

Results: The results demonstrated that the overall alpha diversity of samples collected from different sites exhibited no statistically significant variation. However, significant alterations in alpha diversity among individual diagnostic categories of patients were evident. In contrast, notable alterations were observed in samples collected before and after surgery, and beta diversity analysis revealed a distinct grouping of patients with varying diagnoses, regardless of the collection site.

Conclusion: The findings reveal that the timing and location of sample collection exert a significant influence on the structure of the intestinal microbiota, which may be associated with postoperative complications.

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IN VITRO EVALUATION OF A FORMULATION WITH POSTBIOTICS AND PREBIOTICS AGAINST PATHOGENIC MICROORGANISMS PRESENT IN THE MICROBIOTA OF PSORIATIC SKIN

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Introduction: The skin microbiota is a potential therapeutic target. This study aimed to evaluate the activity of β-glucan-based prebiotics and postbiotics from *Lactobacillus paracasei* and *Saccharomyces cerevisiae*. Additionally, it assessed topical formulations containing these active ingredients against *Staphylococcus aureus* and *Malassezia furfur* in vitro.

Material & Methods: Extracts were collected during the stationary growth phase. The activities of postbiotics and β-glucans were evaluated using broth microdilution and agar diffusion tests. The formulations were assessed using the agar diffusion test against microorganisms.

Results: Extracts of *L. paracasei* and *S. cerevisiae* at a concentration of 500 mg/mL inhibited the growth of microorganisms by 90% and 97%, respectively. Additionally, 0.5% oat-derived β-glucan reduced microorganism growth by more than 90%, while yeast-derived β-glucan inhibited 60% of growth. In the agar diffusion test, the active ingredients interfered with the growth of both microorganisms, except for the yeast-derived β-glucan. However, no antimicrobial activity was observed after incorporating the active ingredients into the formulation.

Conclusion: The results are promising, indicating the potential of these compounds to help rebalance the skin's microbiota in dermatological conditions like psoriasis. Further research is necessary to identify the molecules produced by probiotics and determine the appropriate vehicle for incorporating the active ingredients.

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DISCOVERING POTENTIAL BIOMARKERS RELATED TO TOXICITY CHEMOTHERAPY IN A CRC COHORT

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Introduction: The complex system of microorganisms residing in the human body has emerged as a key factor in the development and progression of colorectal cancer (CRC). Particularly, gut microbiota appears to have an increasing role in this cancer, being associated with the response of oncological treatments [1, 2].

Material & Methods: A cohort study of 36 patients from North West Spain was selected. Fecal samples were recollected post-surgery but pre-treatment, and 16S rRNA V3-V4 amplicon sequencing was performed [3]. A two-class toxicity variable was created under the oncologist criteria based on the rate of side-effects suffered by the patients during chemotherapy treatment. After a preprocessing described in [3], two machine learning algorithms —Random Forest (RF) and Least Absolute Shrinkage and Selection Operator (LASSO) — were employed to process the data and assess their performance metrics in classifying toxicity target variable.

Results: Our findings suggest that microbiota may play a role in the side-effects of chemotherapy specific treatments. Several bacteria as Family_XIII_AD3001_group genera and some species within the genera Eubacterium could have a potential effect on the secondary effects derived from chemotherapy with 5-Fluoracil or/and Oxaliplatin drugs.

Conclusion: This novel insight underscores microbiome's potential in the understanding of personalized treatment approaches.

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USE OF BILE ACIDS AS A REGULATOR OF GUT MICROBIOTA

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Introduction: Gut microbiotas are the typical examples and they are mainly regulated by bile acids. If a certain bile acid is continuously orally-supplied, the ratio of supplied bile acid to the total bile acid is gradually increased due to the continuous enterohepatic circulation.

Aims & Methods: First, we wanted to identify the effects of certain bile acids on each microbiotas. Second, we intended to observe actual intestinal microbial changes through the supply of specific bile acids in animal models. Next generation sequencing(NGS) method was used to analyze the changes of microorganisms according to the supplied amount of UDCA.

Results: Each bile acid formed various inhibitory or promoting regions in target strains. As a result of relative quantitative analysis (RT-PCR) based on the control group, the ratio of Fermicutes to Bacteroidetes was decreased in the group treated with UDCA for 3 weeks. In the cholestatic model, NGS showed changes in the proportion of intestinal microbiota and an increase in diversity after 3 weeks of UDCA supply.

Conclusion: Oral feeding of certain bile acids can be used as a therapeutic treatment for dysbiosis by controlling the microbial environment in the intestinal tract.

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SALIVARY MICROBIOME PROFILING OF HPV+ AND HPV- OROPHARYNGEAL HEAD AND NECK CANCER PATIENTS UNDERGOING DURVALUMAB IMMUNOTHERAPY: A PILOT STUDY

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Introduction: Immunotherapy holds the promise for treating oropharyngeal cancers (OPCs). Our objective was to determine effects of durvalumab immunotherapy on salivary microbiome.

Material & Methods: Early stage OPC patients, 5 positive for human papilloma virus [HPV+] and 12 negative [HPV-], were recruited and treated with durvalumab every two weeks, before surgery. Unstimulated saliva was collected and processed for 16S rRNA gene next generation sequencing and taxa relative abundance determination. Alpha- and beta- diversities were determined for baseline Group A and treatment groups one week apart (Groups B, C, and D). MaAsLin2 R program was used to identify associations with timepoint or HPV status. ROC curves were plotted for species in common between MaAsLin2 analysis and FDR-corrected Mann-Whitney U-test.

Results: Total of 416 taxa were detected. There were no alpha- and beta-diversity differences in longitudinal comparisons. There were beta-diversity differences for HPV status. Mann-Whitney U-test showed Bergeyella sp16471 as significant in A versus BCD (FDR corrected p=0.0396). HPV status was associated with 87 species. MaAsLin2 A versus BCD analysis identified 57 taxa. Leptotrichia spp., present in 63.6% combinational ROC curves (AUC>0.800), were more abundant in HPV+ patients.

Conclusion: This study shows durvalumab immunotherapy as having minimal effects on salivary microbiome composition.

Supported by National Cancer Institute Cancer Center Support Grant award number P30CA012197 to Atrium Health Wake Forest Baptist Comprehensive Cancer Center; Atrium Health Research Fund

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EXPLORING THE ASSOCIATION OF AUTISM SPECTRUM DISORDERS AND GASTROINTESTINAL DYSFUNCTION BY ALTERED GUT MICROBIOTA IN A ANIMAL MODEL OF AUTISM

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Introduction: The aim of this study is to obtain a pseudo germ-free (PGF) animal model with autism spectrum disorders (ASD) with focus on concomitant gastrointestinal symptoms in children with ASD.

Materials & Methods: Within the procedure, following the administration of dysbiotic and healthy faecal microbiota obtained from children's donors, clinical parameters of PGF female mice of BALB/c line were evaluated individually by assigning points according to disease activity index (DAI). Damage to large bowel was evaluated by morphometric analysis of histological sections and on the basis of histological activity index (HAI).

Results: After administration of faecal disbiotic microbiota from children with ASD, there were confirmed histological chnages in the distal section of the large bowel represented by inflammatory infiltrate, degeneration of epithelium, deformation of intestinal crypts, cryptitis and epithelial erosion. The negative influence of administration of faecal microbiota transplant (FMT) from children with ASD on severity of the disease was confirmed by a statistcally significant increase in DAI.

Conclusions: The partial results of our study allowed us to state that we obtained an optimum animal model of gastrointestinal disorders associated with ASD.

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POTENTIAL OF LIMOSILACTOBACILLUS REUTERI E IN PROTECTING INTESTINAL BARRIER AND REGULATING INLFAMMATION

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Limosilactobacillus reuteri E (LRE), a potentially probiotic strain, was the focus of our research aimed at investigating its effects on intestinal barrier integrity and inflammatory processes using in vitro and in vivo models.

The in vitro experiment was conducted in a co-cultivation model using Caco-2 and HT-29 cell lines. Cells exposed to 5-fluorouracil (5-FU) showed a significant decrease in the relative expression of genes encoding tight junction proteins (claudin-1, occludin), aminopeptidase and citrate synthase. The addition of LRE increased the expression of these genes suggesting it´s protective properties against the negative effects of 5-FU.

The in vivo experiment aimed to monitor changes in the inflammatory status of rats fed with a cola beverage along with LRE. High cola beverage consumption increased the expression of the pro-inflammatory interleukins IL-1β, IL-17 and decreased the level of the anti-inflammatory IL-10 in spleen and liver. In contrast, the administration of LRE led to an increase in IL-10 and a decrease in IL-1β, IL-17, indicating it´s positive influence on the regulation of inflammatory processes.

Based on these results LRE may have a beneficial effect on protecting the intestinal barrier and regulating inflammatory processes, making it a promising candidate for further therapeutic research.

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PURIFICATION OF L. PARAGASSERI K7 BACTERIOCINS BY PREPARATIVE CHROMATOGRAPHY AND ASSESSMENT OF POSSIBLE SYNERGISTIC ANTIMICROBIAL ACTIVITY WITH LACTOFERRIN

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Introduction: Gassericins K7 are heterodimeric bacteriocins produced by L. paragasseri K7. Building on the work of Mavrič et al. (2014) and Nilsen et al. (2020), this study focuses on preparative scale purification of gassericins to obtain quantities required to study their effect on the host, their characterisation and synergism with lactoferrin.

Material & Methods: We modified the existing purification protocol to purify gassericins from 10 L MRS culture supernatant (precipitation, amberlite extraction and RP chromatography). We characterised the obtained material and performed synergistic antimicrobial activity assays with lactoferrin donated by Arhel Ltd (Bogovič Matijašić et al., 2020).

Results: Mass spectrometry confirmed 3 (GasK7Aα, GasK7Aβ and GasK7Bα) of the 4 gassericin peptides produced by this strain. We also confirmed synergistic antimicrobial activity between 2 of the gassericins. Partially purified fraction of gassericins showed no synergistic antimicrobial activity with lactoferrin.

Conclusion: Studies on host interaction are hampered by low amounts and purity of gassericins that can be obtained. We obtained enough pure material for further studies, which will include testing the activity of different combinations of gassericins and lactoferrin also on intestinal mucosal cells and immune cells to try to investigate the safety and potential therapeutic alternatives to antibiotics.

Supported by Slovenian Research and Innovation Agency (Ljubljana, Slovenia) through the research program P4-0097 and young researchers program 802-6/2021.

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THE INFLUENCE OF PHENYLKETONURIA ON ORAL MICROBIOME PROFILES: A COMPARATIVE METAGENOMIC STUDY

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Introduction: Phenylketonuria (PKU) is a metabolic disorder characterised by an inborn defect in phenylalanine (Phe) metabolism. Current research suggests that PKU and the Phe-restrictive diet may affect the oral microbiome's composition. This study aimed to compare the diet and oral microbiome profiles of individuals with PKU with healthy controls.

Material and methods: We sequenced oral DNA samples from PKU patients and healthy controls using the 16S rRNA gene (V4) on iSeq100 (Illumina). Metagenomic studies were performed using QIIME 2 (DADA2, Chao1, PERMANOVA) and the Greengenes2 database. Clinical, nutritional, and taxonomic data were analysed using a non-parametric Wilcoxon test (RStudio).

Results: Analysis of food diaries showed differences in consumption of protein, fat, cholesterol, and Phe between the control and the PKU groups. The low protein intake in PKU patients is due to the elimination of meat, fish, eggs, and dairy products (1). Compared to the control group, the PKU group had a statistically significant increase in Lactobacillales, Neisseriales, and fewer Clostridiales and Bacteriodiales in the oral microbiome. Alpha diversity showed reduced microbial diversity in PKU.

Conclusions: The oral microbiota of PKU patients has lower diversity and taxonomic alterations. These data imply that PKU metabolic alterations could affect oral microbial ecology.

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LARGE-SCALE SYNTHESIS AND STABLE PHARMACEUTICAL FORMULATION CONTAINING UROLITHIN A FOR TOPICAL APPLICATION

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Introduction: Urolithin A (UA) is a postbiotic metabolite produced by intestinal microbiota from ellagitannins. UA has well-documented bioavailability, and proven anti-inflammatory properties. However, following absorption in the intestine, UA is metabolized to inactive phase II conjugates. Above limitations related to oral application have led to attempts to utilize its anti-inflammatory potential in a topical formulation applied to the skin. The aim of the study was to develop a large-scale method of UA synthesis as well as stable topical formulation necessary for conducting pre-clinical and clinical studies on UA.

Results: Large scale synthesis of UA commenced with commercially available 2-bromo-5- methoxybenzoic acid which was subjected to demethylation reaction in the presence of excess of AlCl3 in chlorobenzene at 120 °C. The resulting crude bromoacid without further purification mixed, with resorcine in aqueous sodium hydroxide under copper catalysis led to formation of urolithin A (149.0 g; 60%). The topical formulations containing 0.5, 1.0, 2.0, 3.0, and 5.0% urolithin A were elaborated and tested according to ICH, EMA and pharmacopoeia guidelines.

Conclusions: The obtained results indicate the possibility of large-scale synthesis and development of stable pharmaceutical formulation containing urolithin A, which can be applied in topical therapy of skin inflammations of various etiologies.

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Piwowarski, J.P., Granica, S., Sacharczuk, M., Naruszewicz M., UROLITHIN A AND A COMPOSITION CONTAINING SAME FOR EXTERNAL USE IN INFLAMMATIONS OF VARIOUS ETIOLOGIES PCT/IB2019/060337

CHARACTERISING THE GUT MICROBIOME IN TRAUMATIC BRAIN INJURY

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Introduction: Traumatic Brain Injury (TBI) is the leading cause of death and disability among trauma-related injuries worldwide (1). The gut microbiome has emerged as a major determinant of patient outcome in TBI due to the Gut-Microbiome-Brain-Axis (2). In this study, we explore the gut microbial changes in TBI patients.

Material & Methods: Two rectal swabs with visible fecal matter were collected from 16 TBI patients. The first sample was collected within 24 hours of the injury (Day 1), and the second sample, after 72 hours (Day 3). The fecal microbiome was analysed using Illumina NextSeq2000 platform.

Results: The gut microbial diversity had significantly decreased by Day 3. There was a significant decrease in the relative abundances of the phylum Proteobacteria, families Enterobacteriaceae, Oscillospiraceae, and Coriobacteriaceae, and genera Escherichia-Shigella, Collinsella, Agathobacter and Howardella by Day 3. The relative abundances of the family Porphyromonadaceae, and genera Porphyromonas and Peptoniphilus had significantly increased by Day 3.

Conclusion: The gut microbial dysbiosis seen in traumatic brain injury is a potential therapeutic target. Simple interventions like probiotics could speed up recovery and improve overall patient outcome.

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SEX-DEPENDENT ALTERATIONS IN GUT MICROBIOTA GLYCOSYLATION AND COMMUNITY STRUCTURE FOLLOWING LONG TERM TCDD EXPOSURE IN MICE

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Toxic compounds can affect the microbiota of the gut directly or via liver toxicity. Microbiota changes can be studied by 16S sequencing, or by multi-parameter flow cytometry. We asked here, if 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) affects the gut microbiota of male and female mice at low doses (1µg/kg) orally administered for 12 weeks. We assessed the microbial patterns in fecal pellets and cecal samples of TCDD-exposed mice. Surprisingly, while 16S sequencing revealed no differences, flow cytometry identified based on scatter characteristics and DNA-staining significant alterations in the microbiota community profile following TCDD exposure in female mice. In contrast to 16S sequencing, flow cytometry might pick up phenotypic changes such as glycosylation.

We therefore stained the bacteria with a panel of fluorescent sugar-specific lectins. Indeed, this unveiled significant alterations in the glycosylation patterns of TCDD-treated mice versus controls. Again, this was more pronounced in female than in male mice. Dysregulation of glycosylation patterns by toxic compounds is a novel finding, which has implications for the health of the host because glycoconjugates govern biofilm forming, infectious behavior of bacteria, and host immune responses. We posit that changes in glycosylation and bacterial community structure are relevant parameters in assessing the toxicity of chemical compounds.

MODULATING GASTROINTESTINAL SYMPTOMS ASSOCIATED WITH AUTISM SPECTRUM DISORDERS USING MICROBIOME-BASED APPROACH: INSIGHTS FROM A PRECLINICAL STUDY

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Introduction: As a significant proportion of individuals with autism spectrum disorders (ASD) experience gastro-intestinal (GI) disturbances 1, this study aims to elucidate the role of the microbiome in these symptoms and explore a potential microbiome-based therapeutic intervention.

Material & Methods: Pseudo germ-free mice were first administered FMT from girls with ASD and subsequently divided into untreated group (ASD-u) and group treated by administration of L. plantarum CCM 7512, L. reuteri CCM 8617 with flax seeds (ASD-t). Clinical scores, haematology parameters, microbiome composition, and immune cell subsets in spleen, Peyer's patches and colon mucosa were assessed.

Results: Administration of ASD-associated FMT resulted in significant changes in the microbiome composition including an increase in the abundance of several ASD-associated bacterial families (e.g., Fusobacteriaceae, Sutterelaceae). These changes were reversed by the administration of synbiotics. Similarly, gastrointestinal symptoms showed significant improvement after treatment, along with the enhancement of various hematological parameters (e.g., RBC, HCT, HGB). However, we observed only a limited number of significant positive changes in the immune cell subsets of secondary lymphoid organs.

Conclusion: Synbiotic treatment effectively improved GI symptoms and reversed microbiome changes in ASD model, highlighting its potential in managing ASD-related disturbances.

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ENHANCING MICROBIOME RESEARCH WITH A LIQUID-BASED METHOD FOR LONG-TERM BACTERIAL DNA STORAGE

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Introduction: When immediate processing of microbiome samples is not possible, it is crucial to ensure that bacterial DNA is preserved. This study assessed the effectiveness of Copan eNAT®, a nucleic acids preservation medium widely used in microbiome research(1,2), in preserving bacterial DNA at 4°C and 30°C.

Material & Methods: A 0.5 McFarland stock of Neisseria gonorrhoeae (NG) was diluted 10^(-5) in Dubelcco's Phosphate Buffered Saline and filtered real urine. 1mL of this solution was transferred into 1mL of eNAT® medium, preparing a sample immeditealy analyzed and samples stored at 4°C and 30°C, to be analyzed at 7 different time points, up to one year and six months. The samples were analyzed using Cepheid Xpert® CT/NG assay (assay A) and Qiagen Microbial DNA qPCR NG assay kit (assay B).

Results: Compared to Ct values from immediate analysis with assay A (CtNG1=33.3, CtNG2=32.4), average values for samples stored at 4°C were CtNG1=29.7(σ=0.73) and CtNG2=29.4(σ=0.66), and at 30°C, CtNG1=29.8(σ=0.44) and CtNG2=29.5(σ=0.42). For assay B, Ct value from immediate analysis was 33.4, while averages for 4°C and 30°C were 32.7(σ=0.96) and 33.1(σ=0.74), respectively.

Conclusion: Results demonstrate that eNAT® effectively preserves bacterial DNA at 4°C and 30°C for up to a year and six months.

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EFFECT OF ROSEOFLAVIN ON THE ACTIVE MICROBIOTA IN HUMAN SALIVA

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Introduction: Roseoflavin, a structural analog and competitive inhibitor of riboflavin (vitamin B2), has been reported to particularly inhibit the growth of gram-positive bacteria [1, 2]. Thus, it might be possible to use roseoflavin as a microbiome modulator against potential oral pathogens, such as Streptococcus mutans, which is involved in caries formation.

Materials & Methods: We obtained saliva samples from 15 healthy volunteers, which were pooled and incubated with or without roseoflavin for 24 hours, respectively. After DNA and RNA extraction, 16S rRNA (cDNA) and 16S rRNA gene sequencing were used to investigate shifts in the community composition of the present (DNA-based) or active (cDNA-based) saliva microbiota.

Conclusions: Our previous, cultivation-based studies [3] particularly suggest an inhibition of gram-positive oral isolates by roseoflavin. Interestingly, amplicon bands from incubations with roseoflavin were significantly weaker than control amplicon bands. While our analyses are still ongoing, we hypothesize that particularly cDNA-based sequencing will reflect a (relative) increase of gram-negative sequence types, indicating an inhibition of gram-positive species by roseoflavin.

Supported by Baden-Württemberg Stiftung (Microbiome Programme).

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CHARACTERIZING GUT DYSBIOSIS IN PATIENTS WITH CLOSTRIDIOIDES DIFFICILE INFECTION – NEW INSIGHTS BASED ON OWN RESEARCH AND GLOBAL PERSPECTIVE

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Introduction: Clostridioides difficile infection (CDI) is a major cause of hospital-acquired diarrhea, particularly in developed countries. One of the most pressing challenges in managing CDI is the prevention of recurrence, which occurs in about 35% of first-time cases and up to 40-60% of subsequent infections. Fecal microbiota transplantation (FMT) has shown promise in reducing recurrence, with clinical trials demonstrating up to 80% success rates. This study aims to analyze the intestinal microbiota composition in CDI patients and identify the factors contributing to infection development and recurrence.

Material & Methods: The intestinal microbiota of 31 individuals (15 CDI patients and 16 healthy controls) was analyzed using 16S rRNA gene sequencing to assess microbial community differences.

Results: Significant alterations in gut microbiota composition were observed between CDI patients and controls. Controls exhibited higher abundances of most key phyla, families, genera, and species, indicating gut dysbiosis in CDI patients. The Bacteroidetes to Firmicutes ratio was markedly lower in patients (0.0215) compared to controls (0.1382). A notable decrease in Bacteroides and Ruminococcus, genera linked to gut health, was observed in patients, alongside an overgrowth of Escherichia-Shigella.

Conclusion: CDI patients exhibit pronounced dysbiosis. Reestablishing gut balance through targeted microbiota restoration may prevent CDI recurrence.

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ADAPTATION OF PORPHYROMONAS SPECIES TO THE HOST ENVIRONMENT MAY BE THE RESULT OF HEME UPTAKE MECHANISMS' EVOLUTION

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Introduction: The human microbiome comprises several Porphyromonas species that inhabit various host environmental niches in health and disease, with the best-known periodontopathogen, Porphyromonas gingivalis. Porphyromonas species acquire heme from the host as an iron and protoporphyrin IX (PPIX) source, using mainly the Hmu system, with a leading role played by the HmuY hemophore-like protein. HmuY is one of the main P. gingivalis virulence factors, influencing host immune response, and is of high importance in the pathogenic process1. HmuY proteins differ in their heme binding properties1; therefore, we aimed to characterize those produced by the human species concerning their engagement in virulence.

Material & Methods: Theoretical analysis and experimental approaches, such as spectroscopic and electrophoretic methods were used to analyze heme and PPIX binding by chosen recombinant proteins from the HmuY family.

Results: Our main findings revealed that some Porphyromonas species encode more than one HmuY homolog which differ in heme-binding properties, with some HmuY proteins preferring PPIX binding without heme-iron coordination.

Conclusion: We hypothesize that individual HmuY proteins have undergone specific evolutionary modifications to better adapt respective bacteria to survive within the complex human microbiome, contributing to the greater virulence potential of P. gingivalis compared to other Porphyromonas species.

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INDOXYL SULFATE, GUT MICROBIOME-DERIVED UREMIC TOXIN, COUSE DYSLIPIDEMIA AND ALTER GENE EXPRESSION RELATED TO CHOLESTEROL IN THE LIVER OF RATS

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Introduction: Indoxyl sulfate (IS) is uremic toxin derived from dietary tryptophan by the gut microbiome1. Elevated concentrations of indoxyl sulfate in blood plasma are linked to the chronic kidney disease and cardiovascular diseases 2,3 . We hypothesized that IS could play a role in altering lipid homeostasis.

Material & Methods: To investigate the potential impact of IS on cholesterol metabolism, we performed in vitro experiment with HepG2 cells. Next, 10-week-old rats on a standard diet received either a vehicle or two doses of IS (10 and 100 mg/kg/day) for 8 weeks. We then assessed the lipid profile, gene expression, including the PCSK9/LDLR system, HMG-CoA reductaseand and SREBP2 using real-time PCR, Western Blot, and ELISA techniques.

Results: In HepG2 cells, IS significantly decreased PCSK9 mRNA and protein levels while markedly increasing LDLR mRNA and protein levels. Rats administered a dosage of 100mg/kg/day of IS demonstrated significantly elevated levels of total cholesterol, LDL and triglycerides, increase in hepatic HMG-CoA reductase and LDLR protein levels, along with a reduction in serum PCSK9 compared to other groups.

Conclusion: IS appears to upregulate hepatic HMG-CoA reductase and LDLR protein levels while decreasing serum PCSK9, suggesting its potential as a therapeutic target for hyperlipidemia.

The study was funded by the National Science Centre, Poland, grant no. 2020/37/B/NZ5/00366.

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THE INFLUENCE OF GUT MICROBIOTA FROM CHILDREN WITH AUTISM SPECTRUM DISORDER ON SHANK3B-/- OFFSPRING

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Introduction: Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder. The etiology of ASD depends on prenatal co-influence of genetic and environmental factors, including gut microbiota. This is supported by frequent gastrointestinal comorbidities and impairments of the gut microbiota in children with ASD.

Material & Methods: FMT (fecal microbiota transplantation) from children with ASD and controls was applied to mice with genetic defect SHANK3B+/-, a genetic animal model of ASD, or WT. Offspring were followed for microbiota composition (RT-PCR), plasma hormones (ELISA) and behavioral tests.

Results: The gut microbiota of prenatally exposed to FMT mice resembled the donor`s transplant. Further, the maternal microbiota had similar characteristics as in offspring, for example, lower Bacteroidetes/Firmicutes ratio. The gut microbiota from ASD children increased leptin levels only in SHANK3B-/- mice and correlated with their weight. Maternal FMT along with genetic factors influenced food preferences and food stereotypes. For example, Lactobacillus abundance was associated with the animal`s hedonic behavior.

Conclusion: FMT to mice impacted the gut microbiota of offspring, and the changes were in concordance with the gut microbiota of donors. The impact of maternal FMT and genetic background on leptin plasma levels and food intake was established.

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ALTERED GUT MICROBIOME COMPOSITION IN INDIVIDUALS WITH COMPLEX REGIONAL PAIN SYNDROME

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Introduction: CRPS causes severe disproportionate limb pain with autonomic & inflammatory signs. This study aims to investigate the composition of gut microbiota in CRPS individuals in 2 independent cohorts & characterizing its stability over different environments & syndrome severity.

Material & Methods: Individuals with CRPS, and matched controls were recruited in 2 geographically independent centers at Israel and Canada. After confirming diagnosis, participants filled the study questionnaires and gave a stool sample. Microbiome analysis was done using 16S rRNA sequencing.

Results: Overall, a marked depletion of several propionic acid metabolizers was observed along with depletion of Prevotella copri, which has also been found to be depleted in individuals with fibromyalgia. A logistic regression model was used on training cohort from Israel. Several sets of exact sequence variants (ESVs) were identified, which were then tested on an independent validation cohort from Canada, yielding a prediction accuracy of 87.1-90.5%, sensitivity of 83-90.5% and specificity of 85.7-91.1%.

Conclusion: In this study, depleted bacterial species in CRPS are potentially linked to altered metabolic pathways. The identified gut microbiome features serve as a CRPS-specific biosignature. These observations suggest a robust association of gut microbiome in CRPS, warranting further research regarding its role in the syndrome.

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MACHINE LEARNING DECODES THE MICROBIOME: ADVANCING DIAGNOSTIC ACCURACY IN GASTROINTESTINAL DISEASES

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Introduction: The gut microbiome plays a crucial role in human health and disease, offering a promising avenue for non-invasive disease diagnostics. With the increasing complexity of microbiome data, traditional statistical methods often fall short in distinguishing meaningful patterns. Machine learning (ML) techniques, with their capacity to model non-linear, high-dimensional relationships, are transforming the field of diagnostics.

Methodology: In this study, we systematically evaluated and compared the diagnostic accuracy of ML models, and addressed the critical challenges such as batch correction, compositional data transformation, and the influence of taxonomic versus functional profiling.

Results: The results showed that species-level taxonomic features significantly outperformed functional profiles for Crohn's disease where batch correction was often unnecessary. n contrast, for colorectal cancer diagnosis, the ComBat-seq batch correction method improved predictive accuracy, highlighting the disease-specific nature of preprocessing strategies.

Conclusion: The study underscores the critical role of selecting appropriate preprocessing steps and ML algorithms tailored to each disease. Random Forest and XGBoost algorithms were found to be the most effective for their robustness in feature preprocessing and high diagnostic performance. Furthermore, insights into minimizing batch effects and optimizing feature importance analysis offer practical guidelines for improving the accuracy and reproducibility of gastrointestinal diseases diagnostics.

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DETERMINATION OF THE CELLULAR PROLIFERATION RATE AND TELOMERE LENGTH IN CULTURES OF HUMAN ADULT PRIMARY FIBROBLAST TREATED WITH DAIGO METABIOTIC

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Introduction: There has been growing evidence that the lifespan of an individual can be longer by affecting telomere length. In its potential, an individual may live up to 150 years. Many lifestyle factors including stress, gut health imbalance and unhealthy diet influence premature aging and may affect longevity by altering the rate of telomere shortening. Cell replication is one of the main causes of telomere shortening. Progressive telomere shortening leads to senescence and various cell transformation, and shorter telomeres are associated with an increase in the number of diseases. The purpose of this study is to investigate preventive and anti-aging functions of metabiotic and its effect on the rate of cell proliferation and telomere shortening.

Materials & Methods: Three independent conditions were evaluated during the proliferative analysis: 1) Standard cell culture condition 2) Standard cell culture condition + 0.3x dilution of starting concentration (1x) of Daigo; 1/9600 from original concentration 3) Standard cell culture condition + 0.04x dilution of starting concentration (1x) of Daigo; 1/86400 from original concentration. For the measurement of the median telomere length of any cell line is used a high- throughput (HT) Q-FISH technique. This method is based on a quantitative fluorescence in-situ hybridization method modified for cells in interphase.

Results:

1. Proliferative analysis after 4 weeks of In vitro treatments with Daigo under standard conditions:
    - No significant differences were identified in any of the variables measured, between the control and treated groups.

2. Telomere measurement analysis after 8 weeks of In vitro treatments with Daigo under standard conditions:
    - Significant differences in all variables measured were identified between both Daigo[0.3x] and Daigo[0.04x] groups compared to control group
    - After normalizing the data by the population doubling, both concentrations Daigo[0.3x] and Daigo[0.04x] were found to lower the telomere shortening rate significantly as detected by the statistical analysis indicating a protective effect.

Conclusions: The effectiveness in application of metabiotic with preventive and anti-age function has been proven:

- Does not cause an increase in growth of human adult primary fibroblasts
- Telomere Shortening Rate - Standard condition with Daigo is more than 2 times less than without

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ROLE OF ORAL MICROBIOME IN SALIVARY GLAND HYPOFUNCTION

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Abstract

Introduction: Dry mouth may be associated with salivary gland hypofunction (SGH) due to conditions such as *sicca* syndrome, and Sjögren's disease, impacting patient's quality of life. Our objective was to determine salivary microbiome profiles of patients with *sicca* and Sjögren's disease (SGH group) compared to healthy controls (HC group).

Material & Methods: A cohort of SGH patients (*sicca*: N=10; Sjögren's: N=19) and HC subjects (N=38) was recruited. Saliva samples were collected and sequenced using 16S rRNA gene sequencing for determination of relative abundance of bacterial taxa. Alpha and beta-diversities were determined. LEfSe analysis and Mann-Whitney U-tests were used to identify differential species. CombiROC analysis was completed for species with AUC>0.75.

Results: Alpha- and beta-diversities of SGH and HC groups differed (p=0.001 and p=0.0001, respectively). LEfSe analysis identified ten species characterizing SGH group and two species characterizing HC group. Multi-species signatures were identified distinguishing SGH group from HC group using CombiROC analysis. Most significant 'combo' signature consisted of *Prevotella fusca*, *Prevotella Melaninogenica*, *Rothia Dentocariosa*, *Veillonella atypica*, and *Veillonella dispar* (AUC=0.939, Sensitivity=0.947, and Specificity=0.828).

Conclusion: There were significant differences in oral microbiome profiles of SGH *versus* HC groups. The differential species identified, possibly constitute a multi-marker signature.

Introduction

- Dry mouth may be associated with salivary gland hypofunction (SGH) due to conditions such as *sicca* syndrome and Sjögren's disease, impacting patient's quality of life.
- Mechanisms of SGH are poorly understood but it is believed genetic and environmental factors contribute to SGH pathogenesis.
- SGH can affect the quality of saliva and saliva composition including the oral microbiome.

Objective

- To determine salivary microbiome profiles of patients with *sicca* and Sjögren's disease compared to healthy controls.

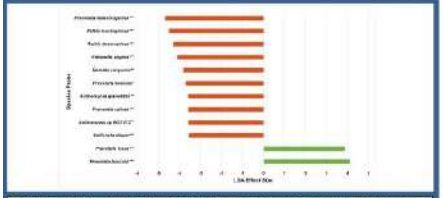
Results

Demographics

	HC[a]	SD[b]	Sicca[c]	SGH[d]
Subject count (M/F)[e]	38 (11/27)	19 (2/17)	10 (1/9)	29 (3/26)
Age[f]:				
Mean	52.66	61.26	49.8	57.31
Median	53.5	62	48	57
Dev	14.02	12.28	11.50	13.19
Range	24-84	26-84	33-69	26-84
Ethnicity count[g]:				
M: C/AA/O	8/0/4	2/0/0	1/0/0	3/0/0
F: C/AA/O	23/2/2	16/1/0	9/0/0	25/1/0
Focus score[h]:				
Mean	NA	2.27	0.188	1.46
Median	NA	1.92	0	1.3
Dev	NA	1.04	0.258	1.29
Range	NA	1.15-4.95	0-0.7	0-4.95

Footnote. Patient demographics based on saliva samples. [a]Healthy control (HC) group (N=38). [b]Sjögren's disease (SD) patient group (N=19). [c]Sicca patient group (N=10). [d]Salivary gland hypofunction group (SGH; N=29) combining the SD group (N=19) with the sicca group (N=10). [e]Total subject count for each group (HC, SD, sicca, or SGH). Male (M) and female (F) counts are shown in parentheses. [f]Subject ages depicted as mean, median, standard deviation (Dev), and range. [g]Subject ethnicity counts where C is Caucasian, AA is African American, and O is other. [h]Focus score of SD and sicca groups depicted as mean, median, standard deviation (Dev), and range. NA is not applicable.

Linear Discriminant Analysis Effect Size (LEfSe)



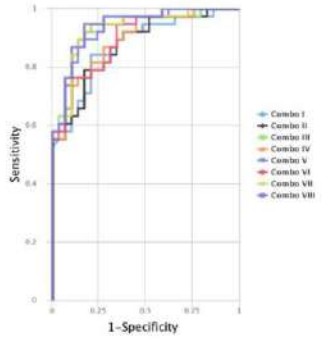
Legend. Linear discriminant analysis (LDA) effect size (LEfSe) analysis showing 10 significant species associated with salivary gland hypofunction (Sjögren's disease and sicca patient samples together, [SGH (burnt orange, n=29)] and two significant species associated with healthy controls [HC (green, n=38)] using oral samples of saliva. Mann-Whitney U-test significant species are depicted by an asterisk (*); * is $p < 0.05$, ** is $p < 0.01$, *** is $p < 0.001$.

Fold Changes of Mann-Whitney U-test Significant Species

Species[a]	Group[b]	p-value[c]	FC_{avg}[d]	Direction[e]
Prevotella melaninogenica	SGH	1.79×10^{-04}	1.92	SGH > HC
Rothia mucilaginosa	SGH	5.72×10^{-04}	1.32	SGH > HC
Rothia dentocariosa	SGH	1.11×10^{-08}	15.26	SGH > HC
Veillonella atypica	SGH	2.22×10^{-07}	6.84	SGH > HC
Gemella sanguinis	SGH	8.10×10^{-03}	0.92	SGH > HC
Prevotella histicola	SGH	1.78×10^{-02}	2.53	SGH > HC
Actinomyces graevenitzii	SGH	1.19×10^{-03}	28.76	SGH > HC
Prevotella salivae	SGH	8.50×10^{-04}	1.40	SGH > HC
Actinomyces sp HOT 172	SGH	1.47×10^{-03}	11.24	SGH > HC
Veillonella dispar	SGH	3.51×10^{-07}	6.31	SGH > HC
Prevotella fusca	HC	1.21×10^{-04}	-1.00	HC > SGH
Prevotella buccalis	HC	3.81×10^{-04}	-1.00	HC > SGH

Footnote. Fold changes of Mann-Whitney U-test significant species probes from saliva samples of Healthy control (HC) samples compared to the salivary gland hypofunction (SGH) group which combines the Sjögren's disease and sicca groups. [a] Significant species probe. [b] Group in which species is a discriminant feature *via* linear discriminant analysis effect size (LEfSe); SGH group (n=10) and HC group (n=2). [c] Mann-Whitney U-test p-value (α=0.05). [d] Fold change of average relative abundance [$FC_{avg}=|B_{avg}-A_{avg}/A_{avg}|$]. [e] Depiction of direction in which group species is greater than.

CombiROC plot



Combination	AUC	SE	SP	OC	Species
Combo I	0.873	0.842	0.793	0.496	*P. fusca*; *V. atypica*
Combo II	0.875	0.789	0.828	0.550	*P. fusca*; *P. melaninogenica*; *V. atypica*
Combo III	0.928	0.868	0.897	0.608	*P. fusca*; *R. dentocariosa*; *V. atypica*
Combo IV	0.893	0.737	0.897	0.590	*P. fusca*; *V. atypica*; *V. dispar*
Combo V	0.936	0.868	0.897	0.565	*P. fusca*; *P. melaninogenica*; *R. dentocariosa*; *V. atypica*
Combo VI	0.902	0.737	0.931	0.647	*P. fusca*; *P. melaninogenica*; *V. atypica*; *V. dispar*
Combo VII	0.929	0.895	0.862	0.456	*P. fusca*; *R. dentocariosa*; *V. atypica*; *V. dispar*
Combo VIII	0.939	0.947	0.828	0.381	*P. fusca*; *P. melaninogenica*; *R. dentocariosa*; *V. atypica*; *V. dispar*

Legend. Multi-Receiver Operating Characteristic (Multi-ROC) curves using the CombiROC online tool showing combined species taxa area under the curve (AUC), specificity (SP), sensitivity (SE), and optimal cutoff (OC) where species taxa had individual AUCs greater than or equal to 0.75 for comparisons the healthy control (HC) group and salivary gland hypofunction (SGH) group consisting of Sjögren's disease and sicca saliva samples combined. Combo VIII consisted of the most significant combination of species taxa.

Methods

- A cohort of SGH patients (*sicca*: N=10; Sjögren's: N=19) and HC subjects (N=38) was recruited.
- Saliva samples were collected and sequenced using 16S rRNA gene sequencing for determination of relative abundance of bacterial taxa.
- Alpha and beta-diversities were determined.
- LEfSe analysis and Mann-Whitney U-tests were used to identify differential species. CombiROC analysis was completed for species with AUC>0.75.

Conclusions

- There were significant differences in oral microbiome profiles of SGH *versus* HC groups. The differential species identified, possibly constitute a multi-marker signature.

Acknowledgments

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Staphylococcus spp. skin colonization in anti-IL-4/IL-13 and anti-JAK-treated atopic dermatitis patients: proposal of a standardized protocol for research and clinical applications.

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Purpose

This pilot study proposes a standardized procedure to monitor *Staphylococcus* spp. skin colonization, in particular that of *Staphylococcus aureus*, in patients with moderate/severe atopic dermatitis, treated with IL-4/IL-13 and anti-JAK inhibitors. The objective is to clarify both the possible drug influence on patient's skin microbiota colonization, and the effect of individual skin dysbiosis at the beginning, at the middle and at the end of the therapy. The results obtained with the proposed protocol could lay the foundations for its possible integration into clinical practice for a more accurate diagnosis, therapy response prediction and therefore a better disease management personalization.

Abstract

Atopic dermatitis (AD) is a chronic inflammatory autoimmune skin disease, characterized by intense pruritus, eczematous lesions, microbiota dysbiosis and skin barrier dysfunction[1], affecting million people worldwide. These alterations facilitate microbial overcolonization and virulence, mainly by the opportunistic pathogen *Staphylococcus aureus* (SA), contributing to the disease severity[2]. Novel biologic systemic treatments, wherein the anti-IL-4/IL-13 Dupilumab antibody and the JAK inhibitor Upadacitinib, changed the therapeutic scenario[3]. Since *Staphylococcus* spp. role in AD is still poorly understood, it must be clarified if the increased levels precede symptoms, contributing to the inflammation onset, or are a consequence. Swabs from lesional and non-lesional skin, collected with validated protocols, are both bio-banked and plated onto selective/differential media for *Staphylococcus* spp. identification and Colony Forming Unit (CFU) assessment. Bacterial DNA is extracted for *Staphylococcus* genus PCR identification and 16SrRNA sequenced for beta-diversity assessment. Finally, *S. aureus* cell-free supernatants are characterized for protein and short chain fatty acid content examination.

Results

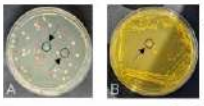
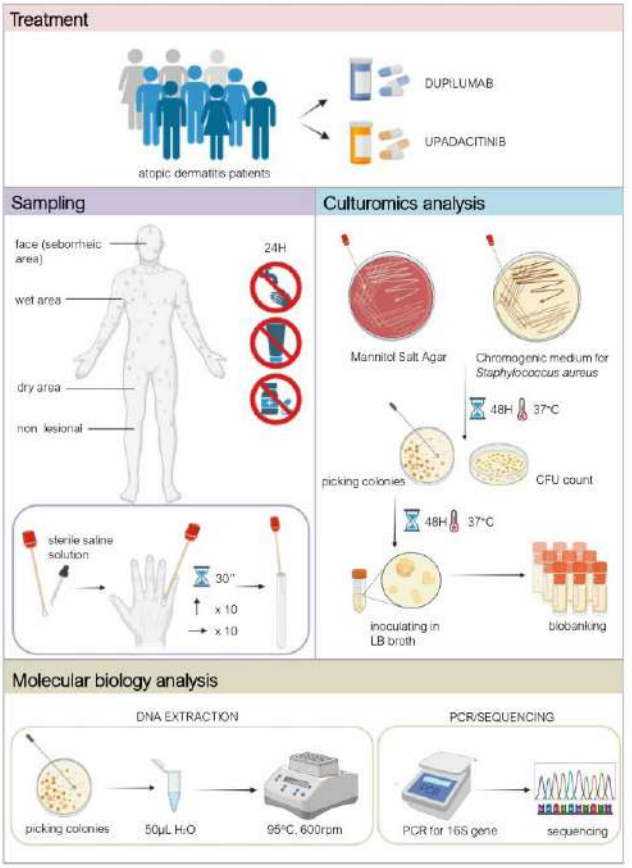


Fig.1: *Culturomic analysis*
A) Chromogenic medium for SA (pink colonies: SA; green colonies: other Staphilococci); **B)** Mannitol Salt Agar (yellow colonies: SA).

Fig.2: *PCR analysis*
A) *Staphylococcus* genus identification by amplification of the 16S gene of 11 skin swab samples (amplified product: 756bp); C+: positive control; C-: negative control.

Methods



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Conclusion

The preliminary results of the ongoing study led to the development of a standardized protocol for skin swab sampling in AD patients. This protocol is ensuring uniform sample collection from specific lesional and non-lesional skin areas, using sterile swabs and optimized storage techniques to preserve microbial viability without environmental contamination. Through the combined use of bacterial culture methods (bacterial isolation and CFU count) and molecular biology techniques (PCR and 16S sequencing), it is possible to identify common microorganisms associated with skin dysbiosis, such as *Staphylococcus aureus* and assess microbiota variation during therapy.

Staphylococcus aureus α-hemolysin interaction with Th17 cells – deep dive into shaping of the immune response

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Introduction

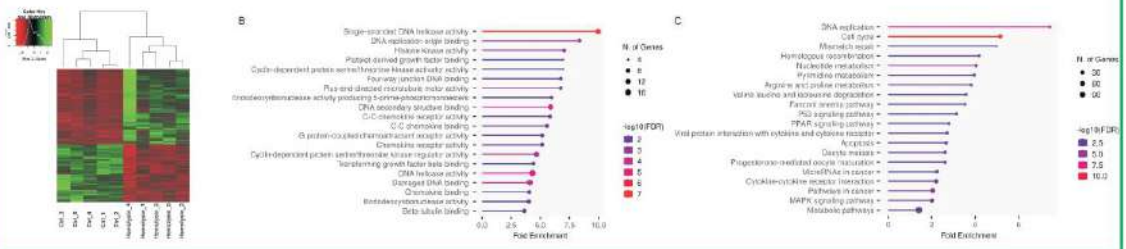
Skin microbiome perturbations have been associated with several immune-mediated diseases. Staphylococcus aureus is one of the common inhabitant of the skin and its enrichment was detected, e.g. in psoriasis and was associated with activation of Th17 response [1]. To better understand the complex nature of interactions between Staphylococcus aureus and the host immune system, we aimed to investigate how its toxin, α-hemolysin, affects differentiation and the transcriptome of Th17 cells.

Material & Methods

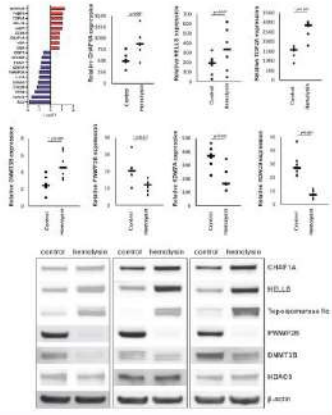
The CD4+ were isolated from buffy coats sourced from healthy, anonymous donors. These buffy coats were procured from the Regional Center for Blood Donation and Blood Treatment in Lodz, Poland. Naive CD4+ cells were induced to differentiate into Th17 lymphocytes in the presence of 200 ng/ml α-hemolysin. After 5 days cells were analysed using RNA sequencing and whole-genome bisulfite sequencing, real-time PCR, Western blot and ELISA techniques.

Results

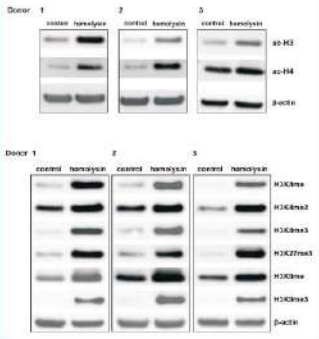
RNA sequencing revealed significant changes in the expression of 1626 genes in the cells treated with α-hemolysin. (B) Gene ontology analysis revealed numerous Gene Ontology terms associated with, e.g. regulation of DNA replication, histone kinase activity, DNA secondary structure binding. (C) Pathway enrichement analysis of the DEGs revealed alteration in pathways related to, e.g. DNA replication, cell cycle regulation, MAPK signaling.



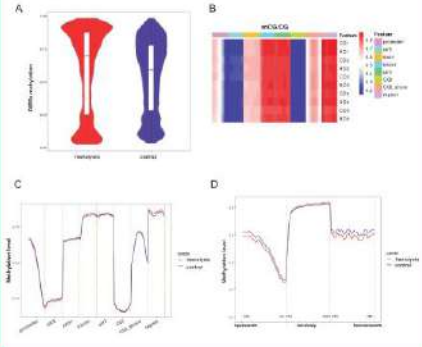
Among DEGs, were found genes known to be part of the regulatory network for Th17 cells and genes involved in epigentic regulation. The expression of the mRNA and protein products of these genes was assessed via quantitative PCR and Western blotting



α-hemolysin increased the acetylation of histones H3 and H4. Moreover treatment with α-hemolysin induced H3K4me, H3K4me2 and H3K4me3 modifications in Th17 cells, which are associated with an active chromatin state. Additionally, the induction of H3K9me3 and H3K27me3 was observed, which are typically considered repressive marks.



We observed that α-hemolysin significantly change genome methylation of human Th17 lymphocytes. (A) Violin boxplots depicting that α-hemolysin led to an increase in CG methylation in DMRs. (B, C) α-hemolysin decreased CG methylation in gene promoter regions, 5'-UTRs, exons, and CGIs while increasing methylation in introns, 3'-UTRs and repeated regions. (D) α-hemolysin decreased the methylation of CGs 2 kb upstream and downstream of gene bodies while increasing methylation within genes.



Conclusions

Our study revealed that α-hemolysin induces changes in histone marks and global methylation, shaping the transcriptome, epigenome, and phenotype of Th17 lymphocytes and attenuating a Th17 response [2].

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Identifying Microbiome Signatures that Distinguish Healthy from Unhealthy Guts

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INTRODUCTION

The microbiome is associated with various diseases, including inflammatory diseases, neurological and autoimmune conditions, and cancer. The human gut microbiota is a complex collection of microbial species. A healthy individual typically has a diverse microbiota and is in a state of eubiosis, whereas an unhealthy gut generally lacks microbial diversity and is in a state of dysbiosis. To develop a microbiome panel that can differentiate healthy versus unhealthy gut, we profiled 96 clinical stool samples on OpenArray based on healthy or unhealthy indicator organisms from the literature.

With 24 healthy control (HC) stool samples and 72 diseased stool samples from *C. diff* infection (CDI), Crohn's disease (CD) and ulcerative colitis (UC) we observed the following:

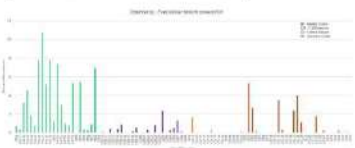
- Greater numbers / diversity of healthy promoting bacterial species in the HC samples versus the diseased controls
- Most diseased samples harbored higher numbers of the *Enterobacteriaceae* (*E. coli*, *Klebsiella*, and other relatives). In addition, several of the "so called" dental pathogens were found in greater amounts in these samples.
- Unexpectedly, we detected significantly higher human RNase P levels from ulcerative colitis and Crohn's disease samples compared to that of healthy controls, suggesting increased RNase P levels, and thus human DNA may correlate with gut inflammation.

MATERIALS AND METHODS

Nucleic acids were extracted from 96 stool samples using the Thermo Fisher Scientific MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit (A42357). After a preamplification step, samples were added to a custom OpenArray panel with 112 microbial targets, all with FAM-MGB TaqMan assays per single through hole. Most assays were amplified with single gene targets, although a smaller set were designed to amplify the 16S rRNA gene. NGS data were collected with The Ion AmpliSeq™ Microbiome Health Research Assay (MHRA).

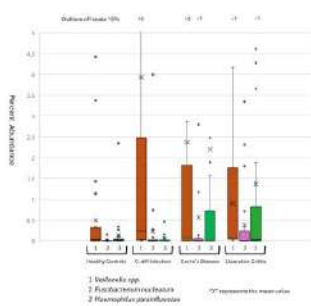
RESULTS

Figure 1. Predominance of *Faecalibacterium prausnitzii* in healthy stool samples vs disease



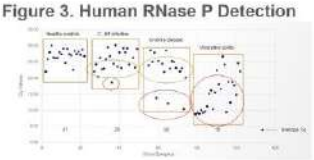
Results showed that healthy control samples predominantly consist of "good bacteria" at abundant levels such *Faecalibacterium prausnitzii* and other small chain fatty acid producers (data not shown).

Figure 2. Predominance of three "dental" pathogens in healthy stool samples vs disease, box and whiskers plot



Results showed that dental pathogens were in greater numbers in the disease gut stool samples vs the healthy controls, especially true for *Fusobacterium nucleatum*, which was found at greater than 0.1% in only one healthy control.

Figure 3. Human RNase P Detection



Unexpectedly, we detected significantly higher human RNase P levels from ulcerative colitis samples compared to that of normal healthy controls, suggesting increased RNase P levels may correlate with gut inflammation.

Figure 4. Selected examples of target abundance: Healthy controls vs disease

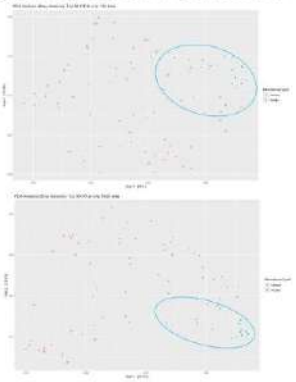
A couple of representative examples from each of the healthy control and disease sample groups show that healthy controls generally have a more diverse and greater number of "good bacteria (green)", primarily the small chain fatty acid produces, whereas disease samples show lower amounts and diversity of these bacteria. The disease groups, generally have higher amounts of the *Enterobacteriaceae* (*E. coli*, *Klebsiella*, and other relatives – blue and salmon colors) which, in the case of *E. coli*, are good at lower amounts, but can cause dysbiosis when they become dominant species in the gut. In addition, the dental pathogens (orange) are often found at much higher amounts in the disease samples.

**The 112 Microbial panel is available upon request.

Figure 5. OpenArray PreAmp vs No PreAmp

We compared the standard OpenArray workflow with a preamplification (preamp) option to check if this extra step can add valuable data. Indeed, we observed a greater number of microbial targets detected with preamp, with about double the number of targets detected. These same targets missed without PreAmp are also missed by NGS.

Figure 7. OpenArray vs NGS: PCA Plots



Comparison of OA and NGS data with Principal Components Analysis (PCA) using the top 50 taxonomic groups. Most healthy controls (blue dots) appear to correlate well in both OA and NGS, while most of the disease gut samples cluster away form the healthy controls, with some exceptions.

CONCLUSION

We have shown that a panel of 112 microbial targets can distinguish a healthy gut from a diseased gut in most stool samples. Results indicated that unhealthy guts could be distinguished from the healthy guts at about 98% accuracy, however with a small number of unhealthy samples showing a healthy microbial profile, the ability to tell if a healthy control profile correlates with a healthy gut is less accurate at around 80-85%.

Additionally, we have seen significantly greater microbial targets amplified with a preamplification step. Finally, we have validated OpenArray results with NGS and observed comparable data.

One valuable feature of the OpenArray panel is the inclusion of several targets beyond those of just bacteria, including fungal, one Archaea, antibiotic resistance genes, and an RNase P control.

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SALIVARY MICROBIOME PROFILING OF HPV+ AND HPV- OROPHARYNGEAL HEAD AND NECK CANCER PATIENTS UNDERGOING DURVALUMAB IMMUNOTHERPAY: A PILOT STUDY

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Abstract

Introduction: Immunotherapy holds the promise for treating oropharyngeal cancers (OPCs). Our objective was to determine effects of durvalumab immunotherapy on salivary microbiome.

Material & Methods: Early stage OPC patients, 5 positive for human papilloma virus [HPV+] and 12 negative [HPV-], were recruited and treated with durvalumab every two weeks, before surgery. Unstimulated saliva was collected and processed for 16S rRNA gene next generation sequencing and taxa relative abundance determination. Alpha- and beta- diversities were determined for baseline Group A and treatment groups one week apart (Groups B, C, and D). MaAsLin2 R program was used to identify associations with timepoint or HPV status. ROC curves were plotted for species in common between MaAsLin2 analysis and FDR-corrected Mann-Whitney U-test.

Results: Total of 416 taxa were detected. There were no alpha- and beta-diversity differences in longitudinal comparisons. There were beta-diversity differences for HPV status. Mann-Whitney U-test showed *Bergeyella sp16471* as significant in A versus BCD (FDR corrected p=0.0396). HPV status was associated with 87 species. MaAsLin2 A versus BCD analysis identified 57 taxa. *Leptotrichia spp.*, present in 63.6% combinational ROC curves (AUC>0.800), were more abundant in HPV+ patients.

Conclusion: This study shows durvalumab immunotherapy as having minimal effects on salivary microbiome composition.

Methods

- ❑ Early stage OPC patients, 5 positive for human papilloma virus [HPV+] and 12 negative [HPV-], were recruited and treated with durvalumab every two weeks, before surgery.
- ❑ Unstimulated saliva was collected and processed for 16S rRNA gene next generation sequencing and taxa relative abundance determination.
- ❑ Alpha- and beta- diversities were determined for baseline Group A and treatment groups one week apart (Groups B, C, and D).
- ❑ MaAsLin2 R program was used to identify associations with timepoint or HPV status.
- ❑ ROC curves were plotted for species in common between MaAsLin2 analysis and FDR-corrected Mann-Whitney U-test.

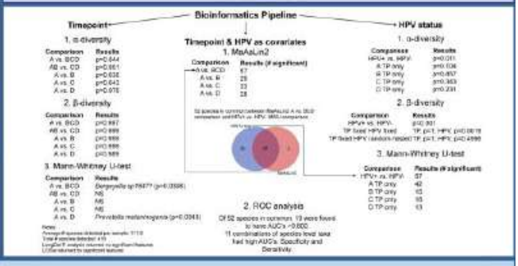
Introduction

- ❑ **There are over 40 different types of HPV able to infect mucosal epithelium.**
- ❑ **Little is known about possible direct or indirect cooperative associations between the oral microbiome and HPV infection.**
- ❑ **HPV16 is responsible for up to 90% of HPV-related oropharyngeal cancers (OPCs).**
- ❑ **Immunotherapy holds the promise for treating OPCs**

Objective

- ❑ **To determine the effects of durvalumab immunotherapy on salivary microbiome.**

Analytical Design



Results

Sample sizes of comparisons and detection of bacterial taxa

Timepoint Comparisons	Samples	Taxa	Samples	Taxa
	A		**BCD**	
A vs. BCD	17	373	47	391
	AB		**CD**	
AB vs. CD	34	400	30	370
	A		**B**	
A vs. B	17	373	17	361
	A		**C**	
A vs. C	17	373	17	347
	A		**D**	
A vs. D	13	373	13	336
HPV Comparisons	**HPV+ Samples**	**Taxa**	**HPV- samples**	**Taxa**
HPV+ vs. HPV- all timepoints	18	299	46	376
HPV+ vs. HPV- A only	5	281	12	326
HPV+ vs. HPV- B only	5	234	12	335
HPV+ vs. HPV- C only	5	247	12	317
HPV+ vs. HPV- D only	3	217	10	314

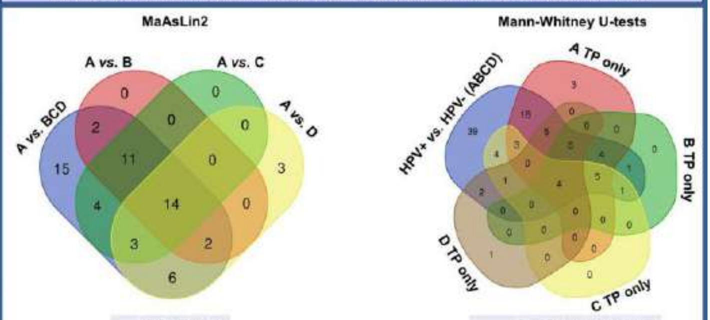
Footnote. Comparisons consisted of longitudinal analyses prior to immunotherapy (A) and during immunotherapy sampled one week apart (B = 1 week; C = 2 weeks; D = 3 weeks) and cross-sectional analysis of HPV positive (HPV+) patients compared to HPV negative (HPV-) patients. Number of samples included in the comparison and bacterial taxa detected are shown. The total number of unique species taxa detected is 416.

Multi-ROC analysis results

Combination	Markers	AUC	SE	SP	Cutoff
Combination [illegible]	Actinomyces massiliensis, Corynebacterium durum, Leptotrichia trevisanii	[illegible]	[illegible]	[illegible]	[illegible]
Combination [illegible]	Actinomyces massiliensis, Actinomyces naeslundii, Corynebacterium durum, Leptotrichia trevisanii	[illegible]	[illegible]	[illegible]	[illegible]
Combination [illegible]	Corynebacterium durum, Leptotrichia trevisanii	[illegible]	[illegible]	[illegible]	[illegible]
Combination [illegible]	Actinomyces massiliensis, Corynebacterium durum, Leptotrichia trevisanii	[illegible]	[illegible]	[illegible]	[illegible]
Combination [illegible]	Actinomyces massiliensis, Leptotrichia trevisanii	[illegible]	[illegible]	[illegible]	[illegible]
Combination [illegible]	Actinomyces massiliensis, Actinomyces naeslundii, Leptotrichia trevisanii	[illegible]	[illegible]	[illegible]	[illegible]
Combination [illegible]	Actinomyces naeslundii, Leptotrichia trevisanii	[illegible]	[illegible]	[illegible]	[illegible]
Combination 1	Actinomyces massiliensis, Actinomyces naeslundii	[illegible]	[illegible]	[illegible]	[illegible]
Combination [illegible]	Actinomyces massiliensis, Corynebacterium durum	[illegible]	[illegible]	[illegible]	[illegible]
Combination [illegible]	Actinomyces massiliensis, Actinomyces naeslundii, Corynebacterium durum	[illegible]	[illegible]	[illegible]	[illegible]
Combination [illegible]	Actinomyces naeslundii, Corynebacterium durum	[illegible]	[illegible]	[illegible]	[illegible]

Legend. Combinational Receiver Operating Characteristic (CombiROC) curves from 10 combinations of species taxa resulting in 11 combinations of species level taxa (Markers) showing the Area Under the Curve (AUC), specificity (SP), sensitivity (SE), and detection threshold (Cutoff). Approximately 64% of CombiROC curves included *Leptotrichia spp.*

Venn diagrams of species in common among the MaAsLin2 and Mann-Whitney U-test results



	AvBCD	AvB	AvC	AvD
AvBCD	NA	29	32	25
AvB	29	NA	25	16
AvC	32	25	NA	17
AvD	25	16	17	NA

	HPV+ vs. HPV-	A only	B only	C only	D only
HPV+ vs. HPV-	NA	37	15	18	12
A only	37	NA	13	12	9
B only	15	13	NA	10	4
C only	18	12	10	NA	5
D only	12	9	4	5	NA

Legend. Species taxa in common between longitudinal comparisons using Multivariable Association with Linear Models (MaAsLin2) (shown above as A vs. BCD; 57 significant) and Mann-Whitney U-tests of taxa relative abundance differences comparing the HPV+ group to the HPV- group (shown above as HPV+ vs. HPV- (ABCD); 87 significant). Tables below each comparison summarize the Venn diagrams.

Fold changes of 10 species with AUC's > 0.800 from MaAsLin2 longitudinal and Mann-Whitney U-test cross-sectional analysis resulting in 52 significant species in common

Species with AUC > 0.800 (n=10)[a]	p-value[b]	AUC[c]	A[d]	BCD_{avg}[e]	FC[f]	BCD increase or decrease[g]	HPV-[h]	HPV+[i]	FC[j]	HPV+ higher or lower[k]
Actinomyces sp4769	<0.0001	0.932	0.0044	0.0063	0.4201	Increase	0.0009	0.0223	23.8868	Higher
NA sp37035	<0.0001	0.906	0.0490	0.0254	-0.4826	Decrease	0.0363	0.0886	1.4429	Higher
Gemella sanguinis	<0.0001	0.903	0.0940	0.0812	-0.1361	Decrease	0.1458	0.2300	0.5776	Higher
Oribacterium sinus	<0.0001	0.89	0.1442	0.0931	-0.3549	Decrease	0.1791	0.2444	0.3645	Higher
Leptotrichia trevisanii	**<0.0001**	**0.837**	**0.0046**	**0.0050**	**0.0492**	**Increase**	**0.0019**	**0.0179**	**8.2892**	**Higher**
Actinomyces naeslundii	**<0.0001**	**0.831**	**0.0043**	**0.0066**	**0.5226**	**Increase**	**0.0118**	**0.0121**	**0.0247**	**Higher**
Veillonella atypica	<0.0001	0.831	0.3840	0.4079	0.0622	Increase	0.8490	0.7587	-0.1063	Lower
NA sp31630	<0.0001	0.818	0.0128	0.0115	-0.0979	Decrease	0.0157	0.0316	1.0059	Higher
Corynebacterium durum	**<0.0001**	**0.813**	**0.0037**	**0.0053**	**0.4249**	**Increase**	**0.0017**	**0.0178**	**9.3332**	**Higher**
Actinomyces massiliensis	**<0.0001**	**0.812**	**0.0015**	**0.0017**	**0.1555**	**Increase**	**0.0017**	**0.0050**	**1.9731**	**Higher**

Footnote. [a]Species (n=10) were determined from the 52 in common between the MaAsLin2 analysis on 416 species in the longitudinal comparison (A vs. BCD) and Mann-Whitney U-tests of 416 species in the cross-sectional comparison (HPV+ vs. HPV-, all timepoints). [b]p-value of Receiver Operating Characteristic (ROC) curve. [c]Area Under the Curve (AUC). [d]Sum of relative abundance for A timepoint. [e]Average sums of the B, C, and D timepoints. [f]Fold change of A timepoint vs. BCD timepoints. [g]Depiction of whether species taxa increases or decreases over time. [h]Sum of relative abundance for the HPV negative (HPV-) group. [i]Sum of relative abundance for the HPV positive (HPV+) group. [j]Fold change of the HPV+ group vs. HPV- group. [k]Depiction of whether species taxa were higher or lower in the HPV+ group. Species taxa shown in **bold** constituted the 11 combinational ROC curves.

Conclusions

- ❑ **This study shows durvalumab immunotherapy as having minimal effects on salivary microbiome composition.**

Acknowledgments

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POSTER-48

CHARACTERISING THE GUT MICROBIOME IN TRAUMATIC BRAIN INJURY

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Introduction:

Traumatic Brain Injury (TBI) is the leading cause of death and disability among trauma-related injuries worldwide.[1] It is a chronic disease with long-term neurological, cognitive and behavioural sequelae.[2,3] Despite advancements, effective treatments remain elusive. Hence, novel therapeutic strategies are the need of the hour.

The gut microbiome has emerged as a major determinant of patient outcome in TBI, mediated through the Gut-Microbiome-Brain-Axis.[4] A thorough understanding of the changes in the gut microbiome following TBI is crucial for the development of targeted therapeutic interventions aimed at positively modulating this axis.

In this study, we characterise the changes in the gut microbial composition in patients with TBI.

Material & Methods:

Type of Study: Prospective cohort study

Study Setting: The study was conducted in the Departments of Microbiology and Neurosurgery in our institute, after IEC approval.

Inclusion criteria:	Exclusion criteria:
✓ Patients admitted with Mild and Severe TBI (GSC ≥13 and <8)	X Aged <18 or >70 years
✓ Willing to participate in the study	X Patients admitted with Moderate TBI
✓ Aged between 18-70 years	X In patient time <72 hrs
	X No visible fecal matter on the swab
	X Antibiotic exposure in the past month

Recruitment of 16 TBI patients for the study

Written informed consent

Collection of 1st rectal swab (a) within 24hrs of TBI (Day 1)

Collection of 2nd rectal swab (b) after 72hrs, and within 96hrs of TBI (Day 3)

Transportation to the laboratory within 20 mins of collection

Storage at 2-8°C (upto 4hrs) till further processing

Fecal DNA extraction using QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany)

16S rDNA PCR

Sequencing on Illumina NextSeq2000 to generate 2x250bp reads/sample

OTU identification

Taxonomy classification using RDP classifier against SILVA-138 database at 97% similarity

Results:

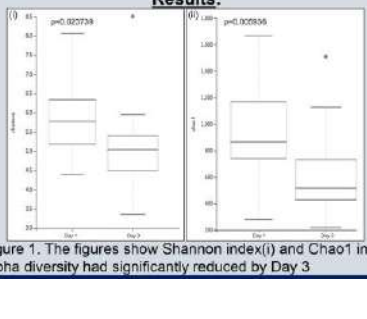

Figure 1. The figures show Shannon index(i) and Chao1 index(ii); Alpha diversity had significantly reduced by Day 3

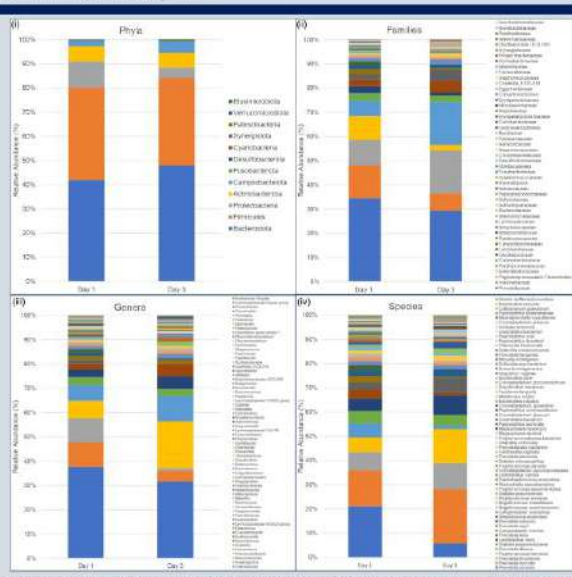

Figure 2. The figures show the changes in Phyla (i), Families (ii), Genera (iii) and Species (iv).

	Decrease	Increase
Phylum	Proteobacteria	
Family	Enterobacteriaceae	Porphyromonadaceae
	Oscillospiraceae	
	Coriobacteriaceae	
Genus	Escherichia-Shigella	Porphyromonas
	Agathobacter	Peptoniphilus
	Collinsella	
	Howardella	
Species	Slackia isoflavoniconvertens	Prevotella timonensis
		Facklamia hominis
		Peptoniphilus lacrimalis
		Peptoniphilus urinimassiliensis
		Peptoniphilus koenoeneniae

Table 1. The table shows the Phylum, Families, Genera and Species which had changed significantly ($p<0.05$) by Day 3

Discussion and Conclusion:

To our knowledge, this is the first clinical study documenting the gut microbiome at different times after TBI. Similar studies have been done in animal models with induced TBI.[5–7]

Systematically induced brain trauma in the in vitro setting cannot be recreated clinically. Any attempt to correlate the gut microbiome with TBI is thus limited by inherent variables such as the severity, type, and extent of trauma, other injuries, and antibiotic exposure.

Traumatic brain injury causes gut microbial dysbiosis. With a thorough understanding of this dysbiosis, simple interventions like tailored probiotics might greatly improve overall patient outcome.

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Targeting Microbiota 2024 – Index of Authors

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Ready to use ***In vitro*** **models** and **Growth enhancer Beads**

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Bac³**Gel** is an Innovation company that **unblocks the full power of microbiota** by providing the complexity of **native ecosystems** in a simple way**.**

WHAT **WE DO**

Our technology is a **tailorable system** that provides the microbiota with the **required conditions to thrive.**

Available Standard Formulations for **different body districts**:

Oral | **Airway** | **Gastric** | **Intestinal** | **Cervicovaginal** | **Universal**

platform for multipurpose bacterial culture and growth

OUR PRODUCTS

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For **boosting proliferation** of hard to grow microbiota *In vitro*. **Its main advantages are:**

- Compatible with bioreactors
- Compatible with extraction and purification protocols of bacteria byproducts
- Easy to use
- Increases production yield
- Promote the growth of hard to grow bacteria

They come in **flasks of 75 units.**
The formulation can be **tailored** to boost the growth of **specific bacteria**.

OUR PRODUCTS

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Models that **mimic** mucus layers from **different body districts**, and can be used with **traditional laboratory supplies** (e.g. extrudable gels in syringes, multiwell systems, transwells, microfluidic systems, 3D printing)**. Its main advantages are**:

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- Stand-alone solution
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- Easy to use
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