



13TH INTERNATIONAL GUT MICROBIOLOGY SYMPOSIUM 2023

P&J LIVE, ABERDEEN, SCOTLAND

13-15 JUNE 2023

13TH JOINT SYMPOSIUM Organised by the Rowett Institute, University of Aberdeen,
and INRAE Clermont Auvergne Rhône Alpes Centre, Clermont Ferrand, France



The Rowett Institute



 abdn.ac.uk/events/conferences/gutmicro2023

 @gutmicro2023 #gutmicro2023

nature awards



THE GLOBAL GRANTS
FOR GUT HEALTH

2023 Call for Applications

There is increasing evidence that altered gut microbiome composition appears to be associated with persistent symptoms in many patients with post-acute COVID-19 syndrome (PACS) or long COVID.

The new Global Grants for Gut Health call for applications in 2023 is seeking research proposals intended to quantify and characterise the phenomena of altered gut microbiome in these patients and particularly those intended to identify mechanisms of action underlying this association and approaches to microbiota modulation to facilitate timely recovery and reduce the global burden of PACS.

The programme, co-supported by Nature Portfolio and Yakult, will make a maximum of three awards available per funding cycle, each of up to US\$ 100,000.

Applications are accepted until 13th September 2023.

Discover more at guthealth-grants.com

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Yakult nature portfolio

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CONTENTS

WELCOME	006
PROGRAMME	007-010
TUESDAY 13 JUNE 2023	008
WEDNESDAY 14 JUNE 2023	009
THURSDAY 15 JUNE 2023	010
GENERAL INFORMATION	011-016
CONFERENCE VENUE	012
SOCIAL VENUES	013
TRANSPORT AND FURTHER INFORMATION	014
INVITED TALKS	017-023
PROFESSOR BÄRBEL STECHER	018
DR SABINA LEANTI LA ROSA	019
PROFESSOR TINE RASK LICHT	020
PROFESSOR WILLEM VAN SCHAİK	021
DR LYNSEY HOWARD	022
DR IRINA SPACOVA	023
OFFERED TALKS	025-045
POSTERS	047-106
AUTHOR INDEX	108-117
MAP	119-120

WELCOME TO...

13TH INTERNATIONAL GUT MICROBIOLOGY SYMPOSIUM 2023

The University of Aberdeen warmly welcomes delegates and invited guests to the 13th Joint Symposium organised by the Rowett Institute, University of Aberdeen, Scotland (UK) and INRAE Clermont Auvergne Rhône Alpes Centre, Clermont Ferrand, (France).

Held at P&J Live on the 13-15th June 2023, the 13th International Gut Microbiology Symposium aims to explore the role of the complex microbial ecosystem present in the digestive tract of humans and animals in maintaining host health. A particular focus will be on microbe-microbe and microbe-host interactions to advance our understanding of the role of these interactions in host welfare and health.

Sessions will focus on topics including colonisation resistance, spread of genes through environmental ecosystems, and successes in translating research into practice. Studies on all other topics related to the gut microbiota, including developing new approaches to study interactions between nutrition and gut microbial ecosystems as well as cross-talk between microorganisms and their host will also be welcomed.

Topics will include:

- Interactions between microbiota, pathogens and host
- Impact of nutrition on gut microbiome and health
- One Health – Linking microbes in the environment, humans and animals
- Beyond the lab: Applying microbiome research in practice
- Other



Local Scientific Organising Committee:

- Karen Scott (Chairperson)
- Sylvia Duncan
- Silvia Gratz
- Petra Louis
- Alan Walker



French Organising Committee:

- Annick Bernalier-Donadille
- Evelyne Forano
- Diego Morgavi
- Pascale Mosoni
- Milka Popova
- Panagiotis Sapountzis
- Lucie Etienne -Mesmin

Event Organisers:

- CPD Services (University of Aberdeen)

For news and updates follow  @gutmicro2023 and use the hashtag #gutmicro2023



PROGRAMME

TUESDAY 13TH JUNE 2023

08:00 – 13:30	Satellite Meeting Gateway to Global Enteric Methane Reduction (Workshop) Co-Organised by Sharon Huws & Matthias Hess	Hilton (TECA)
11:00 – 17:30	Main Symposium Registration	P&J Live, First Floor, Upstairs Right View Area
14:00 – 14:05	Welcome: Karen Scott	Conference Suite 1A
14:05 – 15:30	Session 1: Interactions between microbiota, pathogens and host Session Chairs – Alan Walker and Lucie Etienne Mesmin	Conference Suite 1A
14:05 – 14:50	IT 01: Using the Oligo-Mouse-Microbiota to understand gut microbiota ecology, evolution and pathogen exclusion	Bärbel Stecher
14:50 – 15:10	OT 01: Towards a better description of the interactions of food-borne pathogen Enterotoxigenic Escherichia coli with intestinal mucus and human microbiome	Lucie Etienne-Mesmin
15:10 – 15:30	OT 02: Comparative analysis of 4 enteropathogens in mice with complex and OligoMM12 microbiota	Mathias Klaus-Maria Herzog
15:30 – 16:00	Refreshment Break	Conference Suite 1B/1C
16:00 – 17:20	Session 1 continued...	Conference Suite 1A
16:00 – 16:20	OT 03: An IBD-associated pathobiont collaborates with the NSAID to promote inflammation and cell death in a susceptible host via the caspase-8/NLRP3 axis	Raminder Singh
16:20 – 16:40	OT 04: Revealing the causal relationship between age-associated immune remodelling and changes in the gut microbiome	Selina Stahl
16:40 – 17:00	OT 05: Novel molecular mechanisms by commensal bacteria to inform immunomodulatory microbiome therapies	Jorge Gutierrez-Merino
17:00 – 17:20	OT 06: Designing synthetic communities using a functionally-guided approach complemented with metabolic modelling	Thomas Hitch
19:30 – 21:00	Civic Welcome Reception at Town House, Broad St, Aberdeen AB11 5BU (complimentary shuttle buses from P&J Live to Broad Street, will be provided at 18:00)	

KEY:

IT = Invited Talk

OT = Offered Talk

WEDNESDAY 14TH JUNE 2023

09:00 – 10:25	Session 2: Impact of nutrition on gut microbiome and health Session Chairs – Sylvia Duncan and Milka Popova	Conference Suite 1A
09:00 – 09:45	IT 02: Integrating multi-omics and enzymology to untangle systems for typical and atypical glycan processing in gut microbiomes	Sabina Leanti La Rosa
09:45– 10:05	OT 07: Faecal microbiome mediates the effect of diet on colorectal-cancer risk: comparison of meat based versus pescovegetarian diets	Philippe Gérard
10:05 – 10:25	OT 08: The molecular basis of the degradation of flavan-3-ols by the human gut bacterium <i>Eggerthella lenta</i>	Pascale Mosoni
10:25 – 11:00	Refreshment Break	Conference Suite 1B/1C
11:00 – 12:20	Session 2 continued...	Conference Suite 1A
11:00 – 11:20	OT 09: p-Cresol derivatives interact with the blood–brain barrier and highlight the complex nature of microbiota–host communication pathways associated with the gut–brain axis	Lesley Hoyles
11:20 – 11:40	OT 10: Inter-individual differences in segmental transit time and pH are linked to the human gut microbiome composition and metabolism	Nicola Procházková
11:40 – 12:00	OT 11: Oligofructose Improves Small Intestinal Lipid-Sensing Mechanisms via Alterations to the Small Intestinal Microbiota	Frank Duca
12:00 – 12:20	OT 12: The impact of seaweed-based nutritional manipulation on the rumen microbiome	Alessandra Ferrillo
12:20 – 13:30	Lunch	Conference Suite 1B/1C
13:30 – 15:30	Poster session ODDS with Tea & Coffee from 15:00	Conference Suite 1B/1C
15:30 – 16:45	Session 3: Flash Talks (open topic) selected from poster presentations Session Chairs – Karen Scott and Petra Louis	Conference Suite 1A
15:30 – 15:35	P008: Mitochondrial complex I dysfunction promotes microbial composition that impacts on ulcerative colitis	Leticia Abecia
15:35 – 15:40	P023: A gut microbiome perspective of extracellular vesicle-mediated communication between human cells and bacteria	Simon Swift
15:40 – 15:45	P028: Surf and Turf: The Adaptation of Ruminant Microbiomes to Marine forages	Jeff Tingley
15:45 – 15:50	P032: The use of a continuous flow in vitro model to assess the role of psyllium as a potential mechanism for FODMAP consumption in IBS patients	Hannah Harris
15:50 – 15:55	P035: Linking diet and gut microbiota in obesity: usefulness of a new in vitro human mucosal colon model	Stéphanie Blanquet-Diot
15:55 – 16:00	P072: Microbiome-derive metabolites are powerful biomarkers for anal cancer screening	Jana Seifert
16:00 – 16:05	P077: Understanding polyunsaturated fatty acid provisioning by gut microbiota elements through the lens of an earthworm model	Stephanie Schnorr
16:05 – 16:10	P081: Auxotrophies in human gut bacteria are indicative of microbiome stability	Svenja Busche
16:10 – 16:15	P100: The effect of exercise intervention and cardiorespiratory fitness on the gut microbiome: a Generation 100 cohort study	Natalia Bednarska
16:15 – 16:20	P107: Distinct effects of anaerobe gut fungi on feed lignocellulose composition and structure during its degradation	Jolanda van Munster
16:20 – 16:25	P113: Increasing resolution of mycobiome profiling in the human gut	David Schneider
16:25 – 16:45	Q&A	
16:45 – 17:30	Session 2 continued...	Conference Suite 1A
16:45 – 17:30	IT 03: Impact of diet on microbial metabolism in infants and adults	Tine Rask Licht
19:30 for 20:00 until 23:00	Conference Dinner at Ardoe House Hotel & Spa, South Deeside Road, Blairs, AB12 5YP (return coach transportation will be provided)	

Bus Timings: 18:45 from P&J Live / leaving at 19:10 from Broad Street, Aberdeen, AB11 5BU. **Return:** 22:30 to 23:00 staggered bus returns to Broad Street and P&J Live. Please ensure you arrive 10 - 15 minutes prior to bus departure.

THURSDAY 15TH JUNE 2023

09:00 – 10:25	Session 4: One Health – Linking microbes in the environment, humans and animals Session Chairs – Karen Scott and Diego Morgavi	
09:00 – 09:45	IT 04: Antimicrobial resistance in the human gut microbiome	Willem van Schaik
09:45 – 10:05	OT 13: Resistome characterization in neonatal calves and their environments on dairy farms: Implications for AMR surveillance and risk mitigation	Katie Lawther
10:05 – 10:25	OT 14: RiboTaxa: combined approaches for rRNA genes taxonomic resolution down to the species level from metagenomics data revealing novelties	Oshma Chakoory
10:25 – 11:00	Refreshment Break	Conference Suite 1B/1C
11:00 – 12:00	Session 4 continued...	Conference Suite 1A
11:00 – 11:20	OT 15: Analysis of milk, feces and soil microbiomes composition and structure across Quebec dairy farms	Dominic Poulin-Laprade
11:20 – 11:40	OT 16: Linking environmental pollution and gut microbiome in individuals living in highly contaminated settlements	Francesca De Filippis
11:40 – 12:00	OT 17: Recipient independent high accuracy FMT prediction and optimization in mice and humans	Yoram Louzoun
12:00 – 12:20	OT 18: The human gut microbiome explored at single nucleotide resolution	Falk Hildebrand
12:20 – 13:30	Lunch	Conference Suite 1B/1C
13:30 – 15:30	Poster session EVENS with Tea & Coffee from 15:00	Conference Suite 1B/1C
15:30 – 17:40	Session 5: Beyond the lab: Applying microbiome research in practice Session Chairs – Petra Louis and Simon Roques	Conference Suite 1A
15:30 – 16:15	IT 05: From Bench to Bedside: The journey of EnteroBiotix	Lynsey Howard
16:15 – 16:35	OT 19: Harnessing the gut microbiome to identify novel anaerobic bacteria with biotherapeutic potential	Indrani Mukhopadhyaya
16:35 – 16:55	OT 20: Harnessing diverse prophages of STEC O157:H7 phage-type 8 for rapid identification of fresh produce-associated STEC strains	Janet Nale
16:55 – 17:40	IT 06: Leveraging lactobacilli against respiratory viral diseases	Irina Spacova
17:40 – 18:00	End of conference and announcement of next conference and poster prize winner	
20:00 – 23:00	Conference Ceilidh - with Stovies (food) at 21:15 at the Beach Ballroom, Beach Promenade, Aberdeen, AB24 5NR (return coach transportation will be provided)	

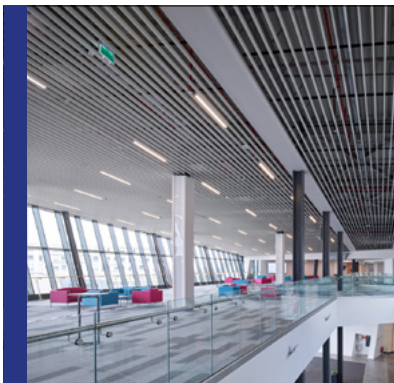
Bus Timings: 19:00 from P&J Live / leaving at 19:20 from Broad Street, Aberdeen, AB11 5BU. **Return:** 23:15 bus returns to Broad Street and P&J Live. Please ensure you arrive 10 - 15 minutes prior to bus departure.

GENERAL INFORMATION



GENERAL INFORMATION

CONFERENCE VENUE



Conference Venue | P&J Live at TECA

TUESDAY 13TH - THURSDAY 15TH JUNE 2023

P&J Live, East Burn Road, Stoneywood,
Aberdeen AB21 9FX

Accessibility Information:

www.pandjlive.com/visiting/accessibility-2/

Toilets: Accessible toilets on all levels

Lift and Escalators: Lift and escalators to both levels

Upstairs View Area (First Floor): Registrations, Help Desk,
Catering overspill area

Conference Suite 1A: Talks

Conference Suite 1B/C: Refreshments, Lunches,
Exhibition Stands and Posters

Taxi rank: Aberdeen Taxis pick-up and drop-off point

Parking: On-site parking, pay on exit

No Cash Machine/ATM: in support of P&J Live's green credentials, all the bars, kiosks and restaurants accept card payments only, this is more efficient, safe and ensures a speedy transaction.

WiFi: FREE WiFi is available at the venue. No need for a code, simply connect via the landing page when prompted.

Smoking: P&J Live is a no-smoking building, this includes the use of e-cigarettes and vaping, but they do have specific outside smoking points for those wishing to smoke outside.

For more FAQs regarding the conference venue visit the P&J Live website:

www.pandjlive.com/faq/#visiting-faq

SOCIAL VENUES



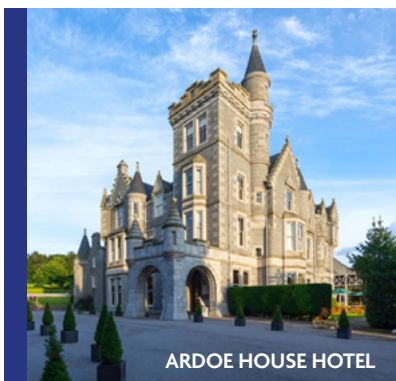
Civic Hosted Welcome Reception | Town House TUESDAY 13TH JUNE 2023

Town House, Broad St, Aberdeen AB11 5BU

19:30 – 21:00 Drinks reception and canapés from 19:30

A complimentary shuttle bus into the town city centre will be provided at 18:00 from P&J Live. More details can be found here:

www.abdn.ac.uk/events/conferences/gutmicro2023/2column-page-2287-2287.php



Conference Dinner | Ardoe House Hotel WEDNESDAY 14TH JUNE 2023

Ardoe House Hotel & Spa, South Deeside Road, Blairs AB12 5YP

19:30 for 20:00 until 23:00

www.ardoehousehotel.co.uk

Dress code: Smart-casual.

A complimentary bus will be provided from the P&J Live and the City Centre, please check the conference website and registration desk for more details:

www.abdn.ac.uk/events/conferences/gutmicro2023/2column-page-2287-2287.php

Timings: 18:45 from P&J Live / leaving at 19:10 from Broad Street, Aberdeen, AB11 5BU. **Return:** 22:30 to 23:00 staggered bus returns to Broad Street and P&J Live.

Please ensure you arrive 10-15 minutes prior to bus departure times.



Ceilidh | Beach Ballroom THURSDAY 15TH JUNE 2023

Beach Ballroom, Beach Promenade, Aberdeen AB24 5NR

20:00 until 23:00 with Stovies (food) at 21:15

www.aberdeencity.gov.uk/beach-ballroom

Coach Transportation will be provided from the P&J Live and the City Centre, please check the conference website and registration desk for more details:

www.abdn.ac.uk/events/conferences/gutmicro2023/2column-page-2287-2287.php

Timings: 19:00 from P&J Live / leaving at 19:20 from Broad Street, Aberdeen, AB11 5BU. **Return:** 23:15 bus returns to Broad Street and P&J Live. **Please ensure you arrive 10-15 minutes prior to bus departure times.**

GENERAL INFORMATION

TRANSPORT & FURTHER INFORMATION



Travelling by Car or Motorcycle

P&J LIVE AT TECA

The P&J Live is easily accessible from the north or the south by the A96 which runs from Aberdeen to Inverness.

The P&J Live is about 5 miles northwest of the city centre.

P&J Live Postcode: AB21 9FX

Latitude/ longitude: 57.1852° N, 2.1917° W

ABERDEEN AIRPORT

Aberdeen International Airport is easily accessible from the A96 and is 6 miles from the city centre, while also being 1.4 miles from the P&J Live.

Aberdeen Airport Postcode: AB21 7DU

Latitude/ longitude: 57.2037° N, 2.2002° W

Taxi Services

Taxis Companies:

- Comcabs - 01224 353535
- Central Taxis - 01224 898989
- Rainbow City Taxis - 01224 878787

Download the Rainbow Taxi UBook app:

www.rainbowcitytaxis.com/ubook/

Should you require assistance in contacting a taxi company please ask one of the event organisers for help.

Fares: All taxi fares are metered.

How to Pay: Cash payments (change given) and many vehicles are fitted with Chip & PIN payment terminals (a transaction charge applies).

Journey Times:

In light traffic, a journey to Aberdeen International Airport from the P&J Live takes about about 5 minutes and costs roughly £6. A journey from the P&J Live centre to the city centre takes roughly 25 minutes and would cost roughly £16.

Fares and journey times will be greater when traffic is heavy. Please ensure you provide plenty of time to book your taxi.



Bus Services

AIRPORT BUS SERVICES

The Jet Service 727 will take you direct from Aberdeen Union Square bus station to Aberdeen Airport terminal. The fleet of Jet 727 buses feature contactless payments, leather seats, free wi-fi and USB charging available onboard.

The service runs on a 10-minute frequency throughout the day Monday - Friday and up to every 20 minutes at the weekend. Running 7 days a week the service starts between 03:00 and 03:29 Monday – Sunday. The last bus leaves the airport at 00:10 from Monday to Sunday.

www.stagecoachbus.com

Fares: correct at time of publication, pricing may vary

- **Aberdeen City Dayrider (valid for unlimited travel on service 727 all day):** Adult- £4.90 / Student - £3.70
- **Aberdeen City 7 Day Megarider (valid for 7 days):** Adult- £16.40 / Student - £12.30

How to Pay: Cash (change given), Contactless Card Payments, Mobile tickets with Stagecoach Bus App: www.stagecoachbus.com/promos-and-offers/national/stagecoachbusapp

GENERAL INFORMATION

TRANSPORT & FURTHER INFORMATION



Currency and Banks

The official currency of the UK is £ sterling. Commonly accepted credit cards are Visa and Mastercard. Display signs are usually visible in all restaurants and shops indicating which cards they accept.

There is a range of banks in Aberdeen City Centre with ATMs. Most banks are only open until early afternoon on Saturdays and closed all day Sunday. There is no ATM at P&J Live.

Restaurants

A selection of restaurants, eateries and bars can be found on VisitAberdeenshire's website www.visitabdn.com/food-and-drink

Smoking

The Smoking, Health and Social Care (Scotland) Act 2005 made it an offence to smoke, or permit someone else to smoke, within premises which are enclosed or substantially enclosed. Scotland P&J Live is a no-smoking building, this includes the use of e-cigarettes and vaping, but they do have specific outside smoking points for those wishing to smoke outside.

Extending your stay

If you are keen to extend your stay and see what Aberdeenshire and the rest of Scotland has to offer, you can find more information at www.visitscotland.com and www.visitabdn.com





INVITED TALKS

IT01

USING THE OLIGO-MOUSE-MICROBIOTA TO UNDERSTAND GUT MICROBIOTA ECOLOGY, EVOLUTION AND PATHOGEN EXCLUSION

PROFESSOR BÄRBEL STECHER

Ludwig Maximilian University of Munich, Germany



Human gut microbial communities harbor hundreds of bacteria that form complex metabolic networks. Both pair-wise and higher-order interactions are the basis of community functionality. The lack of suitable model systems has limited our current understanding how individual community members shape host-microbiota interactions and resistance to infections. To this end, we are using synthetic bacterial communities that we can study in silico, in vitro and in gnotobiotic mouse models. Our recent data reveal how individual strains within a bacterial consortium interact to influence community composition but also complex microbiome functions such as colonization resistance to pathogen infections and provide necessary insight to develop strategies to steer microbial communities towards beneficial interactions promoting human health.

Biography

B. Stecher is a Professor for Hygiene and Microbiology at the LMU Munich and an expert in microbiota-mediated colonization resistance and the pathogenesis of infections with non-typhoidal *Salmonella*, *E. coli* and related pathogens. Since 2019, B. Stecher is the deputy speaker of the DFG CRC1371 “Microbiome Signatures-Functional Relevance in the Digestive Tract” and coordinator of the DZIF TTU Gastrointestinal Infections since 2022. Her work is internationally recognized by several awards, including the Main Award by the DGHM and an ERC Consolidator Grant (2019). Her group uses synthetic microbial communities and gnotobiotic mouse models to mechanistically probe the interplay between the host, pathogens and gut microbiota. The Oligo-MM, a synthetic microbial community developed from preclinical microbiome research in mice is used by >50 research groups world-wide. B. Stecher’s research also focuses on the mechanisms driving microbiome evolution and transmission of antimicrobial resistance in order to develop novel tools for intervention.

IT02

INTEGRATING MULTI-OMICS AND ENZYMOLOGY TO UNTANGLE SYSTEMS FOR TYPICAL AND ATYPICAL GLYCAN PROCESSING IN GUT MICROBIOMES

DR SABINA LEANTI LA ROSA

*Faculty of Chemistry, Biotechnology and Food Science,
Norwegian University of Life Sciences, Aas, Norway*



The community of microbes inhabiting the gastrointestinal tract includes a large variety of bacterial species that collectively influence numerous aspects of host health and nutrition. Firmicutes and Bacteroidetes phyla are typically dominant, with specific symbiotic members supplying an arsenal of carbohydrate-active enzymes for the depolymerization and fermentation of otherwise indigestible complex carbohydrates to short-chain fatty acids. This talk will present some of our recent research, which utilizes methodological toolsets that combine traditional culturing, meta-omics (including next-generation sequencing and functional multi-omics), biochemistry and enzymology, to fully elucidate enzymatic pathways that microbial populations employ for the utilization of nutrients consumed by the host. This includes typical hemicellulosic polysaccharides, such as β -mannans, and atypical glycans such as the food additive xanthan gum. In particular, coupling of detailed knowledge of microbial saccharolytic mechanisms to unique structural features of β -mannans has allowed us to design intervention strategies to selectively engage beneficial microbes at genera/strain level. Additional application of multi-omics has enabled visualization of the impact of β -mannans on the gut microbiota composition and functions, unveiling interactions between key players in degradation of this fiber directly in complex endogenous animal microbiomes and elucidating mechanisms by which these microorganisms affect host biology. We demonstrate that a multi-faceted approach is needed for deciphering and implementing efforts to enhance host health and minimize disease by manipulating gut microbial composition and metabolism.

Biography

Dr Sabina Leanti La Rosa received her PhD in 2014 from the Norwegian University of Life Sciences, working with Prof. Ingolf F. Nes and Dr Dag Brede on the biology of *Enterococcus faecalis*. She then trained with Barbara E. Murray, MD at the University of Texas Health Science Center in Houston (TX, USA), investigating the genetic and biochemical mechanism of enterococcal pathogenicity. After that, Sabina spent three years in the lab of Assoc. Prof. Bjørge Westereng, focusing on the enzymatic mechanisms through which gut commensal Firmicutes degrade beta-mannans. In 2018, Sabina joined the lab of Prof. Phil B. Pope at the Norwegian University of Life Sciences (the Microbial Ecology and Meta-omics group); her current research interests include

- 1) investigating the ability of diet- and wood-derived carbohydrates to modulate the composition and metabolic output of the gut microbiota in humans, monogastric farm animals and fish;
- 2) the use of multi-omic tools to decrypt mechanistic connections between diet, host and its microbiome;
- 3) the mechanism through which gut bacteria break down food additives and human milk oligosaccharides.

IT03

IMPACT OF DIET ON MICROBIAL METABOLISM IN INFANTS AND ADULTS

PROFESSOR TINE RASK LICHT

National Food Institute, Technical University of Denmark



The complex bacterial community inhabiting the human intestine feeds on nutrients provided either by cross-feeding on lysed bacteria, by the host through shedding of epithelial cells and mucus, or by ingested compounds that escape host digestion and reach the gut microbes. While a lot of knowledge has now been gained about the influence of diet on the compositional structure of the bacterial population residing within us, we still know less about the output of the processes related to bacterial conversion of ingested nutrients.

Short Chain Fatty Acids, generated by bacterial metabolism of dietary fibres are well known mediators of host-microbe interactions. However, also other groups of metabolites are likely to play key roles. In recent years, a particular interest in bacterial metabolites derived from aromatic amino acids has emerged, due to their suggested involvement in a long range of interactions relevant for host health and disease.

Evidently, the generation of aromatic microbial metabolites is impacted by the abundance of producing microbes, as well as by the availability of aromatic substrates. However, both of these factors are influenced by complex interactions between dietary components and the intestinal microbiome. Here, I will present some of our findings related to microbial metabolism of aromatic amino acids, and introduce some key factors governing these particular metabolic activities within the bacterial community residing in the gut of infants and adults, respectively.

Biography

Professor Tine Rask Licht is Deputy Head of the National Food Institute, Technical University of Denmark, and leader of the Research Group on Gut, Microbes and Health at this institute. She graduated as PhD in Molecular Microbial Ecology in 1997, and most of her research has revolved around the microbial ecology of the gut.

Today, her group focuses on effects of diet on the intestinal bacterial community, which they study in humans, animal models, and in vitro model systems. The research group has contributed significantly to the understanding of the role of human milk and complementary diet on establishment of the microbiota in young infants, and of the impact of the metabolic activities of specific bacteria on human health. Also, her group identified the link between intestinal transit time and bacterial metabolism in the human gut, and highlighted the impact of human donor variability in faecal transplantation studies with gnotobiotic mice.

From 2012 to 2018, Prof. Licht was heading the research center 'Gut, Grain and Greens', and she is currently heading the major research effort '**PRIMA**' - towards **P**ersonalized dietary **R**ecommendations based on the **I**nteraction between diet, **M**icrobiome and **A**biotic conditions in the gut, funded by the Novo Nordisk Foundation. From 2018 to 2022, she was chair of the panel of Global Grants for Gut Health, supported by Yakult and Nature Research.

IT04

ANTIMICROBIAL RESISTANCE IN THE HUMAN GUT MICROBIOME

PROFESSOR WILLEM VAN SCHAIK

*Institute of Microbiology and Infection,
University of Birmingham, United Kingdom*



The human gut microbiome acts as a reservoir for antimicrobial resistance genes (ARGs), collectively known as the gut resistome. While shotgun sequencing, with either short- or long-read technologies, can determine the presence and abundance of ARGs, it remains a challenge to confidently resolve the microbial hosts of ARGs located on mobile genetic elements. We have used proximity ligation techniques, (meta3C and Hi C), to link bacterial genes to phylogenetic markers can be used to determine ARG-host linkage.

We implemented Hi C to investigate the bacterial hosts of ARGs in 4 human faecal samples. Using binning techniques on metagenomic assemblies from these samples, combined with Hi C data, we were able to link 87 ARGs to their hosts across the 4 samples, out of a total of 119 ARGs identified by shotgun sequencing. ARGs carried on plasmids in an *Acinetobacter pittii* strain that was used as a spike in were correctly linked to their host in all samples. We found that Hi-C was able to link ARGs to multiple contigs in each metagenomic assembly, and the main limiting factor in identifying the bacterial hosts of the ARGs was the success of the binning process and the ability to taxonomically classify the bins.

Following Hi C analysis, hosts of ARGs were enriched by growing in media with antibiotics, isolated (n=20) and whole genome sequenced with a combination of short- and long-read techniques. Genome sequence analyses showed that these strains contained 54 ARGs in total. Of these, 9 ARGs were associated with plasmids and 45 with the chromosome, with evidence for association with Integrative Conjugative Elements for 21 of these. While there was overlap between culture-based and Hi-C approaches, both methodologies also identified ARG-host links that were not uncovered by the alternative approach, highlighting the complementarity of Hi-C and culture-based approaches to fully characterise the gut resistome.

Biography

Professor Willem van Schaik obtained his MSc and PhD degrees at Wageningen University in the Netherlands under the supervision of Professor Tjakko Abee and Professor Willem de Vos. He then did post-doctoral studies on an EMBO Long-Term Fellowship at the Pasteur Institute (Paris, France) on the regulation of virulence gene expression of *Bacillus anthracis* in the laboratory of Dr Agnès Fouet. He then moved to the University Medical Center Utrecht in the Netherlands, where he worked on the comparative and functional genomics of several multi-drug resistant opportunistic pathogens. His group also worked on the gut microbiome of critically ill patients that receive intensive antibiotic treatment.

In 2017, he moved to the University of Birmingham, where he was appointed as Professor of Microbiology and Infection. In the same year, he was awarded a Royal Society Wolfson Research Merit Award. His group studies the role of commensal bacteria in the human gut microbiome as reservoirs of antibiotic resistance genes and the evolution of mobile genetic elements carrying antibiotic resistance genes in opportunistic pathogens. Prof Van Schaik has collaborations with researchers in China and Bangladesh to study the spread of antibiotic-resistant bacteria in low- and middle-income countries. He has been Director of the University of Birmingham's Institute of Microbiology and Infection since Summer 2020

IT05

FROM BENCH TO BEDSIDE: THE JOURNEY OF ENTERBIOTIX

DR LYNSEY HOWARD

EnteroBiotix, Scotland, United Kingdom

EnteroBiotix Limited (EBX) is a patient centred biopharmaceutical company which harnesses microbiota derived directly from the human gastrointestinal tract to treat a broad range of serious diseases and disorders linked to the gut microbiome. On the development journey to create its lead product candidate EBX-102, the company created a bespoke donor management platform (Number2), which rigorously screens healthy individuals to generate a high diversity whole community therapeutic, which then utilises a rapid drying process (AMPLA) to create a stable and convenient product presentation for oral delivery. Through extensive Research and Development work, EBX has developed a suite of best-in-class analytical and manufacturing methods, as well as leveraged know-how into GMP-controlled manufacture of our therapeutic under MHRA license. To evaluate EBX-102 for the treatment of liver cirrhosis and hepatic encephalopathy, EnteroBiotix has initiated a Phase II clinical trial named IMPuLCE. This trial will enrol participants across sites in the UK and evaluate EBX-102 in patients with cirrhosis and HE being treated with standard of care medications.

Biography

Dr Lynsey Howard has over 10 years of experience in the strategy, design and implementation of innovative biopharmaceutical Research along with team leadership, business development and senior management.

Lynsey originally completed her BSc (Hons) in Virology from the University of Glasgow, where she subsequently completed a PhD in novel stem cell and gene therapies for cardiovascular disease within the Institute of Cardiovascular and Medical Sciences. Following this, Lynsey took up a Post-Doctoral Researcher position at the University of Bristol working in the Bristol Heart Institute.

Prior to joining EnteroBiotix, Lynsey was Head of Preclinical Research and Development at Lamellar Biomedical where she created extensive internal and external scientific capability in polymicrobial infection biology, antimicrobial development, respiratory disease, gene therapy, and immunology. Lynsey directed several collaborations with world-leading institutes and commercial partners, as well as led an in-house team of multidisciplinary scientists. Lynsey's work generated valuable intellectual property leading to multiple patent submissions.

At EnteroBiotix, Lynsey heads up the Research team and also performs the role of Biosafety Officer, ensuring the innovative testing and characterisation strategies for all products are best-in-class to create safe and effective medicines for those patients who are living with microbiome-mediated health conditions.

IT06

LEVERAGING LACTOBACILLI AGAINST RESPIRATORY VIRAL DISEASES

DR IRINA SPACOVA

University of Antwerp, Belgium

Globally, viral respiratory tract infections are a leading cause of morbidity and mortality. Clinical studies suggest a link between lactic acid bacteria in the gut and airway microbiome, and respiratory health. Probiotics for the gastrointestinal tract show promise in the prevention of viral respiratory tract infections, and recently the topical application of probiotic lactobacilli in the upper airways is emerging as a protective strategy with local benefits.

Probiotic lactobacilli could impact the course of viral infection through a range of potential mechanisms of action, some of which are strain-specific (Spacova *et al.*, 2021, Trends in Molecular Medicine). These mechanisms include stimulation of mucosal antiviral immune responses, respiratory epithelial barrier maintenance and microbiome modulation, as well as direct viral binding and inhibition of viral infectivity.

To leverage the potential antiviral activity of lactobacilli in the clinic, we have implemented a pipeline to select *Lactobacillaceae* strains based on their safety, applicability and antiviral functionality. Three selected strains *Lacticaseibacillus casei* AMBR2, *Lacticaseibacillus rhamnosus* GG and *Lactiplantibacillus plantarum* WCFS1 were formulated in a throat spray, which was applied in a randomized double-blind placebo-controlled trial in COVID-19 primary care patients. Lactobacilli abundances in nose-throat swabs increased during spray use, and after 3 weeks a trend was observed towards lower SARS-CoV-2 viral loads in the swabs of the treatment group vs. the placebo group. These results show promise for rational selection of lactobacilli to prevent or treat respiratory viral diseases in clinical settings.

Biography

Dr Irina Spacova is a senior researcher at the University of Antwerp, Belgium. She obtained her Bachelor's degree in Biology from Moldova State University and her Master's degree in Bioscience Engineering from KU Leuven, Belgium. Her subsequent PhD dissertation at KU Leuven and the University of Antwerp unraveled the mechanisms through which probiotic lactic acid bacteria prevent asthmatic inflammation. Part of her postdoctoral research work on antimicrobial and immunostimulatory activity of lactic acid bacteria was conducted at the University of Manchester (UK), Seattle Children's Research Institute and the University of Washington (USA).

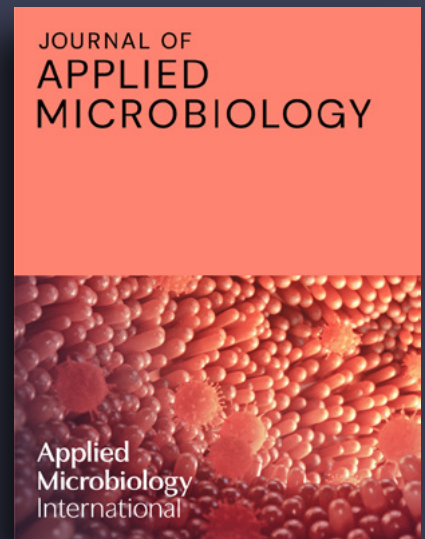
Currently, Dr Irina Spacova investigates how bacteria within the airway and environmental microbial communities modulate common urban respiratory diseases, both experimentally and in clinical trials. Her ongoing research projects funded by the Research Foundation Flanders (FWO) and the University of Antwerp focus on direct interactions of beneficial microbiota members with respiratory viruses, as well as microbial immunomodulation at mucosal surfaces. She has received several awards for her research, including the International Scientific Association for Probiotics and Prebiotics (ISAPP) Early Career Researcher prize in 2021.

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OFFERED TALKS

OT01 | TOWARDS A BETTER DESCRIPTION OF THE INTERACTIONS OF FOOD-BORNE PATHOGEN ENTEROTOXIGENIC ESCHERICHIA COLI WITH INTESTINAL MUCUS AND HUMAN MICROBIOME

Dr Thomas Sauvaitre^{1,2}, Dr Josefien Van Landuyt², Mr Claude Durif¹, Dr Charlène Roussel³, Dr Adeline Sivignon⁴, Dr Ophélie Uriot¹, Dr Florence Van Herreweghen², Prof Tom Van de Wiele², Prof Stéphanie Blanquet-Diot¹,

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⁴Université Clermont Auvergne, UMR 1071 Inserm, USC-INRAE 2018, Microbes, Intestin, Inflammation et Susceptibilité de l'Hôte (M2iSH), Clermont-Ferrand, France

Background: The intestinal mucus layer has a dual role in human health constituting a well-known microbial niche that supports gut microbiota maintenance, but also acting as a physical barrier against enteric pathogens. Enterotoxigenic Escherichia coli (ETEC), the major agent responsible for traveler's diarrhea, is able to bind and degrade intestinal mucins, representing an important but understudied virulent trait of this pathogen.

Objectives: Our work aimed to describe how the mucus microenvironment could shape different aspects of the human ETEC pathophysiology.

Methods: Using a set of complementary in vitro approaches recapitulating the human digestive environment, we investigated the survival, adhesion, virulence gene expression, interleukin-8 induction and interactions with human gut microbiota of the human ETEC reference strain H10407.

Results: Using the TNO gastrointestinal model (TIM-1) simulating the physicochemical conditions of the human upper gastro-intestinal tract, we report that mucus secretion and physical surface sustained ETEC survival facing the upper gastrointestinal tract stresses. The integration of the host part through the Caco2/HT29-MTX co-culture model demonstrated that mucus secreting-cells favored ETEC adhesion and virulence gene expression, without impeding ETEC-induced inflammation. Furthermore, we show that the presence of a mucin-matrix tended to reduce ETEC colonization in a complex gut microbial background simulated by fecal batch experiments. Mucus-specific microbiota was also widely modified upon ETEC challenge suggesting a role in the pathogen infectious cycle. Using multi-targeted in vitro approaches, our work supports the major role played by mucus in ETEC pathophysiology, opening avenues in the design of new treatment strategies.

OT02 | COMPARATIVE ANALYSIS OF 4 ENTEROPATHOGENS IN MICE WITH COMPLEX AND OligoMM12 MICROBIOTA

Mr Mathias Klaus-Maria Herzog¹, Audrey Peters⁴, Nizar Shayya⁵, Monica Cazzaniga^{2,3}, Trisha Arora^{8,9}, Kardokh Kaka Bra², Marcus Claesson^{2,3}, Xavier Domingo-Almenara^{8,9}, Cormac G.M. Gahan^{2,3,7}, Markus M. Heimesaat⁵, Stefan Bereswill⁵, Gad Frankel⁴, Wolf-Dietrich Hardt¹

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Studies in experimental infectious disease research typically focus on one pathogen at a time. Consequently, the ability to compare results across infection models or pathogens is often limited. In an effort to identify universal principles in colonization resistance and disease mechanisms, we have compared the infection kinetics and pathology of four entero-pathogens (*Campylobacter jejuni*, *Citrobacter rodentium*, *Listeria monocytogenes*, *Salmonella enterica* serovar Typhimurium) in the same animal facility using two types of C57BL/6J mice that differ in their microbiome composition. Specifically, we tested C57BL/6 mice with a complex specific pathogen free (SPF) microbiota and gnotobiotic C57BL/6 mice associated with the oligoMM12 microbiota. Upon orogastric infection, we analyzed pathogen loads in stool and internal organs and assessed tissue histopathology. We observed striking differences in colonization resistance comparing the defined/low complexity (oligoMM12) model to the SPF model. All pathogens were able to colonize and trigger various degrees of inflammation in the oligoMM12 model, but only *Citrobacter rodentium* could elicit pathology in unperturbed SPF mice. These data are currently complemented with analyses of microbiome changes over time (16S), stool inflammation markers, host tissue RNAseq, as well as metabolomics. Our results suggest that the oligoMM12 model can be used as a universal model to compare enteropathogen infection and the influence of the microbiome composition on colonization resistance against all four pathogens.

OT03 | AN IBD-ASSOCIATED PATHOBIONT COLLABORATES WITH THE NSAID TO PROMOTE INFLAMMATION AND CELL DEATH IN A SUSCEPTIBLE HOST VIA THE CASPASE-8/NLRP3 AXIS

Mr Raminder Singh^{1,2}, Dr Valerio Rossini¹, Dr Stephen R. Stockdale¹, Mr Gonzalo Saiz-Gonzalo^{1,2,3}, Ms Naomi Hanrahan^{1,2}, Ms Tanya D' Souza¹, Ms Lorraine A. Draper¹, Dr Colin Hill^{1,4}, Dr Ken Nally^{1,3}, Dr Fergus Shanahan¹, Dr Stefan Andersson-Engels^{5,6}, Dr Silvia Melgar¹

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²Department of Medicine, School of Medicine, University College Cork, Cork, Ireland,

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⁴School of Microbiology, University College Cork, Ireland,

⁵Department of Physics, University College Cork, Ireland,

⁶Irish Photonics Integration Centre, Tyndall National Institute

Non-steroidal anti-inflammatory drugs (NSAIDs) are believed to exacerbate inflammation in patients with inflammatory bowel disease (IBD), but the mechanisms regulating NSAID-induced symptoms are unknown. Pathobionts such as adherent-invasive *Escherichia coli* (AIEC) are widely prevalent in the mucosa of patients with Crohn's disease (CD) and are considered relevant to CD pathogenesis. The inflammasomes such as NLRP3 are implicated in the maintenance of gut immune homeostasis and gut injury. Caspase-8 is a protein regulating programmed cell death, intestinal homeostasis, and inflammation. We hypothesise that the presence of AIEC might explain the NSAID-induced symptomatic worsening in IBD. Using IL-10^{-/-} mice, we show an aggravation of colitis in AIEC-colonised mice fed on an NSAID supplemented diet accompanied by activation of the NLRP3 inflammasome, caspase-8 and cell death executors, e.g., caspase-3, PARP and Gasdermin-D. However, IL-10^{-/-} mice colonised with AIEC alone or fed an NSAID supplemented diet alone did not develop colitis, highlighting the synergistic effect of both AIEC and the NSAID. Using small-molecule inhibitors targeting NLRP3 and caspase-8, we show an amelioration in colitis due to a reduction in pro-inflammatory cytokines, M1-macrophages, cell death (apoptosis/pyroptosis) and improved histology. 16S rRNA gene analysis identified an increased fecal abundance in *Clostridium* cluster XIVa species in both inhibitor-treated groups. In conclusion, our findings provide evidence and mechanistic insights into how NSAIDs and an opportunistic CD-associated gut pathobiont can synergise to worsen IBD symptoms and inflammation. The data suggest that targeting the caspase-8 and NLRP3 axis could be a potential therapeutic strategy for IBD patients with NSAID-worsened inflammation.

OT04 | REVEALING THE CAUSAL RELATIONSHIP BETWEEN AGE-ASSOCIATED IMMUNE REMODELLING AND CHANGES IN THE GUT MICROBIOME

Ms Selina Stahl¹, Prof. David B. Haslam², Prof. Hartmut Geiger¹

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²*Cincinnati Children's Hospital, Cincinnati, USA*

As individuals age, the gut microbiome undergoes significant shifts in its diversity and composition, which have been linked to various aging-related tissue dysfunctions and diseases. In order to gain a better understanding of the underlying factors that drive these changes, it is crucial to investigate the relationship between the aging immune system and the gut microbiome. We test the hypothesis that age-related changes in the frequency or function of immune cells in the intestine might be, at least in part, responsible for an altered composition of the gut microbiome. To test this hypothesis, we used an animal model in which we were able to generate an aged or young adaptive immune system in otherwise young mice (young RAG1^{-/-} hosts transplanted with aged or young HSCs). We demonstrated, via metagenome sequencing, that the presence of a young or old immune system resulted indeed in a distinct microbial composition within the gut. Furthermore, our data showed that the presence of an old immune system was associated with a significant decrease in the abundance of microbes that are capable of producing or salvaging vitamin B6, while we could confirm reduced levels of vitamin B6 in serum of aged mice. These results suggest that aging of the immune system may contribute to changes in the microbial composition upon aging which in turn results in reduced vitamin B6 availability in old age. Our findings thus imply a critical connection between the aging immune system, the gut microbiome and changes in levels of vitamins.

OT05 | NOVEL MOLECULAR MECHANISMS BY COMMENSAL BACTERIA TO INFORM IMMUNOMODULATORY MICROBIOME THERAPIES

Mr Selvin Solis¹, Dr Elaina Maldonado¹, Dr Carlos Maluquer de Motes¹, Dr Subhankar Mukhopadhyay²,
Dr Jorge Gutierrez-Merino¹

¹University of Surrey, Guildford, United Kingdom,

²King's College London, London, United Kingdom

The discovery of the human microbiome has revolutionized the field of Medicine. To date, we have access to endless data informing of the microbial diversity present in different organs and body systems, and how this diversity correlates with many medical conditions. However, we are still unsure which of the many commensal microbes that reside the host are the main drivers that restore or protect health from disease. Much little information is concerning the molecules that these key commensals possess to interact with the immune system. In this respect, we have recently reported that certain species of lactobacilli significantly activate the production of type I interferon (IFN-I) cytokines in macrophages, and that this IFN-I activation is predominantly driven by cytosolic DNA sensors. IFN-I cytokines are essential to confer protection against microbial infections and auto-immune disorders. Furthermore, we have observed that lactobacilli encode some surface adhesin proteins with the potential to interact with macrophages for subsequent phagocytosis via non-opsonic scavenger receptors. Therefore, we are focused on determining the role that adhesins play as a port of entry in macrophages and characterize the IFN-I-mediated intracellular signalling initiated by DNA sensing. Elucidating these unknown mechanisms will be important to inform on how specific molecules of commensals modulate or stimulate host responses that, in unhealthy individuals, are exacerbated or inhibited. Overall, our studies will provide a better understanding on the molecular crosstalk between the microbiome and mammalian cells, paving the way for major therapeutic discoveries.

OT06 | DESIGNING SYNTHETIC COMMUNITIES USING A FUNCTIONALLY-GUIDED APPROACH COMPLEMENTED WITH METABOLIC MODELLING**Dr Thomas Hitch**¹, Prof Thomas Clavel¹¹*University Hospital RWTH Aachen, Aachen, Germany*

Host-associated microbiota consist of hundreds of species, each containing thousands of proteins, making them functionally complex. While a few species dominate most microbiota profiles, species occurring at lower abundances are known to contribute important functions. Synthetic communities (SynComs) are currently designed based on the knowledge of those designing it, or using limited functional information to guide design, making each time intensive to create and taxonomically restricted to those the designers consider suitable. To solve this, we developed an automated system for the creation of SynComs based on the functional repertoire of either an individual, or group of input metagenomic samples. The environments profile is then compared to that of a collection of isolates.

This approach involves four major steps; firstly, functions deemed 'core' within the input samples of interest, or differentially present between the groups being studied, are weighted to enhance selection. Secondly, isolates are shortlisted based on the frequency of their selection during sequential creation of SynComs. Thirdly, all combinations of the shortlisted bacteria are compared functionally to the input metagenomes and scored based on the functional repertoire of each community. The final step involves metabolic modelling of the potential SynComs to predict their stability.

Using this method, we can develop SynComs which are condition-specific (healthy Vs diseased), or representative of an entire ecosystem (the human gut) for use as a research model or next-generation probiotics.

OT07 | FAECAL MICROBIOME MEDIATES THE EFFECT OF DIET ON COLORECTAL-CANCER RISK: COMPARISON OF MEAT BASED VERSUS PESCO-VEGETARIAN DIETS

Miss Camille Etienne¹, Dr Carlotta De Filippo², Dr Francesco Vitali², Dr Françoise Guéraud³, Dr Clara Della Croce², Dr Vincenzo Longo², Miss Claire Maudet¹, Dr Lisa Giovanelli⁴, Dr Fabrice Pierre³, Dr Giovanna Caderni⁴,

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Two validated models of colorectal cancer (CRC): Pirc rats, mutated in the Apc gene and thus developing spontaneous colon tumours and Azoxymethane (AOM)-induced rats were fed for 3 months with high-CRC risk diet (meat-based MBD), a normalized CRC risk diet (MBD plus alpha-tocopherol, MBD-T), a low-CRC risk diet (pesco-vegetarian, PVD) and a control diet (CTRL). Pirc rats fed the PVD diet, showed a significantly lower number of colon tumors than rats fed all the other diets. In the AOM-treated rats Mucin Depleted Foci (MDF) were smaller with the PVD and CTRL diets than with the two meat-based diets. Oxidative stress parameters such as fecal TBARS, urinary DHN-MA and urinary 8-iso-PGF 2 α were lower in PVD than in Meat-based diets-fed rats. Microbiota analysis using 16s rRNA sequencing showed that bacterial communities significantly differed based on diet, with the exception of MBD and MBD-T samples which were similar. To determine if the microbiome contributes to the different tumorigenesis associated with the diets, feces from Pirc rats fed the 4 different diets were thus transplanted into AOM-induced germ-free rats fed a control diet for three months. Strikingly, rats transplanted with the MBD-feces had the highest number of MDF compared with all the other diets. In conclusion these results confirm the carcinogenetic activity of MBD- diets and the protective properties of PVD diet. Our results further demonstrate that these impacts of the different diets on carcinogenicity are, at least in part, mediated by the intestinal microbiome.

OT08 | THE MOLECULAR BASIS OF THE DEGRADATION OF FLAVAN-3-OLS BY THE HUMAN GUT BACTERIUM EGGERTHELLA LENTA

Dr Ruben Halifa¹, Dr Agnes Cornu², Dr Claire Dufour³, Dr Carine Le Bourvellec³, Prof. Pierre Peyret¹,
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Flavan-3-ols are among the most consumed polyphenols by humans and have been shown to prevent cardiovascular diseases. They are found in plant as monomers and mainly as oligomers such as procyanidins. The majority reaches the colon where the microbiota converts them into phenolic metabolites likely to participate in their health effects. While the metabolic pathways of the degradation of flavan-3-ols by the microbiota are relatively well described, only a few microorganisms and microbial genes involved in these pathways are known. We have previously shown that *Eggerthella lenta* metabolizes flavan-3-ol monomers and oligomers. Here, our aim was to identify *E. lenta* genes encoding enzymes degrading flavan-3-ols and to determine their prevalence in human gut metagenomes.

By a transcriptomic approach (RNAseq) carried out with the type strain of *E. lenta*, coupled with the heterologous expression of the genes of interest in *Escherichia coli*, we have discovered two genes (*fmber1*, *fmber2*) encoding two benzyl ether reductases cleaving the C ring of the monomers and an operon of two genes (*pber*) catalyzing this reaction on the dimers of type-B procyanidins. Furthermore, two operons of three genes (*cadh*, *ecadh*) encoding enzyme complexes dehydroxylating the B-ring of (+)-catechin and (-)-epicatechin have also been identified. These genes constitute good markers of flavan-3-ol metabolization in the gut. Their prevalence in human gut metagenomes suggested that 27% of individuals cannot convert flavan-3-ols into potential bioactive metabolites. These results raise the question of whether individuals who do not harbor these bacterial genes are at greater risk of developing cardiovascular disease.

OT09 | p-CRESOL DERIVATIVES INTERACT WITH THE BLOOD–BRAIN BARRIER AND HIGHLIGHT THE COMPLEX NATURE OF MICROBIOTA–HOST COMMUNICATION PATHWAYS ASSOCIATED WITH THE GUT–BRAIN AXIS**Prof Lesley Hoyles¹**, Dr Simon McArthur¹¹*Nottingham Trent University, Nottingham, United Kingdom*

Microbial fermentation of amino acids in the large intestine leads to the production of metabolic end-products that can interact with the host at the intestinal and systemic levels. End-products of tyrosine and phenylalanine fermentation include p-cresol. This microbiota-derived metabolite undergoes conjugation in enterocytes and the liver, reaching the systemic circulation as p-cresol sulfate (pCS) and p-cresol glucuronide (pCG). Metabolically healthy individuals can clear pCS and pCG efficiently, but in patients with kidney disease these metabolites accumulate within the blood. pCS is thought to contribute to the impaired cognitive function frequently observed in these individuals.

In vitro and in mice, physiologically relevant levels of pCG prevented the blood-brain barrier (BBB)-permeabilizing effects of endotoxin, acting by antagonizing the LPS receptor TLR4. In contrast, physiologically relevant levels of pCS increased paracellular permeability and disrupted intercellular tight junctions. pCS changed the whole-brain transcriptome, suppressing neuronal activity, transcription and mitochondrial respiration pathways. It also stimulated the epidermal growth factor receptor (EGFR), leading to mobilization of matrix metalloproteinase (MMP)-2/9. In vivo, the deleterious effects of pCS on the BBB were prevented by the EGFR antagonist erlotinib or the MMP2/9 inhibitor SB-3CT. Human hCMEC/D3 endothelial cells exposed to serum from haemodialysis patients, but not from healthy donors, showed an erlotinib-sensitive increase in paracellular permeability that correlated with the total serum pCS content. These data demonstrate the complexity of microbial metabolite–host communication pathways underlying the gut–brain axis, and identify means by which microbiota-associated metabolites can be targeted to improve brain function.

OT10 | INTER-INDIVIDUAL DIFFERENCES IN SEGMENTAL TRANSIT TIME AND pH ARE LINKED TO THE HUMAN GUT MICROBIOME COMPOSITION AND METABOLISM

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⁵VIB Center for Microbiology, Leuven, Belgium

The origin of inter-individual variability in the human gut microbiome is poorly understood. Besides diet, gut environmental factors such as pH and transit time may contribute to variations in gut microbial composition and metabolism.

Here, we conducted a 9-day observational study with 61 healthy participants that included daily registration of participants' habitual diet and bowel habits, daily collection of faecal and urine samples, and two study visits. Segmental transit times and pH throughout the gut were assessed by SmartPill® capsules in 50 participants on day 2 following a standardized breakfast. The urinary and faecal metabolomes were obtained via untargeted liquid chromatography mass spectrometry-based metabolomics whereas the faecal microbiota and bacterial cell counts were assessed by 16S rRNA gene sequencing and flow cytometry, respectively.

The longitudinal measurements showed that faecal pH was a strong individual trait that remained surprisingly stable over the 9 days (CV 2 %). Moreover, large inter-individual variation in segmental transit times and pH including colonic transit times (CTT, 3-64 hours) and colonic pH were observed. Notably, CTT explained 9 % of the inter-individual variation in the urine metabolome. Similarly, stool moisture (a proxy of transit time) explained 3.1 % and 3.6 % of intra-individual variation in the urine metabolome and gut microbiome, respectively. We also identified several microbial-derived metabolites associated with CTT and pH including urinary phenylacetylglutamine. Altogether these results suggest that inter-individual variations in transit time and pH contribute to individual differences in gut microbial metabolism, which may be important for understanding personal responses to foods.

OT11 | OLIGOFRUCTOSE IMPROVES SMALL INTESTINAL LIPID-SENSING MECHANISMS via ALTERATIONS TO THE SMALL INTESTINAL MICROBIOTA

Miss Savanna Weninger¹, Miss Chloe Herman², Dr Greg Caporaso², **Dr Frank Duca**¹

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Upper small intestinal dietary lipids activate a gut-brain axis regulating energy homeostasis that is impaired by high-fat (HF)-feeding. The prebiotic, oligofructose (OFS) improves body weight and adiposity during metabolic dysregulation but the exact mechanisms remain unknown. Furthermore, the role of the small intestinal microbiota has been largely overlooked, despite the physiological relevance of small intestinal nutrient sensing mechanisms. Lastly, no study has examined the impact of OFS on small intestinal microbiota. This study examines whether alterations to the small intestinal microbiota following OFS treatment improve small intestinal lipid-sensing to regulate food intake in HF-fed rats. In rats fed a HF diet for 4 weeks, OFS supplementation decreased food intake and meal size within 2 days, and reduced body weight and adiposity after 6 weeks. Acute (3 day) OFS treatment restored small intestinal lipid-induced satiation during HF-feeding, and was associated with increased small intestinal CD36 expression, portal GLP-1 levels and hindbrain neuronal activation following a small intestinal lipid infusion. Transplant of the small intestinal microbiota from acute OFS treated donors into HF-fed rats also restored lipid-sensing mechanisms to lower food intake. 16S rRNA gene sequencing revealed that both long and short-term OFS altered the small intestinal microbiota, increasing *Bifidobacterium* relative abundance. Small intestinal administration of *Bifidobacterium pseudolongum* to HF-fed rats improved small intestinal lipid-sensing to decrease food intake. In conclusion, OFS supplementation rapidly modulates the small intestinal gut microbiota, which mediates improvements in small intestinal lipid sensing mechanisms that control food intake to improve energy homeostasis.

OT12 | THE IMPACT OF SEAWEED-BASED NUTRITIONAL MANIPULATION ON THE RUMEN MICROBIOME

Ms Alessandra Ferrillo¹, Dr Arturo Vera-Ponce De León¹, Ms Katrine S. Eikanger⁴, Dr Alemayehu Kidane⁴, Dr Cassie Bakshani², Prof William Willats², Mr Emil Sørensen³, Mr Mantas Sereika³, Prof Mads Albertsen³, Prof Phillip Pope¹, Dr Live Hagen¹

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Over the last centuries, methane concentration in the atmosphere has increased exponentially, largely caused by human-related activities. A significant portion of methane emission derives from agriculture, specifically from livestock production. In the rumen of cattle, enteric methane is generated by methanogens which metabolize intermediate metabolites that result from microbial fermentation of plant fibres. Different strategies have been tested to mitigate enteric methane emissions. One of the emerging strategies is the utilization of certain types of red seaweed that contain halogenated compounds (e.g., bromoform) that inhibit key enzymes used by the methanogens during methane synthesis. However, seaweed-based nutritional manipulation is still relatively unexplored, and the effects on rumen microbiome function and host performance are largely unknown. In this context, our overarching goal is to explore how the rumen microbial community responds to red seaweed (*Asparagopsis taxiformis*) added to the feed, particularly seaweed fractions that inhibit methane production. Herein, we report *in vivo* seaweed trials in Norwegian Red dairy cattle. Integrated meta'omics analysis including metagenomics and metaproteomic, of the rumen microbiome, reveal functional important microbial populations through the recovery of metagenome-assembled genomes (MAGs) and their detected protein expressions. The meta'omics data is further linked to comprehensive host-related metadata, including enteric gas production, rumen volatile fatty acids and plant polysaccharide profiles, as well as livestock production measures (e.g., milk composition). We additionally explore under-reported aspects of rumen microbiota such as micro-eukaryotes (i.e., protozoa), providing greater insights into rumen microbiome populations and their functional roles in feed conversion and methane metabolism.

OT13 | RESISTOME CHARACTERIZATION IN NEONATAL CALVES AND THEIR ENVIRONMENTS ON DAIRY FARMS: IMPLICATIONS FOR AMR SURVEILLANCE AND RISK MITIGATION

Dr Katie Lawther¹, Dr Fernanda Godoy Santos¹, Dr Gillian Scoley², Dr Francesco Rubino¹, Dr Linda Oyama², Miss Lucy Dillon¹, Dr Steven Morrison², Dr Nicholas Dimonaco¹, Mr Aaron Brown², Dr Chris Creevey¹, Dr Sharon Huws¹

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One of the biggest threats to both animal and human health is antimicrobial resistance (AMR), which requires a One Health approach to overcome it. Here we aimed to contribute to AMR surveillance within dairy farms by characterising the resistomes associated with neonatal calves and their associated environments.

Ten dairy farms were surveyed for >90 parameters including hygiene practices and antibiotic usage. Both culture-based and culture independent techniques including shotgun metagenomic sequencing were used to characterise the resistomes present in calf faeces and calf house environment including feed equipment, calf feed and milk fed to calves. Samples were tested in vitro for phenotypic AMR against seven antibiotic classes: penicillins, macrolides, phenicols, aminoglycosides, tetracyclines, synthetic and polymyxins. Resistant bacteria were isolated and underwent 16S rRNA gene and genome sequencing, and multidrug resistance (MDR) testing.

The study revealed high AMR diversity and abundance in calf houses, metagenomics showed high resistance gene abundances within faecal samples, with transcripts per million values reaching 13,085, while resistant bacterial CFU/mL reached 9.57e8. Phenotypic resistance to 6 antibiotic classes was detected on all farms, with resistance against neomycin and trimethoprim being highest. Of the 84 anaerobic AMR isolates 16S sequenced, 66 isolates (78.6%) were MDR, while 36.0% of the 75 aerobic isolates were MDR.

This study emphasises the importance of animals and dairy calf houses as reservoirs of AMR, as well as the AMR risks associated with farming practices, including sanitation and milk replacer utilisation, that may contribute to high AMR levels in calf gastrointestinal tracts.

OT14 | RiboTaxa: COMBINED APPROACHES FOR rRNA GENES TAXONOMIC RESOLUTION DOWN TO THE SPECIES LEVEL FROM METAGENOMICS DATA REVEALING NOVELTIES

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Metagenomic classifiers are widely used for the taxonomic profiling of shotgun metagenomic data and estimation of taxa relative abundance. Small subunit rRNA genes are nowadays a gold standard for phylogenetic resolution of complex microbial communities, although the power of this marker comes down to its use as full-length. We benchmarked the performance and accuracy of rRNA-specialized versus general-purpose read mappers, reference-targeted assemblers and taxonomic classifiers. We then built a pipeline called RiboTaxa to generate a highly sensitive and specific metataxonomic approach. Using metagenomics data, RiboTaxa gave the best results compared to other tools with precise taxonomic identification and relative abundance description without false positive detection. Using real datasets from various environments (ocean, soil, human gut) and from different approaches (metagenomics and gene capture by hybridization), RiboTaxa revealed microbial novelties not seen by current bioinformatics analysis opening new biological perspectives in human and environmental health.

Applied to 62 metagenomics data from a study on human gut and extreme longevity, RiboTaxa revealed a richer microbial diversity identifying 2621 species in semi-supercentenarians (aged 105 to 109 years) compared to other groups. Moreover, it detected the presence of an uncultured archaeon in semi-supercentenarians highlighting a new archaeal genus not yet described and 3 new species belonging to the *Enorma* genus that could be species of interest participating in the longevity process.

RiboTaxa is user-friendly, rapid, allowing microbiota structure description from any environment and the results can be easily interpreted. This software is freely available at <https://github.com/oschakoory/RiboTaxa> under the GNU Affero General Public License 3.0.

OT15 | ANALYSIS OF MILK, FECES AND SOIL MICROBIOMES COMPOSITION AND STRUCTURE ACROSS QUEBEC DAIRY FARMS

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Manure and hygiene management measures can impact the transmission of microorganisms between various niches on dairy cattle farms, which can disseminate across the One-Health continuum. The aim of this preliminary study was to evaluate the shared composition and structure of the microbiome (bacteria, fungi and protozoa) in feces, milk, and soil samples collected from five Quebec dairy herds. We sequenced hypervariable regions of the 16S, 18S and ITS rRNA to detect community structures, diversities, and spatial compositions. The bacterial, eukaryotic and fungal composition of the microbiome was significantly different between niches, as indicated by the β -diversity. Bacteria and eukaryotes showed the highest community richness and diversity in fecal samples, whereas fungi were most diverse in soil. The most abundant bacterial genera in feces were also among the most connected nodes in a network analysis. In milk, more than 50% of the bacterial sequences were classified as *Achromobacter* (51.1%), followed by *Prevotella*, *Staphylococcus*, and *Lactobacillus* (>2%). None of the most abundant genera in milk were key components of the network analysis. The predominant fungal genus was *Mortierella*, in both milk (3.3%) and soil (16.3%). *Cystofilobasidium* (4.7%) was the second most prevalent genera in soil, and among the most connected nodes in the fungal network. The microbiome structure in each niche was not significantly different between herds. Hence, we concluded that sample type had the greatest influence on microbiome structure. These results elucidate interactions at the animal-environment interface, informing the need for a better understanding of fungi dynamics within the One-Health continuum.

OT16 | LINKING ENVIRONMENTAL POLLUTION AND GUT MICROBIOME IN INDIVIDUALS LIVING IN HIGHLY CONTAMINATED SETTLEMENTS

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The array of all the genes in the microbiome along with the host genes has been defined as the “hologenome”. While the host genome is highly conserved, and genetically adapt slowly to the changes in the environment, the microbiome genome can rapidly change in response to the environment, and it has been indicated as a possible additional way of boosting evolution. Humans are daily exposed to a wide range of xenobiotics, that may include different classes of molecules and can reach the gut microbiome directly or can be previously metabolized in the liver. We analyzed gut metagenomes in 359 healthy, Italian adults, grouped according to the level of exposure to environmental pollutants, as well as blood levels of dioxins and heavy metals. Analysis highlighted an increase in gene richness in subjects with a high exposure level (HIGH group). In addition, exposure to different classes of environmental pollutants drives the selection of microbial strains able to degrade these compounds in the human gut. Our results highlighted the role of xenobiotics in shaping gut microbiome composition and activity, suggesting the intriguing possibility that it may be implicated in the host response to environmental pressure.

Acknowledgements: This work was supported by the project Linking environmental pollution and gut microbiota in individuals living in contaminated settlements, funded by the Italian Ministry of Health (GR-2016-02362975)

OT17 | RECIPIENT INDEPENDENT HIGH ACCURACY FMT PREDICTION AND OPTIMIZATION IN MICE AND HUMANS

Prof. Yoram Louzoun¹, Oshrit Shtossel¹, Prof. Omry Koren¹

¹Bar Ilan University, Ramat Gan, Israel

Microbiota manipulation or supplementation have been argued to restore microbiome associated with healthy conditions. Fecal Microbiota Transplantation (FMT) is among the most popular microbiome intervention procedures. Current practices are to choose the transplanted microbiome based on the donor phenotype, and not based on the expected recipient phenotype. However, the two differ drastically.

We show that the donor and recipient phenotypes differ widely, and propose a tool to predict the recipient phenotype after the FMT using only the donors' microbiome and when available demographics for transplants from humans to either antibiotic treated mice, or other humans.

We then extend the method to optimize the best-planned transplant (bacterial cocktails) by combining the predictor and a genetic algorithm (GA). We validate the predictor using a de-novo FMT experiment highlighting the possibility to choose transplants that optimize an array of required goals, using less than 30 taxa, opening the way for over-the-shelf FMT.

OT18 | THE HUMAN GUT MICROBIOME EXPLORED AT SINGLE NUCLEOTIDE RESOLUTION.**Dr Falk Hildebrand**^{1,2}, Dr Rebecca Ansorge^{1,2}, Dr Ezgi Ozkurt^{1,2}, Dr Clemence Frioux^{1,2,3}¹Quadram Institute Bioscience, Norwich, United Kingdom,²Earlham Institute, Norwich, United Kingdom, ³INRAE, France

The gut microbiome is essential to the wellbeing and health of its human host, yet most studies can only resolve the gut microbial community at genus or species level. Yet we do know that two bacterial strains of the same species can differ by more than half their genome. Furthermore, in clinically relevant microbes, pathogenicity is often a trait encoded at the strain - not species - level. Therefore, my group develops the technologies to track bacterial strain in metagenomic time series, and to investigate evolutionary pressures.

I will present some of our studies, where we can show the immense persistence of gut bacteria in healthy human cohorts. A significant fraction of microbial species are frequently exchanged among family members, reflected in the selective pressures on their genes. Investigating microbiomes in patients, strain resolved metagenomics becomes even more important, as many difference between healthy and diseased microbiomes can only be detected using strain resolved metagenomics.

OT19 | HARNESSING THE GUT MICROBIOME TO IDENTIFY NOVEL ANAEROBIC BACTERIA WITH BIOTHERAPEUTIC POTENTIAL

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Roseburia intestinalis is one of the most abundant human gut bacteria that produces butyrate from a variety of dietary polysaccharide substrates and plays an important role in maintaining gut health. Individuals with inflammatory and other metabolic diseases frequently harbour lower levels of these bacteria, highlighting its importance in maintaining gut homeostasis. We have used whole genome sequence data from sixteen selected *R. intestinalis* strains isolated from healthy human gut, from different geographical locations, and performed pangenome analysis. We investigated its antimicrobial resistance potential and carbohydrate utilisation capabilities. The results demonstrated that *R. intestinalis* strains exhibited an open pan-genome structure. Phylogenetic analysis of the core genome showed regional clustering of the strains based on their origin of isolation (Asia, Europe and America) indicating geographical stratification. In total, 295 genes were involved in carbohydrate degradation and contained at least one CAZy domain. 96 of these domains were present in all sixteen strains, signifying considerable inter-strain conservation. Interestingly, the majority of the *R. intestinalis* strains (10/16) harboured the tetracycline resistance genes *tet(O)* or *tet(40)*, with the two genes located in tandem on a plasmid for the L1-82 strain. The results showed considerable conservation between the *Roseburia intestinalis* genomes, whilst also revealing region-specific differences indicating that specific expansions have occurred in different habitats. The identification of tetracycline resistance genes in these strains warrants a careful evaluation of their suitability as probiotic and live-biotherapeutic candidates to minimise the risk of transferring resistance to other resident gut bacterial populations. (Funded by PROBI AB)

OT20 | HARNESSING DIVERSE PROPHAGES OF STEC O157:H7 PHAGE-TYPE 8 FOR RAPID IDENTIFICATION OF FRESH PRODUCE-ASSOCIATED STEC STRAINS

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The Shigatoxigenic *Escherichia coli* (STEC) serotype O157:H7 is a priority foodborne pathogen in humans. STEC strains in the same serotype can be classified according to their susceptibility to lysis by a set of bacterial lytic viruses, bacteriophages, termed phage typing. In many parts of the world the majority of STEC outbreaks are now derived from plant-based food, with an apparent association of phage-type (PT) 8 strains. Thus, rapid and reproducible identification system to supplement existing methods is needed for these strains. Here, we took a sequence-based approach to identify specific molecular markers. We identified twenty-five prophages (genome-embedded phages) in a representative STEC O157:H7 serotype, isolate 644-PT8, from lettuce. Seven markers unique to 644-PT8 prophage genes were designed to enable rapid and accurate identification of PT8 strains. These markers had the added benefit of discriminating STEC based on their phage repertoire, allowing for detection of STEC strains from additional sources. The markers comprised of primers sets that each produced a unique single amplicon of varying sizes (169, 306, 507, 798, 1022, 1855, 2371 bp) from the bacterial host genome, 644-PT8, for a multiplex PCR. The markers showed 100% specificity for *E. coli* O157:H7 in public databases and clustered other serotype strains into specific clades, using the BLASTn tool. These molecular markers provide a valuable novel diagnostic tool for the identification of isolates in different niches that could enhance STEC surveillance. Future work will focus on creating an easy-to-use pipeline for STEC identification *in silico*, and test feasibility of adoption from field isolates.

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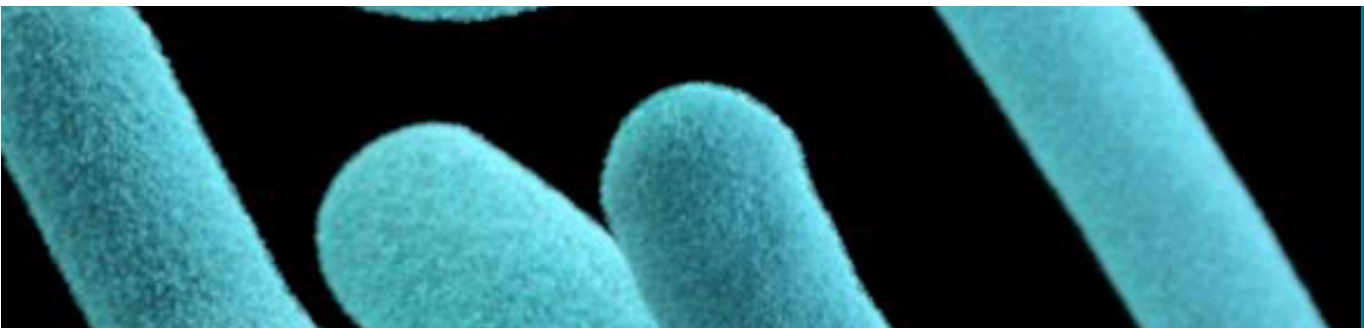
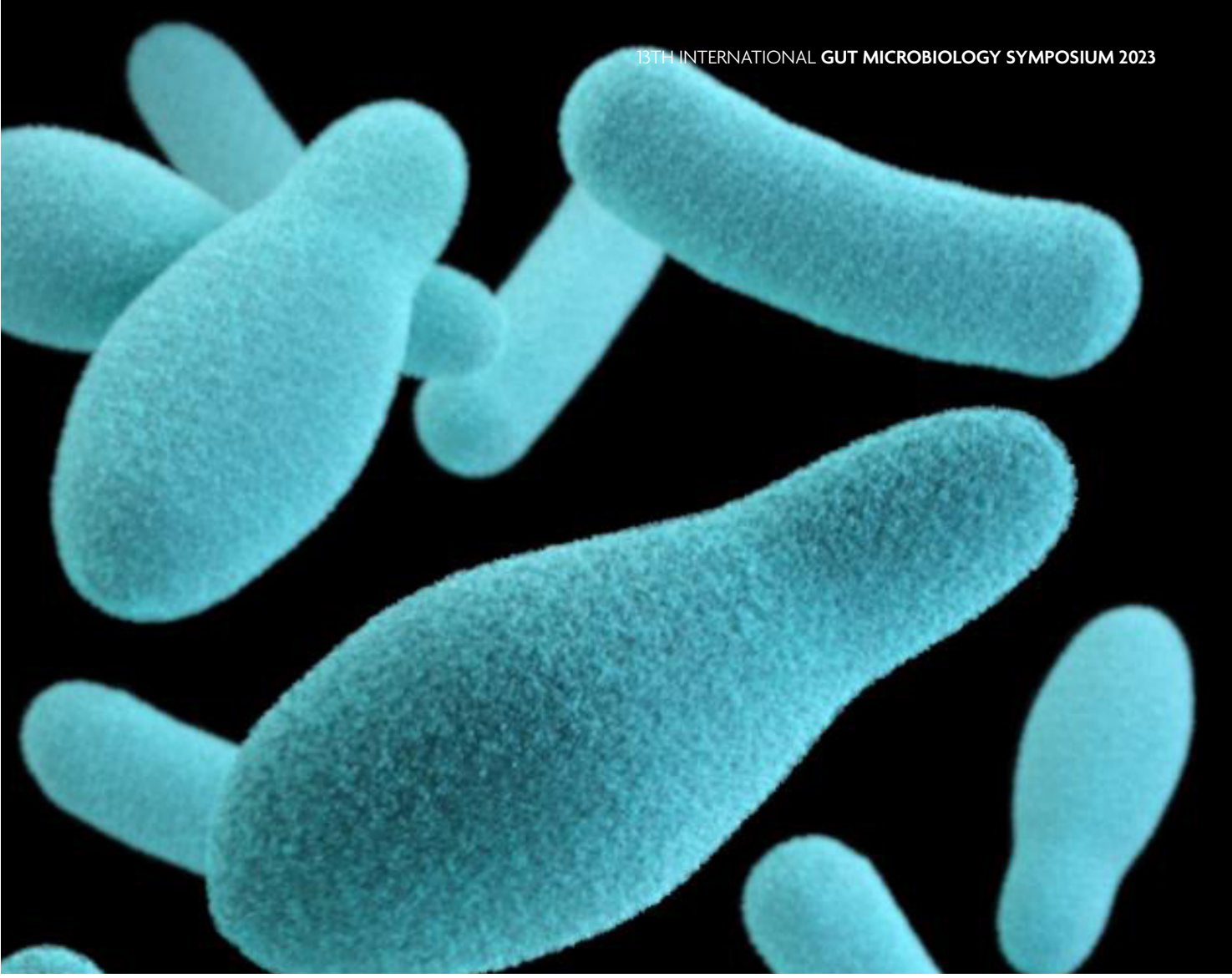
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POSTERS

P001 | CULTURING OF 'UNCULTURABLE' RUMEN BACTERIA USING DILUTION TO EXTINCTION AND MEDIA DIVERSIFICATION

Ms Theano Stoikidou¹, Mr Ziming Wu¹, Mr Corey Roulston¹, Miss Jnana Sree Pakalapati¹, Mr Jack Jordan¹, Miss Maria Buckland¹, Dr Katie Lawther¹, Dr James Pickup¹, PhD Fernanda Godoy Santos¹, Prof. Linda Oyama¹, Dr Conrad Ferris², Dr Sharon Huws¹

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The rumen represents 80% of the ruminant stomach at maturity and it is a complex, dynamic ecosystem comprising mainly anaerobic bacteria, protozoa, anaerobic fungi, methanogenic archaea, and bacteriophages. These microbes interact with each other and have a symbiotic relationship with the host, providing energy from the breakdown of plant cell wall carbohydrates. The understanding of most microbiomes, including the rumen, is hindered by poor culture collections and reduced efforts placed on culturing after the recent boost in the 'omic' technologies; leading to an assumption that many microbes are unculturable, when it is recognised that with enhanced effort they could be cultured. In this study, using the dilution to extinction method, cattle and sheep samples were plated onto Bovine Heart Infusion (BHI) medium, Hobson's M2 medium, and PC basal medium all prepared with and without rumen fluid. Our efforts allowed for the isolation of over 120 ruminal bacteria with profile differences as per medium emphasising the significance of diversification of growth media during isolation. Furthermore, 16S rRNA gene sequencing of the isolates revealed a potent new species of ruminal bacteria; according to the NCBI database, the most closely related species to it is the *Parabacteroides goldsteinii* with 94.38% identity. Characterisation of this new species further may provide insights into their role in the rumen as well as their importance for the host. Overall, our results allow for an improved understanding of the rumen microbiome as well as new research possibilities on its role/function with respect to animal health, productivity, and sustainability.

P002 | MICROBIOTA RESILIENCE AFTER CLOSTRIDIODES DIFFICILE INFECTION TREATMENTS

Ms Elena Montenegro-Borbolla¹, Prof. Claire Bertelli¹, Prof. Benoît Guery¹

¹*CHUV, Lausanne, Switzerland*

Clostridioides difficile (CD) infections (CDI) are the main cause of nosocomial antibiotic-associated diarrhoea and reports from community acquired CDI are also increasing. Antibiotic treatment is the main risk factor for infection, as the disruption of the intestinal microbiota results in an ideal environment for CD to thrive. Recurrent CDI (rCDI) is the most common complication occurring in up to 30% of patients.

Our goal is to identify the microbiota profile after CDI treatment comparing the 2 drugs recommended as standard of care, vancomycin and fidaxomicin. Stool samples are taken at key time points: at CDI diagnosis day (Day 0) prior to antibiotic treatment, at the end of the antibiotic treatment (Day 10), at the end of the period defining sustained clinical cure (week 10), and two long term follow-ups (Months 6 and 12). 16S rRNA amplicon sequencing metagenomics are used to evaluate the microbiota profile.

A total of 39 CDI patients and 8 healthy donors have been recruited as of February 2023. Metagenomics analyses show a lower microbial diversity on all patient samples when compared to healthy donors. Within the patient samples, after an initial decrease due to the antibiotic treatment, the microbiota alpha-diversity is restored to initial levels at week 10. Also, at this latter time point fidaxomicin treatment might better preserve bacterial species richness compared to vancomycin and other antibiotics. This specificity may partially explain the lower risk of recurrence observed with this drug.

P003 | MICROBIOME-METABOLOME INTERACTIONS PREDICT HOST PHENOTYPE**Prof Yoram Louzoun¹**, Oshrit Shtusel¹, Prof. Omry Koren¹¹Bar Ilan University, Ramat Gan, Israel

The effect of microbes on their human host is often mediated through changes in metabolite concentrations. As such, multiple tools have been proposed to predict metabolic profiles from microbial taxa frequencies. Such tools cannot capture the dependence on host demographics or conditions of the microbiome-metabolite relation.

We show that the microbiome-metabolites relation is best predicted as log concentrations. We develop LOCATE (Latent Of miCrobiome And meTabolites rElations), a machine learning tool based on internal latent representation to predict metabolite concentration based on microbiome composition. LOCATEs' accuracy is higher than all current predictors. The metabolite concentration prediction significantly decreases cross datasets, and cross conditions, especially in 16S data.

LOCATEs internal representation can be used to predict the host phenotype better than either the microbiome or the metabolome. This representation is strongly correlated with host demographics. A significant improvement in accuracy is obtained even with a small number of metabolite-samples (~50).

P004 | IN VITRO MODELLING OF ORAL MICROBIAL INVASION IN THE HUMAN COLON

Dr Victoria Meslier¹, Dr Lucie Etienne-Mesmin², Dr Ophélie Uriot², Dr Elora Fournier², Dr Charlotte Deschamps², Mr Sylvain Denis², Mr Aymeric David¹, Ms Sarah Jegou¹, Mr Christian Morabito¹, Benoit Quinquis¹, Ms Florence Thirion¹, Dr Florian Plaza-Oñate¹, Dr Emmanuelle Le Chatelier¹, Dr S. Dusko Ehrlich¹, Dr Stéphanie Blanquet-Diot², **Dr Mathieu Almeida¹**

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Mounting evidence have revealed oral microbial detection in stools of dysbiotic patients. However, potential interactions of these invasive oral bacteria with intestinal microbiota have been poorly investigated.

In this study, we present a new set up of the Mucosal ARTificial COLon recreating the physiochemical and microbial parameters of the human colon combined with a salivary enrichment and whole metagenome shotgun sequencing to simulate oral to gut microbial invasion. The M-ARCOL model was inoculated with fecal samples from healthy donors and saliva from the respective donors was injected. Fermentation was conducted for 11 days with sampling from fresh stools, saliva, and luminal and mucosal compartments. Samples were sequenced by whole metagenome shotgun sequencing to resolve in depth microbial composition of the oral and gut microbiota.

We showed a higher species richness retained in the mucosal compartment of the M-ARCOL during in vitro fermentations, when compared to the luminal samples at each time point. More importantly, while a few oral microbial species were present in the colonic compartments before saliva addition, oral microbial species were detected almost exclusively in the mucosal microenvironment during oral-to-gut invasion simulation, regardless of the richness and gut microbial composition of the donors.

Our study suggests a preference of oral microbial invaders for the mucosal microenvironment, shedding light on the critical importance of including the mucosal set-up when studying microbial dynamic in in vitro models.

This new in vitro model of oral-to-gut invasion can provide useful mechanistic insights into the role of oral microbiome in various disease processes.

P005 | DO EXERCISE AND ENERGY METABOLISM EXERT A SELECTIVE PRESSURE ON GUT MICROBIOME? LESSONS LEARNED FROM THE EXOMIC PILOT CLINICAL STUDY

Mr David Martin¹, Mr Mathis Bonneau¹, Mr Romain Demay¹, Mme Emmanuelle Lecommandeur³, Mme Mathilde Hazon³, Mme Valérie Monbet², Mr Rufin Boumpoutou⁴, Mme Crystèle Cressard³, Mme Pierre Cressard³, Mr Frédéric Derbré¹

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Over the last decade, substantial studies observed that microbial α -diversity and fecal short-chain fatty acids (SCFA) concentrations were higher in elite athletes than sedentary population supporting the hypothesis that highly trained individuals would possess an optimized microbiome extracting more energy from food. However, all of these studies present some important limitations, including control for dietary habits. In this context, we conducted a clinical study (NCT05220657) to characterize the gut microbiome of male participants ranging from sedentary individuals to elite athletes with high (i.e. elite soccer players) or even very high energy requirements (i.e. elite cyclists). Measured metabolic parameters (e.g. VO₂max, carbohydrate and fat oxidation) were then linked to the metagenomic shotgun, metabolomic data and habitual dietary intakes. On contrary to our expectations, diversity showed an inverse U-shaped relationship with cardiorespiratory fitness (VO₂max). The majority of cyclists had a Prevotellaceae enterotype (n = 12/14; 86%), whereas almost all sedentary participants had a Bacteroidaceae enterotype (n = 18/21; 85%). Participants exhibiting a P enterotype were related to the higher fat oxidation values during exercise (p < 0.01). Although P enterotype participants displayed lower gut microbiome α -diversity (p < 0.01), fecal concentrations of SCFAs (i.e. Propionate and Valerate, p < 0.01) were higher compared to B enterotype, probably conferring an advantage for endurance performance. Neither dietary intake of carbohydrates, fat, fibers nor proteins explained the enterotype shift. Further experiments including fecal transplantations in mice will determine if a causal relationship really exists between gut microbiome and energy metabolism during submaximal exercise.

P006 | UNDERSTANDING THE HOST–MICROBIOME INTERACTIONS INVOLVED IN LIVER ABSCESSES FORMATION IN BEEF CATTLE

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Feedlot diets are formulated to provide ~10% dry matter as roughage. Diets low in forage increase the incidence of metabolic disorders, with 20-30% of cattle in feedlots developing liver abscesses. Severe liver abscesses are linked to reduced feed efficiency costing the Canadian beef industry ≈ \$61.2 million annually. The processes involved in the development of liver abscesses are not well understood. We present our efforts to examine the host-microbiome interactions involved in the development of liver abscesses in cattle. To examine the impact abscesses have on liver function, we have employed RNA-seq and found significant alterations in gene expression in abscessed livers. We characterized the rumen microbiome of cattle with and without liver abscesses and did not observe a relationship between the rumen microbial community and the development of a liver abscess. We have also characterized the liver abscess microbiome and found a low diversity community dominated by *Fusobacterium* spp and *Bacteroides* spp. Abscess severity impacted the richness and composition of the abscess microbiome. To further characterize these pathogens we have isolated *Fusobacteria* from liver, abscess and gut samples. *Fusobacteria* spp. were isolated from all samples, including from seemingly healthy liver tissue. Genomic approaches are being used to examine the phylogenomic relationship between *Fusobacteria* isolated from the gut and livers of cattle with abscesses. This work provides novel insight into the host-pathogen interactions leading to the development of liver abscesses in cattle and will help reduce the use of antibiotics in livestock production.

P007 | CHANGES IN MELANOMA PROGRESSION DUE TO THE SKIN MICROBIOME**Ms Aline Rosin**¹, Ms Maya Kissner¹, Mr Tewes Tralau¹, Ms Lisa Lemoine¹¹German Federal Institute for Risk Assessment, Berlin, Germany

The human skin is one of the largest and most versatile organs harbouring millions of microorganisms, namely bacteria, fungi and viruses. Together these are referred to as the skin microbiome. Particularly bacteria are involved in critical cellular processes like pathogen protection and immune modulation. The respective symbiotic relationships contribute significantly to human health.

Dysbiosis refers to a lack of balance among bacterial communities and the host that may lead to skin diseases. Recent studies showed that such dysbiosis is also found in melanoma, the most deadly form of all skin cancers. However, the underlying mechanism is not yet sufficiently understood.

To investigate the impact of the skin microbiome on melanoma progression, we have established a complex co-culture system based on the commercially available MelanomaFTM model from MatTek. This model consists of epidermal keratinocytes, dermal fibroblasts and malignant A375 cells colonized with whole skin swabs.

Colonizing skin models using a skin swab from a healthy volunteer demonstrated an increased release of LDH along with elevated secretion of the melanoma marker S100B. The latter is expressed by melanocytes and indicative of loss in cell integrity and cell death. Further experiments confirmed release of cytokines such as IL-1 α , CD40 ligand and GM-CSF. This is associated with good survival in cancer patients. Taken together these results suggest death of degenerated melanocytes in colonized tumour models and an antitumor effect of skin commensals.

In addition, models are being developed with a defined bacterial community as well as with tumour-associated pathogens.

P008 | MITOCHONDRIAL COMPLEX I DYSFUNCTION PROMOTES MICROBIAL COMPOSITION THAT IMPACTS ON ULCERATIVE COLITIS

Dr Ainize Peña-Cearra^{1,2}, Dr Deguang Song³, Janire Castelo², Ainhoa Palacios², Dr Jose Luis Lavín⁴, Dr Diego Barriales², Iratxe Seoane^{1,2}, Itziar Martín-Ruiz², PhD Ana Maria Aransay², Dr Hector Rodríguez², Dr Noah W Palm³, Dr Juan Anguita², **Dr Leticia Abecia**^{1,2}

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Recent evidence demonstrates potential links between mitochondrial dysfunction and inflammatory bowel diseases (IBD). In addition, bidirectional interactions between the intestinal microbiota and host mitochondria may modulate intestinal inflammation. We observed previously that mice deficient in mitochondrial protein MCJ (Methylation-controlled J protein) exhibit increased susceptibility to DSS colitis. MCJ is a mitochondrial protein that negatively regulates complex I of the electron transport chain, controlling ATP and ROS (reactive oxygen species) production. However, it is unclear whether this phenotype is primarily driven by MCJ-/- associated gut microbiota dysbiosis or direct effects of MCJ-deficiency. Here, we demonstrate that fecal microbiota transplantation (FMT) from MCJ-deficient mice into germ-free mice was sufficient to confer increased susceptibility to colitis. Therefore, an FMT experiment by cohousing was designed to alter MCJ-deficient microbiota. It was observed that mitochondrial dysfunction phenotype was reverted through FMT. Further, we use magnetic activated cell sorting (MACS) and 16S rDNA gene sequencing to characterize taxa-specific coating of the intestinal microbiota with Immunoglobulin A (IgA-SEQ) in MCJ deficient murine model. We show that high IgA coating of fecal bacteria observed in MCJ-deficient mice play a potential role in disease progression. In summary, this study allowed us to identify potential microbial signatures in faeces associated with mitochondrial dysfunction and disease progression. These microbial biomarkers might serve as predictors, permitting the stratification of ulcerative colitis patients into distinct clinical entities of ulcerative colitis spectrum.

P009 | IMPACT OF WESTERN DIET ON FOOD-BORNE PATHOGEN INTERACTIONS WITH HUMAN MICROBIOME IN THE MUCOSAL ARTIFICIAL COLON

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Enterohemorrhagic *Escherichia coli* (EHEC) is a major food-borne pathogen causing human diseases ranging from diarrhea to life-threatening complications. Relatively little data is available on interactions between EHEC and the human gut microbiota. Accumulating evidence demonstrates the involvement of Western diet in gut microbiota shifts that enhance susceptibility to enteric infection, but the effect of diet on EHEC pathogenesis remains unknown. Our research aimed to investigate the effects of healthy versus Western diet on gut microbiota composition and activities and EHEC colonisation in an in vitro human colon model M-ARCOL (Mucosal ARTificial COLon). This model reproduces the main nutritional, physicochemical and microbial (luminal and mucus-associated microbiota) parameters of the colonic environment. Two bioreactors were inoculated with human fecal samples (n=4) and ran in parallel, one receiving a healthy diet, the other a Western diet and infected with EHEC strain EDL933. EHEC survival was determined by qPCR, gut microbiota composition was assessed by 16S metabarcoding and microbial activities were evaluated through gas and short chain fatty acid analysis. Diet, donor and EHEC infection impacted beta-diversity in luminal and mucosal samples. EHEC survival was dependant on both donor and diet in luminal samples. EHEC was more rapidly depleted when treated with a healthy diet compared to a Western diet and eliminated sooner in some donors. EHEC was maintained in mucosal samples without elimination, suggesting a possible niche environment for colonisation and survival. The prolonged EHEC colonisation sustained by a Western diet in vitro could suggest an increased susceptibility to infection in humans.

P010 | A SYSTEMATIC REVIEW: METAGENOMIC INSIGHTS ON THE INFLUENCE OF COMMON PATHOGENS ON POULTRY GUT MICROBIOME

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In poultry production there is accumulating evidence demonstrating the importance of gut microbiota in the optimisation of health and growth efficiency. Metagenomic next-generation sequencing (mNGS) is now increasingly used to characterise the microbial dynamics and communities and its association with other factors such as disease conditions. Therefore, in this research, we systematically reviewed studies that conducted mNGS for gut microbiota profiling of chicken experimentally challenged or naturally infected with microbial pathogens.

Following PRISMA guidelines, we identified 1446 records from January 2011 to November 2022, and 40 records were ultimately included after screening. The influence of supplements on infected broilers was the most frequent rationale for study, with bacteria (53%), specifically *Salmonella* species, as the most tested pathogen. 16S rRNA sequencing using Illumina platforms (80%) was most consistently used, while utilised bioinformatic pipelines and analytical software varied. Caecal digesta (60%) is the most common sample type, while Firmicutes was commonly reported as the predominant phylum in diseased birds.

In around 40% of the studies, determination of alpha and beta diversity metrics, differential taxa analyses, and potential functional profiling of microbial communities were performed; with majority indicating significant differences between diseased and healthy control birds. Generally, decreased abundances of Firmicutes and *Lactobacillus* species and increased abundances of members of Bacteroidetes and Proteobacteria (i.e. Enterobacteriaceae) were observed in the gut profile of infected chicken. These results provide new insights in the importance of the gut microecosystem balance and reduction of pathogens to maintain gut health, food safety and overall chicken performance.

P011 | BREED-DEPENDENT DIFFERENCES OF LACTOBACILLUS DRIVE THE INTESTINAL PROLIFERATIVE ABILITY IN A PIG MODEL

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The potential role of gut microbiota in regulating the proliferation of intestinal epithelial cells in mammals has attracted considerable attention. However, whether the breed-dependent composition of specific intestinal microbes regulates intestinal epithelial proliferation remains unclear. This study, employing the Chinese native breed Meishan and commercial breed LargeWhite barrows in the same physiological stage, revealed higher abundance of Lactobacillus and lactate concentration in the jejunum in Meishan pigs than in LargeWhite pigs. These results were supported by the higher abundance of metabolic enzymes for lactate production (GPI, FBA, TPI, GAPDH, PGK, LDH), which were mainly phylogenetically assigned to Lactobacillus at the genus level as revealed by Metatranscriptome. Further, the Meishan pigs had higher proliferation ability in the jejunum than LargeWhite pigs, with deeper jejunal crypt depth, more PCNA+ TA cells and Lgr5+ active stem cells. Wnt- β -catenin signaling pathway, important in regulating the proliferation of intestinal stem cells, was more activated in the Meishan pigs than in the LargeWhite as. To mechanistically explore the role of intestinal microbial lactate on ISC-mediated porcine epithelial proliferation, we established a porcine jejunal organoids model to and found that lactate activated the Wnt/ β -catenin signaling pathway to promote ISC-mediated porcine epithelial proliferation in a Gpr81-dependent manner. The results suggest that the breed-dependent differences of Lactobacillus drive the intestinal proliferative ability, providing evidence that genetics affect intestinal microbiota composition and function.

P012 | CHARACTERISING THE CANINE AND FELINE GUT MICROBIOME THROUGH 16s rRNA SEQUENCE ANALYSIS

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The gut microbiome is known to have a significant impact on numerous aspects of host health, therefore characterising the canine and feline gut microbiome is key to ensuring a long, healthy life for companion animals. The aim of this project was to characterise a healthy gut microbiome in cats and dogs and explore the key differences between host-species. Fresh faecal samples were collected from a cohort of healthy cats and dogs housed at Waltham Petcare Science Institute and submitted to Illumina amplicon sequencing of the 16s rRNA gene. Cats and dogs were found to have significantly different microbiome compositions (PERMANOVA $F(1, 47) = 31.833$, $p = 0.01$). Dogs had higher alpha diversity in their microbiomes with less variation between samples than that observed in cats. Cats and dogs separated distinctly when analysing beta diversity using Bray-Curtis measure of dissimilarity and partial least squares discriminant analysis (PLSDA). Following PLSDA analysis, the component loading of each bacterial genus was assessed to identify key genera responsible for the difference observed between the canine and feline gut microbiomes. This study highlighted that the composition of the gut microbiome was significantly different between cats and dogs. Currently, particularly in a veterinary setting, cats and dogs receive similar courses of treatment for gastrointestinal disorders which does not account for the key differences shown in the current study. It is therefore important to understand these key differences between host species in order to ensure the best course of host-specific treatment is followed.

P013 | DIARRHEAL-ASSOCIATED MICROBIOTA INCREASED STEC COLONISATION IN AN INFANT ARTIFICIAL COLON

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Background: Shiga toxin-producing *E. coli* (STEC) are major food-borne pathogens with a highly regulated infection process controlled by environmental conditions in the human colon. Previous studies have found different bacterial profiles in stool samples from children infected by Diarrheagenic *E. coli* (DEC) compared to healthy ones.

Objectives: We evaluated in an in vitro model the impact of STEC-associated microbiome and related metabolites on the reference STEC strain EDL-933 survival and virulence.

Material and method: The Artificial COLon (ARCOL) was set up to reproduce the main nutritional, physicochemical and microbial parameters of the infant colon and inoculated with stool samples from children with STEC-positive diarrhea or after the diarrhea episode as a healthy control (n=2). At day 1, STEC reference strain was added to the ARCOL system fermentations were further run for 7 days. Microbiota composition and metabolic activities were followed by 16S metabarcoding and short chain fatty acid dosage, respectively. STEC survival and gene expression (Ipf, stx and ler genes) were followed by qPCR.

Results: We observed a higher level of acetate and a lower level of propionate in DEC conditions compared to the healthy control. Regarding microbiota composition, Firmicutes and at a lower taxonomic level Clostridium were more abundant under DEC condition. Interestingly, a longer STEC survival and virulence gene expression were found under diarrheal versus healthy conditions. These results suggest STEC-associated microbiome could favor STEC survival and virulence in the simulated infant colon. Such data can help for a better understanding of STEC physiopathology in at-risk infant population.

P014 | DESULFOVIBRIO BACTERIA ENHANCE ALPHA-SYNUCLEIN AGGREGATION IN A CAENORHABDITIS ELEGANS MODEL OF PARKINSON'S DISEASE.

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Parkinson's disease (PD) is a common and complex neurodegenerative disorder that affects the movement. The root causes of the disease have remained unknown despite more than 200 years of intensive studies. It has been suggested that genetics, environmental factors and lifestyle play some role in the disease pathogenesis. Accumulation of the neuronal protein alpha-synuclein (alpha-syn) is the key pathological feature of PD. The aggregates have been found not only in the central nervous system, but also in other parts of the human body including gastrointestinal tract. Alpha-syn aggregation, thus, has been suggested to be induced in the gut cells by intestinal pathogens. Furthermore, exposure to various microbial components and products (namely curli-producing *Escherichia coli*) has been shown to trigger alpha-syn aggregation and PD-like symptoms in animal models. Recently, we showed that *Desulfovibrio* bacteria are associated with PD. However, it was not clear how these bacteria contribute to the disease, particularly concerning the emergence of alpha-syn pathology. In this study, by employing a *Caenorhabditis elegans* model that overexpresses human alpha-syn, *Desulfovibrio* bacteria isolated from the feces of PD patients have been demonstrated to increase alpha-syn aggregation in both volume and abundance, statistically more than curli-producing *E. coli*. Additionally, patient *Desulfovibrio* strains were stronger than those isolated from healthy individuals in inducing alpha-syn aggregation and toxicity. Our results suggest that *Desulfovibrio* bacteria, especially patient strains, contribute to PD pathogenesis by inducing alpha-syn aggregation.

P015 | A MULTI-OMICS APPROACH TO IDENTIFY MOLECULAR PATHWAYS ASSOCIATED WITH REMISSION IN PEDIATRIC CROHN'S DISEASE

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Although the etiology of Crohn's disease (CD) is still under investigation, it is thought that an interplay between genetic predisposition, environmental factors and microbiome triggers inflammation. Insight in the mechanisms underlying the transition to remission is of major importance in the development of novel therapies. For this study, 59 pediatric patients (9-21 years) with active CD or in remission (characterized by fecal calprotectin levels), were included. The fecal microbiome was determined by both ITS1 (fungi) and 16S (bacteria) analysis. In addition, metabolomics analysis with HILIC-QTOF-MS was performed on fecal, urine and plasma samples and proteomics with LC-MS/MS on fecal samples. Distinct signatures for the two patient groups were observed for the metabolomes (both fecal and plasma), and proteome. The bacteriomes of patients in remission scored a higher Shannon-diversity index and were more similar to each other, as shown by Bray-Curtis dissimilarity, while fungal data did not reveal large differences between all individuals. Integrating the different datasets in a Manifold Mixing for Stacked Regularization model, specifically designed for multi-omics datasets resulted in a model that separates the interactome of the two patient groups. The bacteriome, fecal and plasma metabolome showed to have the strongest effect on this separation. In active disease an inflammatory profile of proteins was observed which is positively correlated with amplicon sequence variants belonging to the *Hungatella* and *Lachnoclostridium* genera and *Streptococcus oralis/sanguinis/parasanguinis*. Further investigation of the underlying pathways may provide more insight in the mechanisms involved in remission of Crohn's disease.

P016 | GENDER-DEPENDENT HCC DEVELOPMENT IN X/MYC MOUSE MODEL

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Hepatocellular carcinoma (HCC) is among the leading causes of cancer-related death worldwide. HCC is associated with chronic liver disease due to chronic viral hepatitis, alcohol intake and non-alcoholic steatohepatitis (NASH). Epigenetics plays an important role in liver oncogenesis and gut microbiota changes in HCC patients, but the relationship between them during carcinogenesis is not clear. Epidemiological reports indicate that the incidence of HBV-related HCC is higher in males than in females, while the cause underlying this observation is largely unknown.

Aim: To investigate the relationship between the transcriptomic profile and the microbiome during hepatocarcinogenesis.

Methods: WT and X/Myc mice were used to perform a RNA-seq analysis on liver tumor (T) and peri-tumor (PT) and to sequence rRNA 16s of stools at 10 and 22 weeks, corresponding to initiation and progression of liver tumors.

Results: Transcriptome analysis of T and PT indicated that HCC developed by X/MYC mice correlated with the HCC subclass S2, characterized by proliferation and poor prognosis. WT, PT and T tissues showed a different transcriptomic profile depending on the gender. rRNAs 16S analysis confirmed a significant different microbiota composition at both early and late phases of HCC development. Female, while presenting a dysbiosis, were enriched in protective bacterial species, which have been reported to have a role in clinical response to anti-PD-1 immunotherapy. Finally, we defined a network of RNAs and bacterial species that characterized females and males hepatocarcinogenesis.

Conclusions: Our results highlight the existence of a sex-dependent RNA-gut microbiota cross talk during liver tumor development.

P017 | SPECIFIC MICROBIAL FINGERPRINTS ARE ASSOCIATED WITH LOW LEVELS OF LT TOXIN-CARRYING ENTEROTOXIGENIC ESCHERICHIA COLI IN THE GUT

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Post-weaning diarrhoea (PWD) is an economically important gastro-intestinal disease affecting piglets, causing growth retardation, diarrhoea, mortality and therefore, represents one of the main problems for the piglet industry. PWD is a multifactorial disease in which enterotoxigenic *Escherichia coli* (ETEC) strains play a major role. Their pathogenicity is due to the production of enterotoxins and adhesins. However, other factors can contribute to onset of the disease, including host genetics, host immunity, stress factors and/or impaired gut microbiota. Gut microbiota indeed plays important role in porcine health as it affects metabolism, immunity and defense against pathogens. Using 16S rDNA sequencing, we characterized the rectal microbiome and the jejunal, ileal and colonic microbiome of 64 piglets across 8 different farms differentially affected by PWD, starting from 7 days of birth up to 4 days post-weaning. The microbiota composition was analyzed in relation to ETEC shedding pattern and colonization levels in different intestinal segments. We categorized piglets based on their ETEC levels quantified by qPCR. In particular, we focused on the presence of the LT toxin and by using a supervised clustering approach we identified microbiota members associated with low levels of LT colonization post-weaning, in different timepoints pre and post-weaning and different intestinal segments. Our findings contribute into understanding the role of gut microbiota composition and its interaction with the host in the development and resistance against post-weaning diarrhoea. These data can be used for rational development of preventive measures that promote a microbiota composition to reduce ETEC levels in the gut.

P018 | CALF REARING IN DAIRY FARMS: AT THE CROSSROADS BETWEEN MICROBIAL TRANSMISSION AND WELFARE

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Separation of dairy calves from dams at birth increases income of the farm. However, it affects the microbial colonisation of the gut and could have an impact on calves' health. Core microbes colonise rumen in the first days of life, retarded separation could be a trade-off between welfare and farmers' benefits. The aim is to evaluate effect of separation on gut microbial colonization of calves.

We followed three groups of nine calves (0-20 weeks) that were in contact (6 h/day) with dams until weaning at 13 weeks (DAM), reared in isolation with artificial milk until weaning (CONTROL), or reared with dams until 4 weeks and then reared as control group (MIXED). Rumen contents were sampled at 3, 10, 13, 20 weeks to follow establishment of microbiota (metataxonomics).

Rumen microbial community composition (Bray-Curtis index) at 3 weeks was similar between groups in contact with dams (DAM and MIXED) but different in CONTROL. At 10 weeks, the community differed in all groups, but at weaning (13 weeks), DAM and CONTROL clustered together. At 20 weeks of age, dissimilarities were observed for only MIXED and DAM.

These preliminary results confirm that rearing practices affect early-life colonisation and microbial communities later in life. The differences observed in the rumen microbial community in MIXED suggest the effect of the double stress: the separation at 4 weeks and weaning at 13 weeks. Further work is to integrate health data (diseases record, growth...).

P019 | MICROENCAPSULATION AND ITS EFFICACY IN PROTECTING THE VIABILITY OF A NOVEL ANAEROBIC BACTERIUM

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Introduction: Probiotic survival is affected by various factors during storage and consumption, because of exposure to variable temperatures, oxygen gradients, pH, and conditions in the gastrointestinal tract. Consequently, microencapsulation has been extensively evaluated as a potential technique to enhance probiotic viability and function. Published studies concerning probiotic microencapsulation are mainly focussed on lactic acid bacteria and/or Bifidobacterium spp. In contrast, limited studies are available regarding the protective effect against oxygen permeability on the viability of anaerobic bacteria. Here we examined the potential and efficiency of microencapsulation in providing protection to the obligatory anaerobic bacterium, Eubacterium rectale, under harsh conditions including the exposure to high oxygen concentrations and simulated gastrointestinal digestion fluids.

Results: Cells of E. rectale were encapsulated using sodium alginate and calcium chloride solution to form microcapsules that were stored under different temperatures and atmospheric conditions and bacterial survival examined. E. rectale showed significant growth in its rich growth medium after encapsulating with alginate. The optimal storage conditions of the encapsulated bacteria were anaerobic conditions at 4°C. Microcapsules were exposed to simulated gastric digestion fluids to determine the viability of the bacteria under these harsh conditions. The encapsulated bacteria survived each step of simulated gastric digestion, with a gradual decline in numbers.

Conclusions: Microencapsulation within alginate showed promising results in protecting the obligatory anaerobic bacterium E. rectale from exposure to oxygen during storage for up one month at 4°C. Microencapsulation with alginate has potential as a technique to enhance viability of obligately anaerobic bacteria after exposure to harsh conditions.

P020 | THE RUMEN ECOSYSTEM OF SEMI-ADULT GOATS REARED WITH ADULT COMPANIONS ADAPTS DIFFERENTLY TO NUTRITIONAL CHALLENGES

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Rumen development is a complex process that involves substantial changes at anatomical, physiological and microbiological levels, specially at weaning. Several strategies have been suggested to optimize this process, however their long-term effects have not been assessed. Recently, we demonstrated that the presence of adult companions facilitated the rumen microbiological development and the weaning process (Palma-Hidalgo et al. 2021, Front.Vet.Sci. doi: 10.3389/fvets.2021.706592). Here we evaluated the persistency of such intervention in the long term.

Sixteen goat kids were randomly allocated to 2 experimental groups (n=8) after birth. One group (CTL) was kept isolated from adult animals, while the other (ADL) was in continuous contact with non-lactating adults from birth. At 9 months of age, the goats went through two subsequent nutritional trials: one with concentrate-based diet (CON), and one with 100% oat hay (FOR). Each trial lasted 5 days, and samples of orts, feces and urine were taken to assess digestibility. Rumen content was sampled after each trial to determine NH₃-N, volatile fatty acids and protozoal counts. DNA was extracted to perform qPCR on the main microbial groups and 16S sequencing to assess the community structure.

The treatment did not have major effects on the rumen fermentation or digestibility. However, microbial data showed a more abundant prokaryotic community in the ADL kids during the CON trial. Alpha- and beta-diversity analyses showed that the microbial community was substantially different across treatments in both trials, with ADL kids harbouring a more complex and diverse rumen ecosystem, particularly when receiving the FOR diet.

P021 | THE HIGH G+C LACTIC ACID BACTERIA OLSENELLA AND TRACTIDIGESTIVIBACTER ARE IMPORTANT MEMBERS OF THE RUMEN MICROBIOTA INVOLVED IN GLYCYL RADICAL ENZYME-MEDIATED METABOLISM

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High G+C lactic acid bacteria belonging to the genera *Olsenella* and *Tractidigestivibacter* are members of the rumen microbiota that have received little study. Here we examined the genomes of six rumen cultures from these genera in comparison with the type strains of *Olsenella umbonata* and *Tractidigestivibacter scatoligenes* isolated from the pig gastrointestinal tract. These six cultures could be separated into three groups, *O. umbonata*, a novel *Olsenella* species and a novel *Tractidigestivibacter* species. These three groups differed in their complement of carbohydrate utilisation genes indicating that they may play slightly different roles within the rumen. A particular feature was the presence of glycy radical enzyme (GRE) superfamily enzymes in several strains that catalyse reactions rarely found in other bacteria: notably choline trimethylamine lyase which releases TMA for use by methylotrophic methanogens, and arylacetate decarboxylases involved in aromatic amino acid and flavonol metabolism. These activities influence rumen function and the health and environmental impact of the host, making these bacteria important members of the rumen microbial community.

P022 | FUNGAL INFECTION MODELLING USING HUMAN INTESTINAL ORGANOID-DERIVED MONOLAYERS

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Traditional modelling of host-fungal interactions (both commensal and pathogenic) is typically carried out using immortalized cell lines, which are straightforward to manipulate but lack many of the cell types present in human tissue. Recent approaches have introduced multiple cell types in the form of additional immortalized cell lines or manipulate spatial organization of epithelium via organ-chip systems. To build upon these established techniques, human intestinal organoids were assessed for their ability to improve physiological relevance and offer novel insights into host-microbe interactions.

Crypt stem cells, from human bowel resections, were harvested and expanded in 3D as organoids before dissociation and seeding of 2D organoid-derived monolayers (ODMs). ODMs were grown to confluency over two weeks and encouraged to differentiate over one week. Epithelial integrity was monitored during growth and immunofluorescence experiments were performed to confirm presence of different cell types. Fungal coculture experiments with *Candida albicans* (a common microbiome-associated yeast) assessed fungal adhesion, invasion, and translocation of differentiated gut epithelium.

Multiple cell types within ODMs were confirmed via immunofluorescence, including enteroendocrine and goblet cells. Compared to Caco-2 cell line monolayers, ODMs allowed for longer coculture time with *C. albicans*, and three fungal infection metrics were measurable in ODMs with minimal changes to methodology.

Human ODMs offer enhanced physiological relevance of host-microbe models through presentation of multiple cell types and longer experimental timeframes. They can be used in complex experimental conditions, such as models of fungal escape from the gut, and may reduce reliance on animal models to address intricate research questions.

P023 | A GUT MICROBIOME PERSPECTIVE OF EXTRACELLULAR VESICLE-MEDIATED COMMUNICATION BETWEEN HUMAN CELLS AND BACTERIA.

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Extracellular vesicles (EVs) are released by cells of all species. EVs are involved in cell-to-cell communications involving a molecular cargo comprising enzymes, toxins, immune decoys, nutrients, signals that influence gene expression, and/or genetic material. EV communications can be intraspecies, interspecies and interkingdom, but few examples describe EV-mediated communication between human cells and bacteria in the gut. We have investigated the effects of EVs from the human colorectal adenocarcinoma cell line HT29 on pathogenic and probiotic strains of *Escherichia coli*. EVs from cell-free conditioned media collected from a CELLLine bioreactor growing HT29 cells were concentrated by ultracentrifugation and purified by size exclusion chromatography. EVs were verified by the presence of CD81, TSG101, and the absence of GRP94 by Western blot, visualised by TEM and quantified by nanoparticle tracking analysis. EVs added at $\approx 10^{11}$ EVs/mL, but not at $\approx 10^8$ or $\approx 10^9$ EVs/mL, stimulated the growth of both strains of *E. coli* in RPMI 1640 medium. The supplementation of the medium with $10 \mu\text{M}$ FeCl_3 gave a similar stimulation of growth to $\approx 10^{11}$ EVs/mL, and the combination of EVs and $10 \mu\text{M}$ FeCl_3 did not stimulate additional bacterial growth. The physical interaction of EV and bacteria was demonstrated using flow cytometry, cell sorting and confocal microscopy, with EVs stained with CMPTX-red and bacteria stained with Syto-9. A comparative transcriptomics approach was applied to investigate bacterial responses to HT29 EVs at non-nutritional levels, with results supporting a hypothesis where bacteria receiving EV-communications are better able to compete in the colon.

P024 | SCREENING BACTERIOCIN EXTRACTS FROM RUMEN STREPTOCOCCUS EQUINUS STRAINS FOR INHIBITORY ACTIVITY AGAINST A RUMEN METHANOGEN.

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Previous studies have reported that rumen streptococci can produce a range of bacteriocins but few of these have been characterized. Genome sequences of 26 rumen *Streptococcus equinus* strains were screened with the web-based genome mining tools BAGEL4 and antiSMASH 5.0 to detect gene clusters encoding the biosynthesis of possible antimicrobial compounds. All 26 strains contained one or more genes predicted to encode the production of bacteriocins, and/or lanthipeptides. Each genome sequence was checked to examine the genomic context and two different lanthipeptide gene clusters with potential antimicrobial activity were detected. Five strains contain an *ll* gene cluster for the production of the novel nisin variant, nisin E, which has been reported in other *S. equinus* strains. Nine strains encode the production of a second uncharacterized lanthipeptide (pfam 04604, Type-A lantibiotic). Genes for both lanthipeptides are present in three strains (SNO33, B315, GA-1). In order to test if bacteriocins from rumen streptococci could be a potential methane mitigation option filtered culture supernatants were prepared from each of the 26 rumen *S. equinus* strains. These culture supernatants were tested in triplicate for the ability to inhibit growth of the rumen methanogen *Methanobrevibacter boviskoreani* JHIT in a high throughput, 96 well microtitre plate growth inhibition assay. Culture supernatants from all strains showed an inhibitory effect. *S. equinus* Sb13 completely inhibited the growth of the methanogen, and nine strains (35%) showed more than 50% inhibition.

P025 | COMPARATIVE TRANSCRIPTOMIC ANALYSIS OF CELLULOLYTIC RUMEN BACTERIA IN MONO- VS CO- CULTURE

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Understanding cellulolytic bacterial interactions that allow for cooperative degradation of cellulose in high-fiber diets is necessary to improve ruminal fermentation. Therefore, the objective was to investigate changes in transcriptional responses between cellulolytic rumen bacteria in mono- or co-culture to identify potential mechanisms for competitive or cooperative growth. *Ruminococcus albus* 7 and *Fibrobacter succinogenes* S85 were grown in mono- and co-culture over 24 h in a cellobiose-containing media. *R. albus* and *F. succinogenes* co-culture had greater ($P < 0.05$) optical density over 24 h of incubation compared with both mono-cultures. Based on qPCR results, *F. succinogenes* had greater ($P < 0.05$) abundance in co-culture compared with mono-culture at h 4 and 8. However, acetate concentration was similar ($P = 0.08$) among both mono- and co-cultures. Transcriptional pathway analysis showed that *F. succinogenes* had upregulated (> 2 -fold; $P < 0.05$) genes involved with glycolysis, pyruvate fermentation, cell structure synthesis, and amino acid synthesis/degradation when co-cultured with *R. albus*. Similarly, *R. albus* had greater ($P < 0.05$) abundance in co-culture compared with mono-culture at h 4, 8 and 16. Additionally, *R. albus* had upregulated (> 2 -fold; $P < 0.05$) genes involved with fatty acid synthesis, amino acid synthesis/degradation, thiamin biosynthesis, and ammonia assimilation when in co-culture with *F. succinogenes*. Increased expression of genes involved in energy metabolism, fatty acid biosynthesis, and amino acid synthesis suggests a benefit from *F. succinogenes* S85 and *R. albus* 7 being co-cultured. This knowledge can inform new strategies to optimize syntrophic exchanges for enhanced cellulose degradation.

P026 | METABOLIC EXPLORATION OF THE OligoMM19.1 SYNTHETIC COMMUNITY OF MOUSE GUT BACTERIA TO UNDERSTAND HOST-MICROBE INTERACTIONS

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The gut microbiota is a complex community of many known, and unknown species. Due to this, an understanding of host-microbe interactions and underlying mechanisms within the gut can be hard to define. Synthetic microbial communities (SynComs) consisting of known commensal bacteria, such as OligoMM12 and OligoMM19.1, can be used to better understand microbe-microbe and microbe-host interactions, in a standardised environment.

Previously, we have shown that the OligoMM19.1, when compared to germ-free, OligoMM12 and SPF mice, successfully recapitulates the phenotype of a complex microbiota in a number of different parameters, including the size of main organs and specific immune cells within the gut. Targeted metabolite analysis of intestinal content identified significant differences between the groups, such as production of the secondary bile acid 'deoxycholic acid' within the OligoMM19.1 group, and increased concentrations of short-chain fatty acids propionate and isovalerate in OligoMM19.1 mice in comparison to OligoMM12.

We then focused our attention on metabolites produced by the bacteria within the consortium. Using the individual strains of OligoMM19.1, we first bioinformatically predicted the production of metabolites (including short-chain fatty acids). Metabolite production was then confirmed using HPLC-RI analysis. Colonisation of gnotobiotic mice with OligoMM12 plus individual OligoMM19.1 strains is being performed to confirm their contribution to the phenotype differences between OligoMM12 and 19.1 mice.

P027 | CHARACTERISATION OF THE PROBIOTIC POTENTIAL OF LACTIPLANTIBACILLUS PLANTARUM K16 AND ITS ABILITY TO PRODUCE THE POSTBIOTIC METABOLITE γ -AMINOBUTYRIC ACID

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Lactiplantibacillus plantarum has been widely studied due to its beneficial effects on health such as protect against pathogens, enhance the immune system, or produce metabolites like γ -aminobutyric acid (GABA). The objective of this study was the evaluation of the GABA-producer L. plantarum K16 isolated from kimchi. The safety and probiotic characterisation of this strain was performed by analysing carbohydrates fermentation, enzymatic activity, antibiotics susceptibility, and haemolytic and antimicrobial activity. Likewise, GABA production was optimised following a one-factor-at-a-time procedure by changing relevant fermentation parameters like incubation temperature, yeast extract concentration and fermentation time. The results indicated that L. plantarum K16 has the potential to stimulate the digestion and absorption of several nutrients and it could have an inhibitory effect against pathogenic bacteria. The best results for GABA production by this strain was around 1000 mg/L, using 12 g/L of yeast extract, 34 °C of incubation temperature and 96 h of fermentation time.

P028 | SURF AND TURF: THE ADAPTATION OF RUMINANT MICROBIOMES TO MARINE FORAGES

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Growth of cattle feed competes for arable land, water, fertilizer and other resources. Other environmental concerns are related to the inefficient digestion of forage and feeds, which results in emission of enteric methane. Alternative marine-based sources of feed, such as seaweeds, pose promising solutions as an alternate cattle feed; however, the digestibility of marine-based forages by cattle has not been extensively studied to date. Marine forages contain complex, and often sulfated, polysaccharides unique to algal species, including carrageenans and agarose (red seaweed), and ulvans (green seaweed). The gastrointestinal microbiome of cattle metabolize complex cell-wall polysaccharides by depolymerizing them into fermentable simple sugars by the action of carbohydrate-active enzymes (CAZymes). Studying cattle known to forage on feeds enriched in seaweed polysaccharides may provide valuable insight into new microbial pathways involved in seaweed digestion.

Here we used multiomics methods, including whole metagenome sequencing, metaproteomics and metatranscriptomics, along with culture enrichments and fluorescently labelled carbohydrate (FLAPs) to identify microorganisms and unique CAZymes from the fecal samples of marine forage-consuming cattle. We discovered CAZymes within polysaccharide utilization loci predicted to be active on seaweed polysaccharides. Functional characterization of these CAZymes using liquid chromatography–mass spectrometry and capillary electrophoresis-laser-induced fluorescence to identify digestion products of seaweed polysaccharides led to the discovery of several unique CAZyme specificities active on seaweed cell-wall polysaccharides, including ulvan (PL24, PL40) and carrageenans (GH16). These results may have implications for the cattle industry of the future as marine feedstocks represent environmentally sustainable and underutilized solutions for the livestock industry.

P029 | FAECAL MICROBIOME CHANGES IN DAIRY CATTLE GRAZING ON SILVOPASTORAL ARRANGEMENTS AND TRADITIONAL PASTORAL SYSTEM FROM FARMS LOCATED IN HIGH ALTITUDE IN COLOMBIA ANDES.

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Silvopastoral arrangements (SA) consisting of remarkable shrubs species with high nutritional value and/or biomass production, which can be used as livestock fodder. The shrubs were integrated as alternative forage and were examined their effects on milk quality and production, methane emission (CH₄) by Hand Laser Detector, and changes in the faecal microbiota by Illumina Miseq. In this study, thirty dairy cows were randomly assigned to graze on three different SA, SA1) *Cenchrus clandestinus*(90%)+*Sambucus peruviana*(10%); SA2) *C. clandestinus*(95%)+*Baccharis latifolia*(5%) and SA3) *C. clandestinus*(95%)+*Acacia decurrens*(5%) vs Control (*Cenchrus clandestinus* -100%) on three farms located in high altitude (2500 m.a.l.s). In total 3.402.466 paired-end reads were obtained using 16S rDNA gene sequencing. Data were analyzed as a completely randomized design using PROC GLIMMIX of SAS ENTERPRISE 8.3. Results showed that cows in SA1 and SA2 had significantly ($P < 0.05$) higher milk production (+16.6% and +23.8%, respectively) than control. Milk quality and CH₄ only were affected by SA1 compared to control, fat contents increased by +31% and CH₄ was reduced by 19.5%. In overall, The Firmicutes (55%) were the dominant phylum in the three SA, followed by Bacteroidetes (16-29%), Proteobacteria (15%), and Actinobacteria (6-12%). The families predominant into S1 were Prevotellaceae (28%), Lachnospiraceae (14%) and Ruminococaceae (10%); in SA2 and SA3 were Lachnospiraceae(14%), Ruminococaceae (13%) and Peptostreptococcaceae (12%). These results could be providing an insight about some SA could had impact on microbial population and seemingly it impact could be used to improve milk production, decrease methane emissions and drive a sustainable production.

P030 | FIBRE-DEGRADING BACILLUS PROBIOTIC CANDIDATE IMPROVES METABOLIC ACTIVITY OF COLONIC BUTYRATE-PRODUCERS IN AN IN VITRO OBESE MODEL

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The human gut microbiota plays a crucial role in maintaining energy homeostasis and is influenced by our diet. Impaired production of short-chain fatty acids (SCFAs) and depletion of fibre-degrading-SCFA-producing bacterial members have been linked to metabolic syndrome. Probiotics have emerged as a promising approach to restore gut homeostasis. This study evaluated the impact of combining dietary fibre and a probiotic candidate with fibrolytic properties on the gut microbiota and metabolites in obese individuals.

Faecal bacteria from obese donors were cultivated in chemostats with a high-fat diet before switching to a medium supplemented with various dietary fibres and a *Bacillus* probiotic candidate. SCFAs were quantified by gas chromatography, and gut bacteria composition was investigated by shotgun metagenomics.

The study found that switching from a high-fat diet to an enriched-fibre diet increased SCFAs and fibre-degrading-SCFA-producing bacterial members in obese microbiota. Additionally, intake of the fibrolytic probiotic candidate in combination with the enriched-fibre diet significantly increased butyrate production in-vitro. The abundance of fibre-degrading and SCFA-producing bacterial members was characterized by dietary fibre and probiotic ability to modulate the balance of gut bacteria in obese individuals.

In conclusion, combining functional fibres and a fibrolytic probiotic *Bacillus* strain could be an effective strategy to modulate the microbial community of obese individuals. The findings of this study have implications for the development of dietary interventions for metabolic syndrome and other related disorders

P031 | PHENOLIC COMPOUNDS INCREASED PHENOLIC UTILIZATION BACTERIA ABUNDANCE WHEN METHANOGENESIS WAS INHIBITED IN VITRO

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Two previous in vitro studies found that phloroglucinol and pyrogallol decreased ruminal dihydrogen accumulation and increased total volatile fatty acid production when methanogenesis was inhibited. The present work builds upon the previous study describing the effects of these phenolic compounds on the bacterial community when methanogenesis was inhibited with rumen inoculum from cows and goats. There were four treatments in the cow's inoculum experiment: CTL (control), BES (3 mM 2-bromoethanesulfonate, methanogenesis inhibitor), PHL (36 mM phloroglucinol), and BES+PHL (3 mM 2-bromoethanesulfonate + 36 mM phloroglucinol). There were six treatments in the goat's inoculum experiment: CTL (control), AT (2% *Asparagopsis taxiformis*), AT+PHL1 (2% AT + 6 mM phloroglucinol), AT+PHL2 (2% AT + 36 mM phloroglucinol); AT+PYR1 (2% AT + 6 mM pyrogallol), and AT+PYR2 (2% AT + 36 mM pyrogallol). In both experiments, PHL and PYR at 36 mM decreased bacterial α -diversity. Most bacteria taxa with increased abundances (including Lactobacillaceae, Bifidobacteriaceae, and Lachnospiraceae) were known with the phenolic compounds utilization, while the abundance of cellulolytic bacteria (including Fibrobacteraceae, Ruminococcaceae, and Succinivibrionaceae) decreased with this supplementation. Although the structure of rumen bacterial community differed between cows and goats inocula, about half of the bacterial clades whose abundance was affected by 36 mM PHL in the cow's inoculum experiment were equally affected by 36 mM PHL and PYR in the goat's inoculum experiment. These findings suggest that nutritional supplementation with phenolic compounds could modify the rumen bacterial community towards a more efficient rumen dihydrogen metabolism and ultimately as a methane mitigation strategy.

P032 | THE USE OF A CONTINUOUS FLOW IN VITRO MODEL TO ASSESS THE ROLE OF PSYLLIUM AS A POTENTIAL MECHANISM FOR FODMAP CONSUMPTION IN IBS PATIENTS

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Irritable bowel syndrome (IBS), is a syndrome associated with gastrointestinal discomfort and is often managed by removing Fermentable Oligosaccharides, Disaccharides, Monosaccharides, and Polyols (FODMAPs) from the diet. Another treatment option for IBS patients is psyllium husk, the gelling properties of which can improve symptoms of gastrointestinal discomfort. Inulin is a prebiotic fibre, and a FODMAP which has many beneficial health effects, is also associated with increased colonic gas, making it unsuitable for individuals who have IBS. In previous research, we have shown that combining psyllium with inulin can prevent increases in colonic gas production and the associated discomfort in IBS patients (Gunn et al 2022, doi: 10.1136/gutjnl-2021-324784). Using an in vitro model of the colon mimicking the terminal ileum (Vessel 1, pH 7), proximal (Vessel 2, pH 5.5) and distal colon (Vessel 3, pH 6.8) in a continuous cascade the effect of psyllium on markers of gut health and microbiome composition was assessed. The models were seeded with faecal inoculum two days prior to the addition of inulin alone or inulin plus psyllium which was added as a single bolus to vessel 1. Psyllium reduced and delayed the peak in metabolite production compared to the inulin alone. Fermentation with inulin and psyllium also altered the metabolite profile, by increasing propionate compared to inulin alone. These results indicate that psyllium delays fermentation of FODMAPS, reduces the peak of fermentation, and leads to increased propionate production. This may explain why FODMAPs are more acceptable in IBS patients when combined with psyllium.

P033 | INFLUENCE OF ZINC OXIDE NANOPARTICLE ON THE RUMINAL BACTERIAL MICROBIOTA OF SHEEP

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Introduction: Microbial colonization of the rumen is a complex process and occurs simultaneously with animal development and maturation of the host immune system. Zinc plays as an essential trace mineral for ruminants, improving immunological functions, antimicrobial activity and utilization of cellulose. This element is used as a supplement diet in livestock as organic chelates or inorganic salts. Supplementation with zinc oxide nanoparticles (Zn-NP) are presented as alternative sources of dietary zinc to provide controlled release of Zn.

Aim: This study aimed to evaluate the effect of Zn-NP in the selected microorganisms from ruminal microbiota of sheep. We grouped eight rumen-fistulated assigned to a 4 × 4 Latin square (4 treatments and 4 periods) fed diets with varying concentrations of Zn-NP (0, 50, 100, 150, mg/animal/day). Rumen fluid samples were taken for DNA extraction and copy number of total Bacteria, Fibrobacter succinogenes, Ruminococcus flavefaciens, Ruminococcus albus, Clostridium sp, Prevotella ruminicola, Ruminobacter amilophylum and Selenomonas ruminantium were quantified by real-time quantitative PCR analysis (qPCR).

Results: The results showed a positive linear regression model between ZN-NP levels with copy number of Selenomonas ruminantium ($y=0.0029x + 4.1$, $r^2=0.27$ $p=0.0033$), and total Bacteria ($y=0.00058x + 7.70$, $r^2=0.11$, $p=0.1026$). However, no effect was observed on the Fibrobacter succinogenes, Ruminococcus flavefaciens, Ruminococcus albus, Clostridium sp, Prevotella ruminicola, Ruminobacter amilophylum communities.

Conclusion: Zn-NP supplementation increased the abundance of total Bacteria and Selenomonas ruminantium, suggesting that Zn-NP may modulate the ruminal bacterial microbiota in a positive way, however further research is needed.

P034 | EFFECT OF DAIRY FERMENTATES ON INSULIN STATIC SECRETION IN AN IN VITRO MODEL OF HUMAN EndoC-βH1 CELL LINE

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There is growing evidence of connections between gut microbiota dysbiosis and suboptimal health conditions, such as type I and type II diabetes, among others. It has been suggested that the development and progression of these conditions might be to some extent associated with changes in dietary habits in Western societies. Therefore, there is a need for healthier food options that could help and/or alleviate conditions resulting from an unbalanced gut microbiota.

Fermented foods and fermentates (powdered ingredients generated through fermentation) are attracting attention as healthier alternatives to Western diets and their consumption has been proposed as a strategy to help and/or alleviate conditions arising from an unbalanced gut microbiota, including diabetes.

Here, we investigated the impact of six dairy fermentates on the bacterial communities in an ex vivo model of the human distal colon. We analysed the changes in the relative abundance of bacterial populations as well as their functional profile and ability to produce short chain fatty acids after exposure to two concentrations of each dairy fermentate. Additionally, we evaluated the effect of the cell free supernatants produced in these colonic environments on the insulin secretion response in a static in vitro model using EndoC-βH1, a cell line validated for studying β-cell pancreatic response in humans. To the best of our knowledge, this is the first report on the impact of dairy fermentates, via the gut microbiota, on pancreatic function using EndoC-βH1 cells.

P035 | LINKING DIET AND GUT MICROBIOTA IN OBESITY: USEFULNESS OF A NEW IN VITRO HUMAN MUCOSAL COLON MODEL

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Obesity is a complex, multifactorial and highly prevalent disease, strongly associated with nutritional disorders and gut microbiota perturbations. For technical, regulatory, ethical and cost reasons, in vitro models simulating the human digestive tract can be a relevant alternative to in vivo assays, provided they are fully validated against in vivo data in humans. To date, no relevant in vitro model reproducing the nutritional, physicochemical and microbial parameters of the obese human colon has been described.

An intensive literature review was performed to adapt the Mucosal Artificial Colon (M-ARCOL) model to the specific colonic environment of obese patients (pH, retention time and composition of ileal effluents). Stools from 9 donors (4 healthy and 5 obese) were used to inoculate two bioreactors ran in parallel, set-up to reproduce either healthy or obese parameters.

When applying obese parameters on healthy stool, significant shifts in microbiota activity and composition were observed, in accordance with in vivo data ($P < 0.05$). Less methane but more short chain fatty acids and associated energy were produced. An increase in obesity-associated marker populations (Prevotellaceae, Veillonellaceae) and a decrease in healthy-associated marker populations (Archaea, Akkermanciaceae, Rikenellaceae and Christensenellaceae) were also observed in lumen and mucus-associated microbiota, together with a lower bacterial diversity. Interestingly, when applying healthy parameters on obese stools opposite trends were obtained demonstrating gut microbiota resilience.

Obese M-ARCOL model can represent a powerful platform as an alternative to in vivo animal assays in preclinical trials to perform mechanistic studies and evaluate nutritional strategies aiming to restore gut microbiota eubiosis.

P036 | MECHANISMS UNDERPINNING THE EFFECT OF RESISTANT STARCH FROM PEAS ON HEALTH USING HUMANISED MICE

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Starch is composed of two types of glucose polymers: 10 to 30% of amylose, a linear polymer, and 70 to 90% of amylopectin, a branched polymer. Starches high in amylose are generally more resistant to digestion by host amylases than amylopectin rich starches. Food processing such as milling or cooking can also affect starch digestibility.

Previous work reported that a natural mutation (rr) in *Pisum sativum* L. (pea) altering starch amylose content improved glucose homeostasis in humans. Here, we addressed the role of gut microbiota composition and metabolism in this process. Mice humanised with gut microbiota from human donors were fed pea starch from both wild-type and rr peas, milled at two different particle sizes. We showed that starch concentration varied in the ileum of mice based on the diet. Shotgun metagenomics showed 1) drastic changes in microbiota composition as early as 3 days after the diet switch and 2) an increased proportion of *Bacteroides intestinalis*, a bacterium potentially able to grow on raffinose, in mice fed rr pea starch. Metatranscriptomics and metabolomics provided additional insights into the effect of resistant starch and food structure on microbial and host metabolism.

P037 | THE GASTROINTESTINAL TRACT SHAPE MICROBIAL ECOLOGICAL STRUCTURE OF LLAMAS FED ON NATIVE GRASSLANDS IN THE ARGENTINE ANDES

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Llamas are versatile and robust animals that have adapted to the extreme climatic conditions of the Andes in South America and are raised primarily for their meat and fiber production. This study aimed to explore the microbiota community composition at five gastrointestinal tract (GIT) sites in llamas fed on native grasslands and supplemented with concentrate. The experiment was conducted at 3,500 m.a.s.l. (Jujuy, Argentina). Eighteen young llamas (*Lama glama*) of 68.8ffl0.6 kg body weight (BW), were randomly allocated to two dietary treatments: G, native grassland (mainly *Festuca* sp) and GS: G + concentrate (1.5 % BW; 80:20, corn: soybean ratio) for 45 days. Sampling of compartment 1 (C1), compartment 3 (C3), small intestine (SI), large intestine (LI) and feces were after slaughter. Meta-taxonomy analysis was performed to explore microbiota composition. Microbial community structure differed between GIT sites and between diets ($P < 0.001$). Diversity and richness indices were higher at C1, C3, LI and feces compared to SI ($P < 0.001$). Regardless of diet, Bacteroidota (58.5ffl18.5%) and Firmicutes (31.4ffl17.1%) phyla predominated in the C1 and C3; in contrast, Firmicutes (86.2ffl13.6%) predominated in the SI, and in the LI and feces both phyla predominated, although Firmicutes (65.2ffl15.1%) were more abundant. Notably, Actinobacteria (4.1ffl2.5%) and Euryarchaeota (3.1ffl1.8%) phyla were detected in the SI, and Proteobacteria (1.8ffl1.2%) were detected only in C1 and C3. Our results suggest the divergent composition of the microbial structure is shaped by the GIT and diets; this could be due to the GIT environmental conditions and substrate supplied to the microbiota.

P038 | THE PREBIOTIC EFFECT OF A RED SEAWEED ON BACTERIAL ABUNDANCE AND SHORT CHAIN FATTY ACID PRODUCTION IN A SIMULATED GUT MODEL

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The community of bacteria that reside in the human gut and their production of short chain fatty acids (SCFA) impacts the overall health and immune status of the host. The ingestion of prebiotic components by the host can enhance the abundance of bacterial species and increase their SCFA production. A South Australian red seaweed was assessed for its potential use as a novel prebiotic. The whole, dried seaweed thallus (WH), polysaccharide (PS) and polyphenol (PP) extracts were digested with gastric enzymes and fermented in a simulated anaerobic gut model with human faecal inoculum. SCFA produced were quantified by gas chromatography, and the relative abundance of bacteria by 16S rRNA sequencing. Inulin (INU) and epigallocatechingallate (EGCG) were used as positive polysaccharide and polyphenol controls.

After 24 hr, total SCFA (predominantly butyric, acetic and propionic acids) produced by samples fermented with red seaweed PS (213.20 $\mu\text{mol/mL}$), WH (183.94 $\mu\text{mol/mL}$), and PP (156.17 $\mu\text{mol/mL}$) were significantly greater than samples fermented with INU (71.05 $\mu\text{mol/mL}$) and EGCG (7.76 $\mu\text{mol/mL}$). WH, PS and PP significantly improved the Firmicutes/Bacteroidetes ratio compared to INU and EGCG, and the abundance of *Barnesiella* species, which are positively associated with regulation of the microbiota composition. WH and PS extracts (but not PP) increased species diversity, richness, and the abundance of Lactobacillales, Faecalibacteria, Roseburia, Butyrivococcus, and Blautia which are associated with butyric and lactic acid production, and immune function. We conclude that extracts from this red species may have potential as prebiotic functional foods to maintain a healthy gut bacterial composition.

P039 | AGRICULTURAL BYPRODUCTS AS POTENTIAL FEED ADDITIVES FOR ENTERIC METHANE MITIGATION

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Greenhouse gasses (GHG) from livestock, specifically methane (CH₄) which has 28 times the global warming potential of carbon dioxide (CO₂), contribute a significant portion of GHG from anthropogenic activities. Methane emission from enteric fermentation in ruminants contributes to 2-12% of gross energy loss, negatively affecting the feed efficiency of the animal. Numerous feed additives that reduce the production of enteric fermentation have been identified, including the red seaweed *Asparagopsis taxiformis*. However, many of these feed additives, including *A. taxiformis* have limitations, such as year round availability and easy global access. These limitations prompted us to investigate other options, more specifically compounds that are readily available on a large scale. An example of these compounds are byproducts from local and regional plan-based agriculture. Many byproducts from agriculture can not be sold as food and are considered waste by the producer. Examples of byproducts are pomace from fruits and vegetable processing. These leftover byproducts still contain high levels of energy which can be utilized by ruminants as a food source as well as microbially active compounds, such as tannins and saponins. Here we will provide an overview of currently underappreciated byproducts that might hold the potential to be used as feed additives for methane mitigation.

P040 | THE RICHER THE BETTER: 16S METABARCODING ANALYSIS OF GUT MICROBIOTA OF LAYING HENS IN RELATION TO FEED EFFICIENCY AND ADAPTATION TO DIET CHANGE

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In the egg industry, feed cost represents the majority of total production costs and breeding efforts are ongoing to improve feed efficiency of laying hens. The gut microbiota is known to play an important role in energy harvest and is likely to affect feed efficiency. In this study, we analysed the composition of the caecal microbiota of 31 week old hens by 16S metabarcoding sequencing to characterise its composition, interactions with the host and influence on phenotypes of interest. As an animal model, we used hens of the R+ and R- lines divergently selected for high (low feed efficiency) and low (high feed efficiency) residual feed intake values respectively, that were fed either a commercial wheat-soybean diet (CTR) or a low-energy corn-sunflower diet (LE). Our results show a line effect on the microbiota composition with the CTR diet, whereas in the LE diet, the microbiota was primarily affected by the diet change. A line x diet interaction was observed: the high efficient R- line presented a greater microbial richness and a reduced impact of the diet change compared to the low efficient R+ line. Interestingly, common taxonomies and/or predicted functions were highlighted between R+ and CTR diet, and between R- and LE diet, which could suggest that common microbiota mechanisms between feed efficiency and adaptation to nontraditional feedstuffs exist. These results provide insight into the role of the microbiota in laying hen feed efficiency and the impact of diet composition on microbiota.

P041 | IN DEPTH-EXPLORATION OF THE INTERACTIONS BETWEEN PREBIOTICS AND A PANEL OF GUT BACTERIA OF HEALTH INTEREST

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The gut microbiota plays a fundamental role in various biological functions and contributes significantly to host health. The provision of dietary substrates for resident microorganisms to modulate the composition and the metabolic activities of the gut microbiota incorporates the concept of prebiotics. These non-digestible food ingredients, through their bacterial metabolization, provide essential nutrients and confer beneficial effects on the host physiology. A dietary supplementation of prebiotics constitute a nutritional strategy to restore and/or maintain the equilibrium within microbial communities. Nevertheless, it is still unclear how the dynamic interplay between prebiotics and microbes maintain the intestinal homeostasis. The current challenge is to understand the interactions that link prebiotics to digestive health and wellbeing. In this study, we explored the relationships between a panel of putative health-promoting bacteria and a range of indigestible food ingredients present in our diet. Genomic analyses allowed the characterisation of enzymatic repertoires involved in the metabolization of extremely diverse polysaccharides providing substrates for the gut microbiome. These predictions were further investigated through single-carbohydrate cultures to evaluate their prebiotic metabolization. Short Chain Fatty Acid production profile suggested complementary phylum-dependent levels of commensal bacteria to metabolize prebiotic ingredients. Each bacterial species showed different degrees of dietary carbohydrate utilisation, which seemed driven by their enzymatic capabilities. Finally, a transcriptomic approach was used to identify the genes involved in the metabolism of prebiotics. Replenishing health promoting bacteria through prebiotics represents a prerequisite for personalized nutrition and an opportunity to tailor dietary interventions.

P042 | THE EFFECTS OF GUT PROBIOTIC SUPPLEMENTATION ON BRAIN HEALTH AND COGNITIVE FUNCTION IN A PRETANGLE TAU ALZHEIMER'S DISEASE ANIMAL MODEL

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The gut microbiota is recognized for its connection to Alzheimer's disease (AD) with decreased diversity and increased dysbiosis in patients. Gut-associated microbes and cytokines can permeate the gut and blood-brain-barrier (BBB) to promote tau hyperphosphorylation and neuroinflammation in AD. Our laboratory has developed a pretangle tau model, seeding hyperphosphorylated human tau (htauE14) in the rat locus coeruleus (LC), recapitulating the key preclinical features defined by Braak. Using this model, we aim to explore the therapeutic potential of increasing gut health through probiotic supplementation on pre-tangle tau pathology. Additionally, we test whether modulating the gut microbiome can either ameliorate stress induced cognitive impairment or enhance the positive effects of enrichment on cognition. Tyrosine hydroxylase (TH)-Cre rats were infused in the LC region with AAVs carrying htauE14 gene 2-3 months postnatally. GFP only AAVs served as a control. Probiotic supplementation or normal diet was given to rats (9-12mo), followed by a battery of behavioral testing (12mo). Fecal and blood samples were collected to measure gut microbiome alterations and biomarkers. SD rats were put through chronic stress or enrichment paradigms (9-10mo) to explore the effect of probiotic supplementation (3-6mo) on cognition. Motility, stress, hedonic behavior, spatial and odor discrimination were tested. Gut microbiomes contain higher levels of Lactobacillus and Bifidobacteria post-feeding. Spatial memory deficit observed in htauE14 groups and late-stress groups can be rescued through probiotic supplementation. Increasing gut health resulted in higher levels of exploratory behavior when paired with enrichment. Measurement of gut microbiome composition, blood and brain markers are ongoing.

P043 | METABOLIZATION OF FLAVAN-3-OL OLIGOMERS BY HUMAN GUT BACTERIADr Ruben Halifa¹, Dr Agnes Cornu², Prof. Pierre Peyret¹, Dr Claire Dufour³, Dr Carine Le Bourvellec³,**Dr Pascale Mosoni¹**¹UMR454 MEDIS, Université Clermont Auvergne, INRAE, Clermont-Ferrand, France,²UMRI213 Herbivores, VetAgro Sup-Université Clermont Auvergne, INRAE, Clermont-Ferrand, France,³UMR408 SQPOV, INRAE, Avignon Université, Avignon, France

Flavan-3-ols are a largely consumed subclass of flavonoids and are involved in the prevention of cardiovascular diseases [1]. The contribution of phenolic metabolites produced by the gut microbiota in the health effects of polyphenols (including flavan-3-ols) is currently an open field of investigation. Although a few bacterial species metabolize flavan-3-ol monomers [2] no bacterial isolates active on oligomers (called procyanidins) have been described. Knowing that the gut microbiota hydrolyzes procyanidins [3], our aim was to identify bacteria degrading flavan-3-ol oligomers and the degradation products.

From human stools of three healthy individuals, culturomic approaches combined with screening for the metabolic activity of bacterial isolates by HPLC-DAD allowed us to obtain four strains of *Eggerthella lenta* and one strain of *Flavonifractor plautii* degrading (+)-catechin and (-)-epicatechin. The activity of these strains was then tested on B-type (DP2 to 4) and A-type (DP2) procyanidins and the metabolites generated were characterized by LC-ESI-MS / MS. These two species co-metabolized (+)-catechin and (-)-epicatechin into hydroxyphenylvaleric acid derivatives. Only *E. lenta* converted procyanidins while *F. plautii* alone or in co-culture with *E. lenta* did not show any activity towards procyanidins. The reaction catalyzed by *E. lenta* on dimers (B-type and A-type) corresponded to the opening of the C-ring of the terminal unit. This work is the first report of flavan-3-ol oligomers metabolization by the human gut bacterium *E. lenta*.

1. Fraga et al (2019). *Food Funct.* 10, 514-528.2. Braune & Blaut (2016), *Gut Microbes*, 7, 216-2343. Le bourvellec et al (2019) *Nutrients*, 11, 664.**P044 | EVALUATION OF A COLOMBIAN OREGANO OIL (LIPPIA ORIGANOIDES) COMBINED WITH A NOVEL YEAST PRODUCT (MEYEROZYMA GUILLIERMONDII) ON RUMEN FERMENTATION AND BACTERIAL COMMUNITIES IN THE RUMEN SIMULATION TECHNIQUE****Dr Eva Ramos-Morales^{1,2}**, AM Sierra-Alarcón³, Dr JM Palma-Hidalgo², Dr O Mayorga³, Dr CJ Ariza-Nieto³, Prof. CJ Newbold³¹CSIC, Spain, ²SRUC, United Kingdom, ³Agrosavia, Colombia

It has been suggested that combinations of feed additives with complementary mechanisms of action may synergistically or additively decrease methane production without any adverse effects on feed digestion or fermentation. Yeast supplementation could modulate the antimicrobial effect of a high thymol containing oregano oil, allowing a more efficient feed utilization. In this study different doses (0.08, 0.16 or 0.25%) of essential oil from *Lippia origanoides* Kunth, native to Colombia, were combined with *Meyerozyma guilliermondii* (0.5 g/L), inactivated yeast from the germplasm bank of Agrosavia (Colombian Agricultural Research Corporation), to study their long term effect on rumen fermentation and methanogenesis whilst also characterising their effect on bacterial communities. None of the treatments had an effect on nutrient utilization or methane production; however, the combination of yeast with oregano oil at 0.16 and 0.25% decreased ammonia production by 22-26%. Overall, treatments had a great effect on the bacterial community structure, with the highest doses of essential oil having a stronger effect. At the phylum level, treatments increased the relative abundance of Firmicutes, without affecting that of Bacteroidetes. Within the Firmicutes phylum, the greatest effect was observed for Lachnospiraceae, Ruminococcaceae and Christensenellaceae, which increased. The inclusion of *Meyerozyma guilliermondii* and oregano oil from *L. organoides* in the diet could potentially lead to an increase of the nitrogen use efficiency also reducing the losses of nitrogen excreted in urine. Further studies are needed to both test higher doses of oregano oil combined with the yeast and to elucidate their mode of action.

P045 | FAECAL MICROBIOME MEDIATES THE EFFECT OF DIET ON COLORECTAL-CANCER RISK: COMPARISON OF MEAT BASED VERSUS PESCO-VEGETARIAN DIETS

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Two validated models of colorectal cancer (CRC): Pirc rats, mutated in the Apc gene and thus developing spontaneous colon tumours and Azoxymethane (AOM)-induced rats were fed for 3 months with high-CRC risk diet (meat-based MBD), a normalized CRC risk diet (MBD plus alpha-tocopherol, MBD-T), a low-CRC risk diet (pesco-vegetarian, PVD) and a control diet (CTRL). Pirc rats fed the PVD diet, showed a significantly lower number of colon tumors than rats fed all the other diets. In the AOM-treated rats Mucin Depleted Foci (MDF) were smaller with the PVD and CTRL diets than with the two meat-based diets. Oxidative stress parameters such as fecal TBARS, urinary DHN-MA and urinary 8-iso-PGF 2 α were lower in PVD than in Meat-based diets-fed rats. Microbiota analysis using 16s rRNA sequencing showed that bacterial communities significantly differed based on diet, with the exception of MBD and MBD-T samples which were similar. To determine if the microbiome contributes to the different tumorigenesis associated with the diets, feces from Pirc rats fed the 4 different diets were thus transplanted into AOM-induced germ-free rats fed a control diet for three months. Strikingly, rats transplanted with the MBD-feces had the highest number of MDF compared with all the other diets. In conclusion these results confirm the carcinogenetic activity of MBD- diets and the protective properties of PVD diet. Our results further demonstrate that these impacts of the different diets on carcinogenicity are, at least in part, mediated by the intestinal microbiome.

P046 | MICROALGAE CELLS AS A NOVEL DIETARY SUPPLEMENT FOR FINISHING LAMBS: A MICROBIOME-BASED APPROACH

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Modulating ruminal fermentation by diet supplementation is one of the most used strategies for improving feed efficiency. Schizochytrium sp., microalgae rich in docosahexaenoic acid (DHA), is an alternative ruminant dietary supplement that could modulate the ruminal fermentation by fatty acid biohydrogenation pathways potentially reducing methane emission and with a consequent increase of omega-3 content in the meat. In this study, we investigated the impact of different Schizochytrium sp. cells supplement levels on methane emission, ruminal fermentation, lipid meat content and the gastrointestinal microbiome. Fifty-six male finishing Texel/Scottish black-face lambs were randomly allocated into four groups according to age and body weight. Each group was assigned to one of the four treatments, with the following microalgae levels (DM basis): 0% (Ctrl), 1.2% (Alg-Low), 2.4% (Alg-Medium) and 3.6% (Alg-High) for 35 days pre-slaughter. Diets were isoenergetic and consisted of grass silage: concentrate in a 50:50 ratio. Individual dry matter intakes and body weights were daily and weekly recorded, respectively. Methane emissions were estimated by respiration chambers. Lamb's carcasses were collected and stored following the regular meat processing procedures. During the slaughter, the gastro tract intestines were collected and dissected for sample collection (rumen, small and large intestine). Meat samples were submitted for fatty acid analyses using the GC method, and the ruminal microbiome was characterized by shotgun metagenomics sequencing. The content of DHA, the main PUFA found in Schizochytrium sp., increased in loin samples corresponding to the microalgae diet supplementation levels. Preliminary results suggest microbiome changes among the treatments.

P047 | INFLUENCE OF LEGUME PROTEINS ON HUMAN HEALTH AND GUT MICROBIOME: A META-ANALYSIS

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Plant-based diets (PBDs) that prioritize fruits, vegetables, legumes, whole grains, and limit meat intake, have been linked to better health outcomes, lower mortality rates, and a reduced environmental impact. In PBDs, legumes are the primary source of proteins. Recent research has demonstrated that the quantity and source of dietary proteins can have a significant impact on the composition and function of the gut microbiota.

Therefore, a comprehensive literature screening on the impact of legumes and their proteins on human gut microbiome was carried out.

Consumption of legumes has been associated with several health benefits, including improvements in cardiovascular risk factors, oxidative stress and inflammation. Human trials have shown that legumes consumption has the potential to positively affect the abundance of some beneficial gut bacteria as well as the microbiome diversity and richness. In contrast, current research on the impact of proteins from legumes on microbiota composition and function is currently limited to studies on animal models, with only a few clinical trials.

Therefore, ad-hoc designed trials evaluating the addition of legumes and/or their proteins to diet are necessary to fully understand how they may impact on human health and modulate the gut microbiome composition and activities.

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P048 | CAPTIVE POLAR BEARS (URSUS MARITIMUS) EXHIBIT A MORE DIVERSE FECAL MICROBIOTA THAN WILD POLAR BEARS, BASED ON 16S rRNA ANALYSIS

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Polar bears are among the most vulnerable species in the Arctic marine ecosystem due to climate change and declining sea ice, which they rely on for hunting and movement. Understanding their gut microbiota and its interaction with diets is crucial for gaining insights into host intestinal health, dietary shift due to habitat change, and ultimately, species conservation. Gut microbial variation between wild and captive polar bear fecal samples (Canadian origin) was investigated using 16s rRNA sequencing technology. Dropped fecal samples were collected from wild polar bears in Churchill (MB), Fort Severn (ON), captive bears (Cochrane Polar Bear Habitat, ON) and the same captive bears fed seaweed (*Fucus* spp.; 200g / day added to their diets) collected from a beach where wild polar bears were observed eating it (Hudson Bay, Churchill, ON). Captive bears exhibited a significantly more diverse fecal microbiota than wild bears. The difference was likely due to significantly increased Firmicutes, Campilobacterota and Fusobacteriota, decreased Actinobacteriota ($p < 0.05$), and absent Bdellovibrionota and Verrucomicrobiota. Individual variation was the main driver of fecal microbiota composition in the captive bears. Seaweed consumption did not alter the diversity or change the composition of the microbiota, but this does not rule out dietary influences on differences between the two groups. This is the first study comparing gut microbiota between captive and wild polar bears and reveals distinct differences between the two groups, which could result from many factors, including available food sources. Findings could serve as a benchmark for measuring dietary changes in polar bears.

P049 | THE RUMEN EPIMURAL BACTERIAL COMMUNITY AND ITS ROLE IN RUMEN DEVELOPMENT

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In the first 4 months of life, the young calf goes through a development from monogastric to ruminant, which requires morphological and functional development of the rumen. The rumen microbiome plays a profound role in this functional development. In this study, the rumen bacterial community of calves at the age of 16 weeks was investigated. Fifteen male Holstein Friesian calves were euthanized and the bacterial composition of both rumen fluid and rumen wall samples were analyzed by 16SrRNA amplicon sequencing (V3-V4 region, Illumina technology). Rumen fluid and rumen wall samples showed separate clustering of the bacterial composition on the NMDS distance matrix. The Chao1 diversity metrics showed a significant higher species richness in rumen fluid samples ($p < 0.001$), whilst Shannon and InvSimpson diversity indices did not show any difference between sample types. In rumen fluid samples, Bacteroidetes was the most dominant phylum, followed by Firmicutes and Proteobacteria. In epimural samples however, Firmicutes was the most dominant phylum, followed by Bacteroidetes and Campylobacterota, which is in contrast with adult Holstein cattle where Proteobacteria were described as the most dominant phylum in epimural samples (De Mulder et al., 2017, Pacifico et al., 2021). This difference between the present and these previous studies could be explained by the fact that Campylobacterota is a newly proposed phylum, and forms a combination of Epsilonproteobacteria (formerly included in Proteobacteria) and Desulfurellales. Campylobacter, a member of the phylum Campylobacterota, is described as a common genus in the rumen epimural fraction, with a role in protein metabolism and oxygen scavenging.

P050 | EFFECT OF DIET ON STRUCTURAL RESILIENCIES OF RUMEN MICROBIOTA IN COWS

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Improving the ruminant digestive physiology by manipulating the rumen microbiota requires understanding the structural and functional resiliencies of microbiota in adult animals and the role of the host's phenotype. Six rumen-cannulated dry dairy cows were fed a corn silage (CS) diet for 5 weeks followed by a hay (H) diet for 5 weeks. Then, during a week labelled w0, the host-microbiota link was experimentally disrupted by collecting, pooling and homogenizing the rumen contents of the 6 cows and refilling each rumen with the homogenized microbiota mixture. The cows were kept on the H diet for 12 additional weeks and finally on the CS diet for 5 weeks. Using 16S-rDNA sequencing and FROGS pipeline, rumen microbiota composition was analyzed immediately after disruption at w0 and twice at the end of the 4 diet periods (w-6; w-1; w+11, w+16). Microbiota α -diversity (richness) was higher on H diet than on CS diet, was highest just after disruption (w0) and stabilized on H diets at a higher level at w+11 than at w-1. In contrast, richnesses were similar at the end of the two CS diet periods. PCoA based on Bray-Curtis distance at ASVs-like level revealed along the first two principal coordinates a clear clustering by week and diet. Microbiota communities were clearly modified when diets were switched. After disruption, community structures did not return to the initial state when cows were fed the H diet but were restored with the CS diet. It suggests that structural resilience of the microbiota is diet-dependent.

P051 | DIETARY FIBER DEPLETION AFFECTS THE INTESTINAL BARRIER AND PROVOKES LASTING GUT MICROBIOTA RESTRUCTURATION IN MICE

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The reduced presence of dietary fibers in western diets has dramatic effects on gut microbiota composition and impacts host health through degradation of the intestinal barrier and ensuing low-grade inflammation. As these factors closely interact, we asked whether a diet deprived of fermentable fibers could create a vicious cycle of bacterial-host interactions that would drive the system towards a new equilibrium. To answer this question, we fed mice with a diet lacking fermentable fibers for 3 weeks, followed by a standard chow diet for 6 weeks. We monitored gut microbial composition (through fecal 16S rRNA gene sequencing) and host health (through fecal markers) over time. Our results show that feeding mice a diet without fermentable fibers led to the rarefaction of several bacterial taxa, some of which remained at an undetectable level 6 weeks after the return to a chow diet. Some mucolytic and sulphate-reducing bacteria, on the contrary, thrived in the absence of fermentable fibers, possibly through cooperation in mucus harvesting. We furthermore observed that the Muribaculaceae family, known to contain mucin monosaccharide foragers, underwent a lasting reorganization following the fermentable fiber free diet. On the host part, we report transiently higher quantities of host DNA and of the inflammation marker lipocalin-2 in feces during the interruption of fermentable fiber intake. Our results indicate that a diet poor in fibers causes lasting gut microbial community restructuring that is potentially deleterious to host health, and highlight the importance of consuming a diet rich in microbiota-accessible carbohydrates.

P052 | EFFECT OF USING DIFFERENT FEED ADDITIVES ON RUMEN FERMENTATION OF DAIRY BEEF CALVES PRE AND POST WEANING

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¹CSIC, Granada, Spain

During the pre-weaning period, dairy beef calves are highly susceptible to enteric and respiratory diseases due to an immature immune system and undeveloped digestive tract. It has been suggested that some nutritional management strategies could have a great influence in the development of immunity, growth and gut maturation and potentially with life-long impact on health and production. This study evaluated the effect of different feed additives on body weight and rumen fermentation of Montbeliarde dairy beef calves at weaning, and their residual effect two months after weaning. Treatments were applied from week two of life until weaning and consisted of a control diet without any additives (CTL), or with a mix of feed additives used in the commercial farm (essential oils and yeast probiotics, MIX), a blend of essential oils (EO) or a synbiotic yeast probiotic (SYN). Weight was monitored throughout the trial and samples of rumen digesta were collected at weaning and two months after weaning to determine fermentation parameters. No effect of the treatments on weights was observed. At weaning, a decreased acetate/propionate ratio and increased branched-chain volatile fatty acids were observed with the MIX and SYN diets, respectively. Two months post-weaning the concentration of total VFA was increased with MIX, EO and SYN diets and a tendency to a greater butyrate proportion was also observed. This study suggests that the tested feed additives could potentially increase microbial activity and the development of papillae in the rumen epithelium, with implications for the long-term development of the animals.

P053 | IN VITRO INTERACTIONS OF PLANT EXTRACTS DESIGNED TO PREVENT NON-ALCOHOLIC HEPATIC STEATOSIS WITH HUMAN GUT MICROBIOTA

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Non-alcoholic fatty-liver is a highly prevalent condition that, if untreated, can progress to non-alcoholic steatohepatitis (NASH). An interplay of western diet and gut microbiota has been reported to be involved in its development, but the role of the gut microbiota remains poorly described. In this context, plant extracts, rich in a great diversity of bioactive molecules, appear promising in a multitargeted strategy. In this study, we investigated the interactions of Totum-448, a combination of plant extracts designed to prevent NASH, with gut microbiota from human origin, using batch colonic fermentation assays.

Fresh stool from five healthy donors were used to inoculate batch vessels, treated or not with 1g/l Totum-448, and 24h fermentations were run. Microbial activity was followed by gas and short-chain fatty acid (SCFA) measurement, while composition was followed by qPCR analysis of selected bacterial populations. Totum-448 metabolites produced by microbiota metabolization were analyzed by UPLC-UV-MS.

No significant effect of Totum-448 on total gas and SCFA production was observed, together with no differences in gas profile and concentrations in acetate, propionate and butyrate. Total bacteria, gamma-Proteobacteria, Bacteroidetes and Firmicutes were not impacted by plant extracts. However, a very efficient metabolization of Totum-448 occurred whatever the stool donors, leading to the production of several metabolites from the plant extract precursors.

These data suggest that Totum-448 has a limited impact on microbial activities, but confirm a key role of gut microbiota in plant extracts bioaccessibility, that needs to be further investigated in a more complex model of the human gut microbiome.

P054 | AN INTEGRATED UNDERSTANDING OF THE METABOLIC BENEFITS OF A NOVEL DOUBLE-TARGETED INTERVENTION USING GENETICALLY ENGINEERED PROBIOTIC EXPRESSING ALDAFERMIN WITH DIETARY CHANGES ON NAFLD

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Lifestyle changes toward a healthy diet and increased physical activity are the cornerstone interventions in the treatment of non-alcoholic fatty liver disease (NAFLD), the most common liver disease worldwide. However, due to its increased prevalence, new therapeutic approaches targeting the gut-liver-axis such as the use of microbial therapeutics and gut-hormonal interventions have been suggested. The present study introduces a seven-week double-targeted intervention using genetically engineered probiotic *Escherichia coli* Nissle 1917 to continuously express aldafermin (a non-tumorigenic analog of a human intestinal peptide hormone, fibroblast growth factor 19) along with dietary change (EcNA). The safety, efficacy, and mechanisms of action of the EcNA intervention were demonstrated using a high-fat-diet-induced NAFLD mouse model. The beneficial effects of the EcNA intervention were evidenced by the decrease in body weight, liver steatosis, and plasma concentrations of aspartate aminotransferase and cholesterol. Comprehensive integrated transcriptomics and non-targeted metabolomic analyses further revealed alterations in NAFLD-related genes and metabolites from the host and gut-microbial origin; along with a switch in amino acid, lipid, and their associated receptor signaling pathways. These results suggest the potential efficacy of EcNA in ameliorating NAFLD by decreasing insulin resistance, steatosis, oxidative stress, and maintaining gut-liver axis homeostasis; and highlight the potential of exploring multi-targeted interventions combining microbial therapeutics with the diet for NAFLD.

P055 | IMPACT OF AGEING ON THE FAECAL FIBROLYTIC MICROBIOME**Mrs Marylou Baraille**^{1,2}, Dr Marjorie Buttet², Mr Samy Julliand², Prof. Véronique Julliand¹¹Université de Bourgogne-Franche-Comté, Institut Agro Dijon, PAM UMR A02.102, Dijon, France,²Lab To Field, Dijon, France

The recommended intake of dietary fibres is higher for seniors than adults. Fibre degradation is driven by the large intestine (LI) microbiota in monogastric mammals. As changes of microbiota richness, diversity, and composition have been reported with ageing, fibrolysis may be decreased and consequently impair the seniors' nutrition and health. To assess whether the LI microbiota fibrolytic function was altered during ageing, we focused on equids, a monogastric herbivorous which can live very old.

Faeces were collected in 8 senior (20-29 years) and 8 adult (7-8 years) healthy horses fed the same controlled high-fibre diet for 1 month. Bacterial diversity, *Fibrobacter* sp. and *Ruminococcus flavefaciens* relative abundance, cellulolytic bacteria concentration, carboxymethyl-cellulase (CMCase) activity, short-chain fatty acids (SCFAs) concentration, and pH were determined. Age effect was assessed using multidimensional descriptive analysis (PCA), correlation of paired data, and analysis of variance.

On the PCA graph, age was positively associated with faecal SCFAs concentration, *R. flavefaciens* relative abundance, and CMCase activity, and negatively with faecal pH and bacterial diversity. Age was positively correlated with CMCase activity ($p=0.0368$) and negatively with pH ($p=0.0003$). However, only faecal pH differed significantly between groups (seniors: 6.35, adults: 7.05; $p=0.0010$). These results suggest that the LI fibrolytic function becomes more efficient with ageing. This supports *in vitro* data concomitantly obtained from the same individuals that demonstrated a higher degradation of hay fibres by the senior faecal ecosystem compared to adults. Further studies should be conducted to confirm whether this first surprising finding is reproducible within other senior populations.

P056 | EFFECT OF DROUGHT ON MICROBIAL COLONISATION OF LOLIUM PERENNE, FESTULOLIUM AND CENCHRUS CLANDESTINUS IN THE RUMEN**Dr Eva Ramos-Morales**^{1,2,4}, Juan Vargas³, Laura Lyons⁴, Olga Mayorga⁵, Claudia Janeth Ariza-Nieto⁵, Charles James Newbold^{2,4}¹CSIC, Granada, Spain, ²SRUC, , United Kingdom, ³University of Florida, , United States, ⁴Aberystwyth University, United Kingdom, ⁵Agrosavia, , Colombia

The global climate is changing; it is likely that environmental stresses, including drought, will affect cell wall biomass quality traits as well as overall plant physiological traits. However, little is known currently about the effect on ruminal microbial colonization and animal performance: if forage has altered composition because of environmental stress, how does this translate into rumen transformations, microbial colonisation, and microbial interactions during fermentation of forages in the rumen and the subsequent emissions?

We investigated the effect of drought on the colonisation of *Lolium perenne*, *Festulolium* and *Cenchrus clandestinus* in the rumen. Swards, established in replicate trays (21 x 34 x 5 cm), were maintained at growth conditions of 22°C/18°C day/night temperature, 13 h photoperiod with a light level of 300 $\mu\text{mol m}^{-2}\text{s}^{-1}$, and either watered regularly or at 18% of the pot capacity. Grass was harvested by cutting herbage with scissors approx. 5 cm above soil level. Batch *in vitro* incubations in rumen fluid were terminated at 2 and 12 h and microbial colonisation determined as previously described (Belanche et al., 2017).

There was a significant effect of time on colonisation of plant material, and particularly at 12 h there were differences in the colonisation of different grasses with a clear time x grass interaction. The imposition of drought condition did not seem to affect the overall colonisation of plant material, but some phyla (specifically Spirochaetes) and a number of genera (including *Pseudobutyribrio*, *Treponema*, Streptophyta and *Anaerobivrio*) appeared to be affected by the reduction in the water supply.

P057 | RECONSTRUCTION OF HIGH QUALITY GENOMES FROM THE COMPARTMENT 1 OF LLAMA USING SHOTGUN METAGENOMICS. PRELIMINARY RESULTS.

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Llamas (*Lama glama*) are versatile and robust animals that have adapted to the extreme climatic conditions and are raised primarily for their meat and fiber production in the Argentinean Andes. Llamas are pseudo-ruminants, where the Compartment 1 (C1) section of the stomach is the main compartment involved in fiber degradation, as occur in ruminants. At the present, bacteria residing in the C1 of llama are poorly characterized. To gain knowledge about the metabolic potential of these microorganisms in lignocellulose degradation and volatile fatty acids fermentation, we used shotgun metagenome sequencing. We took samples from five animals living at 3,500 m.a.s.l. and fed with native grasslands. Samples from C1 were immediately collected post-slaughtering and DNA from the gauze filtered content was obtained using commercial kits and sequenced on an Illumina NovaSeq (read length 2x150 bp).

A total of 119,468,322 reads were obtained, from which 89 % were retained after quality control using Trimmomatic. Reads were assembled using MetaWat, which resulted in 638,505 over 1kbp. Genome binning was performed with MetaBat and quality was assessed with CheckM. A total of 520 bins were obtained, including 12 high quality bins (>90% completeness and <5% contamination) and 103 draft genomes (>70% completeness and <5% contamination). The most represented phyla was Bacteroidetes, accounting for 33% of the genomes recovered, followed by Firmicutes, representing around 15% of all genomes. To our knowledge, this is the first report on llamas' forestomach tract genome reconstruction. A deeper analysis of the reconstructed genomes is underway.

P058 | IMPACT OF THE METHANE INHIBITOR BOVAER® (3-NITROOXYPROPANOL) AND DIET ON GAS EMISSIONS, ANIMAL PERFORMANCE, RUMEN MICROBIOME AND FERMENTATION CHARACTERISTICS OF LACTATING DAIRY COWS

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Effects of diet and Bovaer® (3-NOP) on gas emissions, dry matter intake (DMI), milk yield (MY), milk composition, fermentation characteristics and the rumen microbiome were investigated. Eight Holstein-Friesians were assigned to a replicated 4x4 Latin square and fed grass silage-based (GS) or corn silage-based diets (CS) diets (67% forage:33% concentrate), supplemented with placebo (CTL) or 80 mg 3-NOP/kg DM (3-NOP). Each period consisted of 14d adaptation followed by 4d in climate respiration chambers (CRC). On the last 2d of adaptation, rumen fluid was collected -1, 1, 2, 3, 4, and 6 h after feeding for VFA analysis, and at 3 h for Illumina sequencing. Rumen pH was recorded continuously. During the CRC period, DMI, MY and emissions (methane, CO₂, hydrogen) were measured. Significance was declared at P<0.05.

Results show 3-NOP significantly decreased methane emissions (g/d) by 15% (GS) and 24% (CS). Conversely, 3-NOP significantly increased hydrogen (g/d) 7-fold (CS) and 9-fold (GS). Although 3-NOP decreased DMI in GS and CS, MY and composition was unaffected. Significant increases in ruminal pH and changes in VFAs were observed with 3-NOP on both diets, with propionate increasing at the expense of acetate. Diet had a greater effect than 3-NOP on the microbiome. Lactobacilli, Fibrobacteraceae and Bifidobacteraceae were more abundant on CS whereas Prevotella were more abundant on GS. 3-NOP decreased the relative abundance of Methanobacteraceae in both diets. Although supplementation with 3-NOP led to phenotypic differences in the host, only minor effects on the microbiome were observed.

P059 | IMPACT OF AGEING ON THE FAECAL FIBROLYTIC MICROBIOME**Mrs Marylou Baraille**^{1,2}, Dr Marjorie Buttet², Mr Samy Julliand², Prof. Véronique Julliand¹¹Université de Bourgogne-Franche-Comté, Institut Agro Dijon, PAM UMR A02.102, Dijon, France, ²Lab To Field, Dijon, France

The recommended intake of dietary fibres is higher for seniors than adults. Fibre degradation is driven by the large intestine (LI) microbiota in monogastric mammals. As changes of microbiota richness, diversity, and composition have been reported with ageing, fibrolysis may be decreased and consequently impair the seniors' nutrition and health. To assess whether the LI microbiota fibrolytic function was altered during ageing, we focused on equids, a monogastric herbivorous which can live very old.

Faeces were collected in 8 senior (20-29 years) and 8 adult (7-8 years) healthy horses fed the same controlled high-fibre diet for 1 month. Bacterial diversity, *Fibrobacter* sp. and *Ruminococcus flavefaciens* relative abundance, cellulolytic bacteria concentration, carboxymethyl-cellulase (CMCase) activity, short-chain fatty acids (SCFAs) concentration, and pH were determined. Age effect was assessed using multidimensional descriptive analysis (PCA), correlation of paired data, and analysis of variance.

On the PCA graph, age was positively associated with faecal SCFAs concentration, *R. flavefaciens* relative abundance, and CMCase activity, and negatively with faecal pH and bacterial diversity. Age was positively correlated with CMCase activity ($p=0.0368$) and negatively with pH ($p=0.0003$). However, only faecal pH differed significantly between groups (seniors: 6.35, adults: 7.05; $p=0.0010$). These results suggest that the LI fibrolytic function becomes more efficient with ageing. This supports *in vitro* data concomitantly obtained from the same individuals that demonstrated a higher degradation of hay fibres by the senior faecal ecosystem compared to adults. Further studies should be conducted to confirm whether this first surprising finding is reproducible within other senior populations.

P060 | ESTABLISHING THE DIFFERENT ROLES OF HUMAN GUT MICROBES IN PECTIN BREAKDOWN**Mrs Freda Farquharson**¹, Dr Michael Solvang¹, Dr Jesper Holck², Dr Birgitte Zeuner², Dr Petra Louis¹¹University of Aberdeen Rowett Institute, Aberdeen, United Kingdom,²Technical University of Denmark, Lyngby, Denmark

Plant-derived dietary fibre is a major nutrient source for the human gut microbiota in the lower intestine. The fibre constituent pectin makes up approximately one-third of the plant cell wall of many fruits and vegetables. Pectin is a complex polysaccharide comprised of numerous monosaccharides and linkages. It consists of three main domains: homogalacturonan (HG; galacturonic acid residues, which can be methylated or acetylated), rhamnogalacturonan-I and rhamnogalacturonan-II (RG-I & RG-II). RG-I has a backbone of alternating galacturonic acid and rhamnose residues and contains side chains that are mainly made up of galactose and arabinose. RG-II (usually a minor pectin component) has a galacturonan backbone and a highly complex side-chain arrangement consisting of a variety of constituents. Thus, the complete degradation of pectin requires many different carbohydrate-active enzymes belonging to several families of glycoside hydrolases, polysaccharide lyases and carbohydrate esterases.

Several gut bacteria that are able to grow on pectin or its breakdown intermediates have been identified, but further work is required to elucidate their specific roles in pectin breakdown to better understand the complex competitive and cross-feeding interactions within the gut microbiota during pectin fermentation.

We will present results from pure culture growth studies of a range of gut bacteria on apple pectin and several pectin components, including backbone poly- and oligosaccharides and RG-I side chains. Our study elucidates the specific ecological niches occupied by different gut microbes in pectin fermentation, which will ultimately aid the development of personalised nutritional approaches to promote human health.

P061 | IN VITRO APPROACH TO ASSESS THE IMPACT OF IRON AND LACTOFERRIN ON THE PRE-WEANING INFANT AND ADULT GUT MICROBIOTA

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Iron deficiency is the most common micronutrient malnutrition globally due to the poor absorption rate (~20%) or dietary iron, despite most staple food items being fortified with iron. Lactoferrin is an iron-binding protein that is naturally rich in colostrum. It is claimed not only to enhance the iron absorption, but also to have a potential prebiotic effect in recent studies. This study aimed to assess the effect of iron and lactoferrin (individually and together) in realistic doses (i.e. health supplements and breast or formula milk) on both infant and adult gut microbiota by in vitro fermentation models that mimics the human colonic environment biochemically and mechanically. The models were inoculated with healthy pre-weaned infants (n=4) and adults (n=4). After 24-hour fermentations in the proximal colon settings, the relative abundance of Lactobacillaceae increased more with lactoferrin in both infants (9.39 Log₂ fold change [Log₂FC], p<0.001) and adults (12.28 Log₂FC, p<0.001) than with iron (5.57 Log₂FC, p<0.001 for infants; 5.82 Log₂FC, p<0.001 for adults). The microbial diversity significantly decreased after the fermentation in both infants and adults regardless the treatment, possibly due to an inevitable adjustment for bacteria to the in vitro system. However, lactoferrin showed less reduction than iron, suggesting that lactoferrin could potentially maintain the diversity in the gut microbiota. In order to take the investigation on the effect of iron and lactoferrin on the gut microbiota further, an in vitro three-stage (the proximal, transvers, and distal colon) continuous fermentation work is on progress.

P062 | ADAPTATION TO PROLONGED DIETARY CHALLENGE IN CATTLE: THE RESILIENCE POTENTIAL OF THE RUMEN

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The rumen is a complex organ hosting a multitude of microorganisms distributed over different ecological niches, with distinct composition and metabolic capacities. Feeding easily-fermentable carbohydrates can impair the rumen microbiome composition, as well as the structure of the organ itself. The aim of this study was to investigate the response of the rumen as a whole ecosystem to continuous high-grain (HG) feeding (4 weeks). We evaluated microbial shifts in three ruminal niches (solid/liquid/epimural) using 16S rRNA gene sequencing and targeted liquid chromatography-mass spectrometry, as well as the ruminal epithelium response (histology and gene expression analysis) in nine Holstein cows fed a 65% HG diet. We observed variations in the microbiota composition in all three niches analysed, with a drastic reduction in diversity and species richness especially in the digesta (solid and liquid). This, in combination with increasing concentrations of potentially harmful metabolites, such as kynurenine and 5-aminovaleric acid, between the second and the third week of HG feeding, suggested a disruption of the ruminal homeostasis. However, the ruminal ecosystem seemed to adapt to the challenging conditions by the last week of experiment, with a return to microbial diversity and composition similar to the baseline, especially in the liquid fraction. Despite an increased gene expression associated to both cell proliferation (IGF-1, IGF-1R, EGFR and TBP) and inflammatory pathways (IFN- γ , MyD88, CD14 and TLR-4), the epithelial structure seemed to remain unharmed. We conclude that, within the timeframe of our study, the ruminal ecosystem showed a high resilience potential towards a HG challenge.

P063 | EXPLORING THE GUT MICROBIOTA FOR POSSIBLE INTERVENTION STUDIES IN PELVIC CANCER PATIENTS

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The gut harbours an ecosystem of microorganisms (microbiota) that function in metabolic and regulatory processes that impact the body's immunity and disease development. The gut microbiota ferment dietary fibre (e.g., inulin and psyllium) to produce short-chain fatty acids (SCFAs), including acetate, butyrate and propionate beneficial to gut health.

We investigated the effect of modified diet on tumour control in mice following ionising radiation and then looked to determine relationships with the gut microbiota found in cancer patients.

Faecal samples were collected from pelvic cancer patients pre-treatment and from tumour-bearing mice fed on high-fibre (psyllium, psyllium+inulin, inulin+resistant starch) or low-fibre (0.2% cellulose) diets and treated (n=10) or not (n=5) with ionising radiation. Bacterial communities were profiled using 16S rRNA gene sequencing and SCFA were analysed from the samples.

Analysis of phylum-level gut microbiota composition showed a more similar bacterial profile in cancer patients and mice fed a low-fibre diet than the high-fibre diet groups, with higher Firmicutes and lower Proteobacteria abundance. Lachnospiraceae family was associated with better tumour control in psyllium+inulin in mice. A broad range of SCFA levels was found among cancer patients. Lachnospiraceae family abundance in cancer patients was also positively correlated with the total concentration of three major SCFAs: acetate (R²=0.068, p=0.022), butyrate (R²=0.051, p=0.049) and valerate (R²=0.102, p=0.005) concentrations, but not formate or propionate.

The similarities between the human and mouse low fibre-related microbiota/SCFA concentrations imply that we may exploit dietary fibre supplements in humans to improve outcomes following radiotherapy.

P064 | DECREASED RELATIVE ABUNDANCE OF THE METHANOBACTERIACEAE FAMILY IN FEEDLOT LAMBS WITH POLYHERBAL ADDITIVE

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Herbal additives have been used in animal nutrition to improve welfare, health and productive performance, as well as the quality of derived products. Polyherbal mixtures are rich in secondary metabolites that have a direct effect on ruminal microbial communities, promoting the proliferation of some bacterial groups and the decline of others. In this study, changes in ruminal microbiota were analyzed in response to dietary inclusion of a polyherbal additive (*Withania somnifera*, *Tinospora cordifolia*, *Ocimum sanctum* and *Embllica officinalis*) that provides natural phenols and antioxidants. Ten lambs were assigned in a completely randomized design in two dietary concentrations: 0.0 and 0.14% of polyherbal additive (dry matter). The experimental period lasted 25 days, recording individual liveweight changes and daily feed intake. Ruminal fluid samples were collected from each lamb at the end of the experiment and metagenomic DNA was extracted, from which the V3-V4 region of the 16S rRNA gene was sequenced on the Illumina® MiSeq™ platform. Composition and structure of ruminal microbial communities (bacteria and archaea) were analyzed. The dietary inclusion of the polyherbal additive caused a significant decrease (p ≤ 0.10) regarding to the control on the percentages of relative abundance of methanogenic Archaea (72.8% of the control), bacterial Families XIII of Clostridiales (60%), Christensenellaceae (91.5%) and Ruminococcaceae (70.5%), while Succinivibrionaceae showed an increase (167.6%). Results indicate that dietary incorporation of this polyherbal additive would result in a reduction of methane emissions in feedlot lambs.

P065 | CORRELATION BETWEEN RUMEN MICROBIOTA AND NITROGEN USE EFFICIENCY OF DAIRY COWS UNDER DIFFERENT DIETS

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Nitrogen use efficiency (NUE) is of prime importance for dairy production systems as a measure of how efficiently can dairy cows convert feed protein into milk protein. A reduced dietary crude protein (CP) concentration leads to an increased NUE. Growing evidence also suggests there is animal variation in NUE that may be partly attributable to rumen microbiota. In this study, we evaluated the effects of contrasting levels of CP and animal variation on NUE of lactating cows in relation to the rumen microbiome.

Forty-two mid-lactation cows were randomly allocated to either a high CP (18%) or low CP (14%) diet and fed for 9 weeks following a 3 week 16% CP diet period. NUE was assessed based on daily DM and N intakes and daily milk yield and weekly milk composition. Samples of rumen fluid were taken at the end of the covariate period and at 3, 6 and 9 weeks from there. 16S sequencing was performed to determine repeatability of microbial community structure and relations of microbial taxa with NUE by splitting the animals in two groups based on NUE values.

As hypothesized, NUE was significantly higher in the low CP group across all timepoints. The microbial community composition was similar for both dietary treatments. However, the rumen microbial community structure tended to be different when individual animals were split according to the ranking of NUE values irrespective of dietary treatment. Furthermore, some microbial taxa showed different abundances based on this grouping and had significant positive correlations with NUE.

P066 | INVESTIGATION OF THE RUMEN MICROBIAL COMPOSITION IN 16 WK-OLD HOLSTEIN FRIESIAN (HF) AND BELGIAN BLUE (BB) CALVES FED CORN SILAGE (CS) AT DIFFERENT INCLUSION AGES

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A randomized controlled trial was performed to assess the impact of CS inclusion time in HF and BB calves on calf performance and rumen function. Seventy five newborn HF calves were randomly assigned to 3 treatments: CS included in the pre- (4w), weaning (10w) or postweaning period (16w). Forty BB calves were randomly appointed to: CS included in the pre- (6w) or postweaning period (16w). In HF, inclusion time of CS did not influence calf performance. In BB, inclusion of CS from the preweaning period decreased DMI compared to calves receiving CS after weaning (1.66 kgDM/d vs 1.86 kgDM/d, respectively, $P < 0.001$), resulting in a lower ADG in CS-6w (0.83 kg/d vs 0.98 kg/d; $p < 0.001$). To investigate this difference in CS utilization between breeds, rumen fluid microbial composition was examined by performing 16s rRNA amplicon sequencing. NMDS-analysis showed a separate clustering according to CS inclusion time ($p < 0.05$). Early inclusion of CS induced in both HF and BB a trend to shift from Prevotellaceae and Lachnospiraceae to Succinivibrionaceae, Ruminococcaceae and unidentified families as an adaptation for starch degradation. Rumen samples of HF showed a higher microbial richness than samples of BB calves ($p = 0.01$), explained by differences in 8 low abundant genera ($p < 0.05$). However, evenness did not differ between breeds. In conclusion, both HF and BB showed adaptation of the rumen fluid microbial composition in order to digest CS. The lower ADG in BB could only be explained by the lower DMI capacity of BB and not by interbreed differences in microbial composition.

P067 | ASSESSING DIET, THE FAECAL MICROBIOME AND TRYPTOPHAN METABOLITES IN THE DOMESTIC CAT

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Cats are obligate carnivores and therefore don't require dietary fibre. However, it is commonly included in pet food. Previous research showed when cats consume a high protein diet lacking dietary fibre (<1% crude fibre on a dry matter basis), their faecal microbiome, faecal output, and defecation rate is altered. Adding dietary fibre (plant or animal derived) to the high protein diet increased faecal output. Tryptophan metabolite serotonin is involved in colonic transit and may be implicated in the changing defecation rate. Serotonin is released in response to changing mechanical pressure, bacterial interaction, and concentration of metabolites in the gut lumen. Therefore, interactions between host, diet and tryptophan metabolites were investigated.

Cats were fed one of three diets a high protein diet (Raw), with hydrolysed collagen (Raw+HC) at 4% or 6% (as is), or Raw with inulin and cellulose (Raw+PF: 2% of each). The faecal metagenome was analysed by shotgun sequencing. Functional genes were assigned using the KEGG database. Organic acids were analysed using gas chromatography, and tryptophan metabolites using targeted liquid chromatography mass spectrometry.

Faecal output was lowest in the cats consuming Raw+HC. Bacterial genes involved in tryptophan metabolism were significantly greater in cats consuming the Raw+HC diets compared to Raw+PF. However, circulating concentration of serotonin was not significantly different. These findings suggest the microbiome responded to the changes in tryptophan availability in the colon, but not through the serotonin pathway. Further investigation is required to better understand tryptophan metabolite interactions between the microbiome and host.

P068 | EFFECT OF ZINC OXIDE NANOPARTICLE ON THE RUMINAL PROTOZOA AND FUNGAL MICROBIOTA OF SHEEP

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Introduction: Zinc salts is an important microelement and has been used for animal health and productivity, very little work has been done to discern whether this benefit is exerted solely on the host organism, or whether there is some effect of dietary Zn upon the gastrointestinal microbiota, particularly in ruminants. The zinc oxide nanoparticles (Zn-NP) are presented as alternative sources of dietary zinc supplementation to provide controlled release of Zn.

Aim: Considering the importance of the ruminal microorganisms for animal development, the objective of this study was investigate the effect of Zn-NP on rumen microbiota of sheep.

Methods: Eight rumen-fistulated were assigned to a 4 × 4 Latin square (4 treatments and 4 periods) fed diets with varying concentrations of Zn-NP (0, 50, 100 and 150 mg/animal /day). Rumen fluid samples were taken via a ruminal cannula for DNA extraction and the copy number of ciliate Protozoa and Fungus microbiota were evaluated by real-time quantitative PCR analysis (qPCR).

Results: Using a quadratic regression model our results indicated that Fungus abundance was increased until 63.89 mg of Zn-NP ($y = -4.73x^2 + 604.48x + 36182$, $r^2=0.28$, $p=0.0189$) on the other hand Protozoa abundance has not changed with Zn-NP supplementation ($p>0.05$).

Conclusion: Zn-NP supplementation did not interfere with the protozoan population, but reduced the fungus population. Further research is required to elucidate the relevant impact of Zn-NP on rumen microbiota, and provide more insights into the application of Zn-NP in ruminants.

P069 | INVESTIGATION OF TUMOUR CYTOTOXIC EFFECTS OF GUT BACTERIA GROWN IN AN INULIN-SUPPLEMENTED MEDIUM

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Pelvic cancer is a major public health problem worldwide and conventional treatments include surgery, chemotherapy and radiotherapy. However, these are associated with serious adverse effects, so there is an urgent need to find methods to alleviate these.

It is well documented that diet can impact physiological functions by targeting the gut-microbiota. Members of the gut-microbiota ferment undigested dietary carbohydrates to produce host-health promoting metabolites, including acetate, propionate and butyrate. Recent animal studies in our lab have manipulated the gut-microbiota composition through diet, resulting in improved tumour control following ionising radiation. Further investigations are required into the mechanisms underlying tumour cytotoxic and radiosensitising effects of the gut-microbiota. The current study aims to assess the cytotoxic effects of bacterial supernatants, from *Bacteroides acidifaciens* alone and from co-culture of *B. acidifaciens*+*Faecalibacterium prausnitzii* (cultured in inulin-supplemented broth), on UPPL1591-mouse bladder tumour cells.

UPPL1591-cells were treated with a range of concentrations of supernatants (0, 5, 10, 20 or 40 μ L/200 μ L) from inulin broth, inulin-supplemented culture of *B. acidifaciens* and co-culture of *B. acidifaciens*+*F. prausnitzii* (in triplicate). Cells were incubated for 48-hours and viability of UPPL1591-cells was assessed by MTT assay.

The extracts from *B. acidifaciens* and the co-culture of *B. acidifaciens*+*F. prausnitzii* significantly reduced the cell viability ($p<0.0001$) compared to the inulin-supplemented broth (control); the impact of co-culture was high than for *B. acidifaciens* alone.

This study has demonstrated that the gut bacteria supplemented with inulin reduced the viability of UPPL1591-tumour cells. However, more work is needed to further elucidate these interactions.

P070 | THE POTENTIAL OF SYNBIOTIC TO REDUCE THE PREVALENCE OF ANTIBIOTIC RESISTANCE IN THE GASTROINTESTINAL TRACT OF BROILER CHICKENS

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Four studies were conducted in the USA, Belgium, Germany, and Brazil to evaluate the effect of synbiotic nutritional supplementation on the resistome in broilers.

Study USA: The aim of this scientific study was to evaluate the effect of a synbiotic supplementation and ampicillin on the prevalence of antibiotic-resistant *E. coli* in the ceca of broilers. Administration of ampicillin in broilers led to a significant increase in the abundance of *E. coli* strains resistant to several antibiotics. The prevalence of ceftriaxone resistant *E. coli* was significantly lower for synbiotic group.

Study Brazil & Belgium: The aim of these studies in commercial conditions was to evaluate the effect of the synbiotic supplementation on the prevalence of antibiotic resistome compared to control without antibiotics. Supplementation of broilers with the synbiotic significantly decreased the number and abundance of antibiotic resistant genes compared to the negative control groups in both trials.

Study Germany: The aim of the study was to evaluate the effect of the synbiotic and antibiotic supplementation on the prevalence of antibiotic resistome in broilers in the farm in Germany. Nutritional supplementation of the synbiotic resulted in the significantly lower number and abundance of antibiotic resistant genes in comparison to the group received antibiotic. Treatment of broilers with the antibiotic and synbiotic resulted in the numerical decrease of resistant genes compared to the group received antibiotic.

In summary, the findings from this experiment provided more evidence on the potential of synbiotics to reduce the prevalence of antibiotic resistome in broilers.

P071 | A SUPERFOOD BASED ON MEDITERRANEAN DIET SELECTS FOR HEALTH-PROMOTING SPECIES AND METABOLIC PATHWAYS IN A SIMULATOR OF HUMAN INTESTINAL MICROBIAL ECOSYSTEM (SHIME)

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Mediterranean diet (MD) is recognized as a healthy dietary pattern, promoting a microbiome able to produce beneficial metabolites. Typical MD food products, containing high levels of dietary fibers, polyphenols, flavonoids and glucosinolates are natural precursors of key microbial metabolites. This study is a preliminary part of the project “Microbiome-tailored food products based on typical Mediterranean Diet components” that aims to develop a superfood simulating the composition of the MD and containing these beneficial precursors. The superfood or a placebo were fed to the Simulator of Human Intestinal Microbial Ecosystem (SHIME®) for 14 days and the microbiome was monitored by shotgun metagenomics at baseline, at 7 and 14 days, as well as after a 7-days wash-out period. Samples of lumen content and mucosa were collected. Results indicate that the superfood was able to change the gut microbiome composition, promoting the development of beneficial taxa (e.g., *Faecalibacterium*, *Ruminococcus*), that are recognized as able to produce health-promoting metabolites, and selecting for genes and metabolic pathways involved in biosynthesis of beneficial compounds such as short-chain fatty acids (SCFAs) and isothiocyanate. Also, analysis of the Carbohydrate-Active Enzymes (CAZy) highlighted an overall boosted potential carbohydrate-degrading activity in both lumen and mucosa. Taken collectively, our results show the ability of the MD components-enriched food product to drive the gut microbiome composition and functional potential.

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P072 | MICROBIOME-DERIVE METABOLITES ARE POWERFUL BIOMARKERS FOR ANAL CANCER SCREENING

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The risk of anal cancer is markedly increased in people with HIV. The low specificity of the current screening strategy for the detection of high-grade squamous intraepithelial lesions (HSIL) hinders anal cancer prevention. We investigated in anal swabs microbiota-associated markers of HSIL.

213 individuals were recruited, which were mostly men who have sex with men undergoing HSIL screening including high-resolution anoscopy and biopsies to confirm HSIL. Subjects were splitted in a discovery cohort included 167 individuals and a validation cohort included 46 individuals. We extracted the bacterial DNA, proteins, and metabolites from anal swabs, where we performed 16S rDNA sequencing, mass spectrometry, and targeted metabolite quantification.

Subjects with HSIL exhibited an increased abundance of *Prevotella copri*, while *Streptococcus periodonticum* and *Sneathia sanguinegens* were depleted. HSIL-associated bacteria overexpressed proteins that converged in the production of succinyl-CoA and cobalamin, which levels were consistently increased in subjects with HSIL. The combination of succinyl-CoA and cobalamin overperformed the anal cytology, improving sensitivity from 91.2% to 96.6% and specificity from 34.1% to 81.8%. While the anal cytology classified correctly only 59.9% of individuals, the combination of both biomarkers improved the classification up to 87.7%. This test overcame internal (adjusted AUC 0.877) and external validation. From 98 false-positive cytologic results, the metabolic test reclassified to true negative results 49 (81.9%).

Cobalamin and succinyl-CoA are bacterial products overexpressed in the anal microbiome of subjects with HSIL and show an excellent diagnostic capacity, that could improve the current strategy for anal cancer screening.

P073 | SEQUENCE AND STRUCTURE COMPARISONS OF TETRACYCLINE RESISTANCE PROTEINS: IMPACT ON RESISTANCE PHENOTYPE

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Tetracycline resistance in bacteria is frequently conferred by ribosome protection proteins (RPPs), which prevent tetracycline binding to the ribosome and disrupt protein synthesis. We compared the effects of changes in the amino acid sequences of wild-type RPPs Tet(O) and mosaic Tet(O) from *Campylobacter* isolates, with a special focus on the electrostatic surface potential.

Whole genome sequences of 6,996 isolates of *Campylobacter jejuni* and *Campylobacter coli* cultured from various sources were analysed. Specific genes encoding RPPs were identified in these genomes using bioinformatic analyses: tet(O), tet(O/32/O), tet(O/M/O), tet(O/W/32/O), tet(W) and tet(O/W/O). Forty protein sequences were compared in silico, and *Campylobacter* isolates containing these sequences were tested for MICTET.

There were 77 variable amino acid positions between Tet(O), Tet(O/32/O), Tet(O/M/O) and Tet(O/W/O) (77/639), 20 of which were present in less than five sequences. The calculated electrostatic surface potentials of mosaic proteins showed conservation compared to Tet(O), with the exception of the mosaic Tet(O/M/O). This protein had a significantly altered electrostatic surface potential and conferred the highest MICTET in *Campylobacter*.

In summary, all RPPs but one investigated were highly conserved in sequence, predicted protein structures and electrostatic surface potentials. Variations in the electrostatic surface potential was predicted to affect RPP binding and thus localisation with ribosomes. This changed the interaction with tetracycline, and resulted in different tetracycline resistance phenotypes. The most distinctive protein, encoded by the mosaic tet(O/M/O) gene, confers the highest level of tetracycline resistance.

P074 | EFFECTS OF NOVEL OXIDISING METHANE INHIBITORS ON IN VITRO EMISSIONS AND THE RUMEN MICROBIAL COMMUNITIES USING THE RUMEN SIMULATION TECHNIQUE

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Enteric methane (CH₄) emissions from livestock is one of the leading causes of anthropogenic greenhouse gases. One of the most promising CH₄ mitigation strategies is the incorporation of anti-methanogenic agents into feed additives.

This research introduces novel CH₄ inhibitors based on the control of rumen oxidation-reduction potential (ORP) using mild oxidising agents such as urea hydrogen peroxide (UHP) and magnesium peroxide (MgO₂). The rumen simulation technique (Rusitec), a well-established system for assessing potential feed additives, was used to investigate the effects of these treatments on CH₄ emissions, digestible organic matter (DOM) and volatile fatty acid (VFA) content. The rumen microbial community was investigated using 16S and 18S rRNA profiling on the Illumina MiSeq platform, alongside quantification of total bacteria, fungi, protozoa and methanogens using quantitative PCR.

Treatments consisted of: Control (no treatment); MgO₂ alone; UHP alone and two doses of UHP in combination with potassium iodide (KI). The in vitro diet consisted of 50:50 grass silage:concentrate on a dry matter basis. All 4 treatments reduced CH₄ emissions in terms of CH₄ volume, CH₄ percent and CH₄mmol/gDOM. Reductions in CH₄mmol/gDOM of between 51% and 72% were observed in UHP treated vessels relative to the controls (p<0.05). Results from microbiome analysis reveal differences between treated and control samples that offer a first insight into how these highly effective methanogenic inhibitors alter microbial community dynamics. The positive outcomes from this in vitro experiment has facilitated the appropriate selection of oxidising methane inhibitors for further testing in various in vivo ruminant trials.

P075 | UNCOVERING MICROBIOME ACTIVITIES ASSOCIATED WITH POLYSACCHARIDE DEGRADATION AND HYDROGEN METABOLISM IN LEAN-TYPE AND OBESE-TYPE PIGS

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Obese and lean individuals harbor significant differences in the gut microbiome, and these microbial differences may be related to energy harvest. Colonic microbes break down complex polysaccharides to produce short chain fatty acids and H₂, and the efficient removal of H₂ in turn could improve fermentation efficiency. However, the microbial activities involved in polysaccharide degradation and H₂ metabolism in obese and lean individuals are not clear. Here, using 16S rRNA gene sequencing, metatranscriptomic and metabolomic approaches, we profiled the composition, activities and metabolic pathways of colonic microbiome in lean breed Yorkshire pigs and obese breed Meishan pigs (n=6). Primary polysaccharide degrader (*Bacteroides* and *Treponema*) and reductive acetogen (*Blautia*) were enriched in Meishan pigs, whereas sulphate-reducing bacteria (*Desulfovibrio*) was enriched in Yorkshire pigs. Meishan pigs harbored higher abundances of carbohydrate-active enzyme genes than Yorkshire pigs, mainly including those contributing to arabinoxylan and pectin degradation (e.g., GH43 and PL1). Meanwhile, the enzymes related to acetate production and propionate production (e.g., *pta* and *ackA*) were enriched in Meishan pigs. Furthermore, Yorkshire pigs had greater hydrogen-producing [FeFe]-hydrogenases and respiratory [NiFe]-hydrogenases. Distinct hydrogen consumption pathways were also observed: enzymes involved in sulfate reduction (*AprA* and *AsrA*) were enriched in Yorkshire pigs, while enzymes of fumarate reduction (*FrdA*) and acetogenesis (*AcsB*) were enriched in Meishan pigs. Subsequently, the higher concentrations of acetate and propionate were detected in Meishan pigs. These findings suggest that obese-type pig exhibits the higher efficiency of polysaccharide degradation and hydrogen disposal in the colonic microbiome than lean-type pig.

P076 | A GLOBAL ANALYSIS OF THE ENZYMATIC MECHANISM OF ANAEROBIC FUNGI (NEOCALLIMASTIGOMYCETES) IN LIGNOCELLULOSE DEPOLYMERIZATION

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The Anaerobic Fungi (AF) is the first and primary colonizer of lignocellulosic biomass in the rumen of ruminants. Even AF accounts for 8% of rumen microorganisms, but they can degrade up to more than 50% of untreated biomass through invasive growth and enzyme secretion. And it is worth noting that the co-cultivation of AF and methanogens can further improve the lignocellulosic degradation effect of AF. However, the mechanism of lignocellulose degradation by mono-culture and co-culture AF remains unclear.

The first experiment studies the lignocellulose-degrading capacity of AF at the genomic level. An analysis of the genomes of all AF downloaded from JGI Microcosm's website revealed that the genome of AF contains 10% to 20% of the genes for CAZymes that degrade lignocellulose. Secondly, a *Pecoramyces ruminantium* F1 strain, which has been deeply studied in various characteristics, and the lignocellulose substrate were selected as representatives to study at the transcription level. Transcriptome studies were also performed on representative cellulose and hemicellulose substrates, sampled at the end of logarithmic growth when strain F1 is most metabolically active. A similar study was also conducted on the co-cultivation of AF *Pecoramyces ruminantium* F1 and methanogens *Methanobrevibacter thaueri* F1.

In co-culture, free glycosidases from the GH1 family that did not appear in mono-culture participated in cellulose degradation. Co-cultivation of AF with methanogens improved hemicellulose degradation more than cellulose. Specifically, the free enzymes involved in xylan and the cellulosomal enzymes in arabinose and ferulic acid mainly increased during co-culture hemicellulose degradation.

P077 | UNDERSTANDING POLYUNSATURATED FATTY ACID PROVISIONING BY GUT MICROBIOTA ELEMENTS THROUGH THE LENS OF AN EARTHWORM MODEL**Dr Stephanie Schnorr**¹, Jan-Philipp Wittlinger¹, Dr David Berry¹¹University Of Vienna, Vienna, Austria

In-land production of long-chain polyunsaturated fatty acids (PUFAs) is primarily from eukaryotic microorganisms that inhabit freshwater ecosystems. The scenario by which strictly terrestrial organisms obtain PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is incompletely understood. These observations have translated into a booming supplements industry to market EPA- and DHA-containing oils from marine organisms. Soil bacteria such as members of the γ -Proteobacteria and certain myxobacteria contain genetic competences to denovo synthesize PUFA using an iterative type I fatty acid synthase (FAS)/polyketide synthase (PKS)-like synthase system. More bacterial candidate producers are unearthed as metagenomic data informs on uncultured environmental microorganisms. Microbial and faunal food webs in soils are linked by earthworms. Curiously, their gut-soil and muscle tissue have increasing concentrations of EPA and DHA relative to surrounding bulk soil, implicating microbial synthesis within the earthworm intestinal tract. Therefore, we have interrogated earthworm metagenomic datasets and GenBank repositories, and generated a novel dataset on earthworm microbial composition and gene expression from collected organisms to explore the connection between microbial PUFA synthesis activity in terrestrial ecosystems that overlaps with animal host microbiomes. These data provide insights about how the terrestrial trophic chain is supplied by bacterial producers, and explores the hypothesis that PUFA production is activated by stochastic stress, and constrains trait dispersal. The understanding of this process and extent of producer networks is important for answering open questions in mammalian evolution, as well as for biotechnological efforts to more sustainably source nutritional PUFAs.

P078 | TARGETING METHANOL FORMATION FOR REDUCING METHANE EMISSIONS**Dr Nikola Palevich**¹, Dr Vincenzo Carbone¹, Ms Kerri Reilly¹, Mr Peter Reid¹, Ms Carrie Sang¹, Dr Linley Schofield¹, Dr Ron Ronimus¹, Dr William Kelly¹, Dr Graeme Attwood¹¹AgResearch Ltd., Grasslands Research Centre, Palmerston North, New Zealand

Pectin is a complex polysaccharide that forms a substantial proportion of the plant cell wall of forage ingested by grazing ruminants. Methanol derives from methoxy groups released from pectin by the action of pectin methylsterases (PMEs), and is subsequently used by rumen methylotrophic methanogens, such as members of the genus *Methanosphaera*, that reduce methyl-compounds to methane (CH₄). *Butyrivibrio* are key pectin-degrading rumen bacteria that contribute to methanol formation as well as having important roles in fibre breakdown, protein digestion and biohydrogenation of fatty acids. The aim of this study is to investigate pectin breakdown in *Butyrivibrio* and to find ways to inhibit these activities to reduce methanol release from pectin. Comparative genomics identified candidate *Butyrivibrio* strains containing genes encoding pectin degradation and methanol release. The growth of *Butyrivibrio* strains was tested on high- and low-methoxylated pectin and their fermentation products were analysed to confirm their predicted pectin-degrading (pec+/-) and methanol-forming (MeOH+/-) phenotypes. To investigate metabolic coupling of high methanol producing *Butyrivibrio* strains with rumen methanogens, we performed a time-series RNA-seq experiment on co-cultures of pec+/MeOH+ *B. proteoclasticus* B316T and *B. fibrisolvens* DIT with a methylotrophic methanogen *Methanosphaera* sp. ISO3-F5 grown on pectin. Structures of selected *Butyrivibrio* PMEs have been modelled with bound substrate to identify the amino acid residues best suited for inhibitor binding and inhibitor library screening. With selected compounds synthesized for upcoming in vitro screening, this strategy represents an alternative approach to methane mitigation which currently inhibit methanogenesis directly.

P079 | UNCOVERING CATTLE-ASSOCIATED MARKERS OF FAECAL POLLUTION THROUGH 16S rRNA GENE ANALYSIS

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The cattle industry generates substantial amounts of faecal waste. While cattle manure can be beneficial as biofertilizer, it can also introduce faecal pathogens to agricultural lands and surrounding waters, posing a significant threat to human, animal and environmental health. Identifying the source of contamination is vital for effective remediation and identification of problematic agricultural areas. Our study aims to identify bacterial DNA sequences unique to or strongly associated with cattle faeces and manure which could serve as Microbial Source Tracking (MST) markers.

A total of 430 faecal samples from 49 wild and domestic animal species, including cattle, were collected in Slovenia. Additionally, five samples of cattle manure were obtained. The samples underwent 16S rRNA gene sequencing, targeting the V3-V4 hypervariable region, and analysis of zero-radius operational taxonomic units (ZOTU). The bacterial composition of different sample types was compared using pairwise-permutation multivariate analysis of variance (pairwise PERMANOVA). Subsequently, each ZOTU was split into shorter k-mers, shifting one base at a time. The k-mers discovered in the faecal matter of cattle and manure were compared to those found in other animal species to identify distinctive sequences associated with cattle.

Our analysis revealed a significant difference in bacterial composition between cattle faeces and manure compared to faeces from other domesticated and wild animals (confirmed by pairwise PERMANOVA with $P < 0.05$). Through k-mer analysis of the 16S rRNA gene, we successfully identified several cattle-associated regions of interest. These regions may serve as MST markers for identifying faecal contamination originating from cattle faeces and manure.

P080 | MICROPLASTICS IN THE ENVIRONMENT: IMPACT OF ORAL INGESTION ON HUMAN MICROBIOME AS ASSESSED BY IN VITRO GUT MODELS

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Microplastics (MPs) are recognized as a global threat due to their prevalence in natural environments and food chain. The gastro-intestinal tract (GIT) is the front door of MPs, but to date, the fate and potential effects of MPs in the human digestive environment remain largely unknown, especially for at-risk populations such as infants. This study aimed to investigate the effects of chronic ingestion of virgin MPs of polyethylene (PE), the most manufactured plastic polymer worldwide, on adult and infant microbiota using the Mucosal Artificial Colon model, which recreates the main physicochemical and microbial (luminal and mucus-associated microbiota) parameters of gut environment. The indirect impact of gut microbe metabolites after exposure to PE MPs on the intestinal barrier was evaluated using a co-culture of Caco-2 and mucus-secreting HT29-MTX cells. We report that PE MP impact on the gut microbiota was donor-dependent and resulted in an increase abundance of potential pathobionts, like Enterobacteriaceae, regardless of age conditions. Exposure to PE MPs was associated with a significant decrease in butyrate production in infants, while skatole levels were significantly increased in adults. Conversely, no significant impact of PE MPs on the intestinal barrier, as mediated by changes in gut microbial metabolites, was evidenced. This pioneering work provided significant insights into PE MPs interactions with human gut microbiota and intestinal barrier of two age groups, under relevant human colonic conditions. Next steps will be dedicated to study the impact of MPs on at-risk populations such as pathological situations associated with gut microbial dysbiosis (e.g obesity).

P081 | AUXOTROPHIES IN HUMAN GUT BACTERIA ARE INDICATIVE OF MICROBIOME STABILITY**Svenja Busche**¹, Danielle Harris^{1,2}, Konrad Aden², Silvio Waschina¹¹*Institute of Human Nutrition and Food Science, University of Kiel, Kiel, Germany*, ²*Institute of Clinical Molecular Biology, University of Kiel, Kiel, Germany*

Auxotrophies are defined by an incapability to synthesize essential nutrients and are known to influence the ecology of microbial systems. Since several human diseases are associated with microbial dysbiosis, studying the ecology of the human gut microbiome is essential. However, the influence of auxotrophies on the human gut microbiome remains obscure.

Here, we applied genome-scale metabolic modelling to predict amino acid auxotrophy frequencies with human gut microbiome data from a large population cohort study and statistically tested for associations with the diversity and metabolome data. The impact of auxotrophic bacteria on microbiome stability in the gut was analyzed with data from a longitudinal study.

As a major result, we observed a more stable microbiome structure with increasing frequencies of auxotrophic bacteria. Additionally, higher frequencies of auxotrophic bacteria in the gut were associated with a more diverse gut microbiome, and auxotrophic bacteria were linked to human metabolome data. Among all proteinogenic amino acids, tryptophan auxotrophies were predicted to have the highest abundance in the human gut microbiome. In sum, the results show that auxotrophic bacteria are common in the human gut microbiome and indicate a potential influence on stability.

P082 | EFFECT OF FAT, NITRATE AND 3-NITROOXYPROPANOL (3-NOP) AND THEIR COMBINATIONS ON THE RUMEN MICROBIOTA OF LACTATING DAIRY COWS**Dr Samantha Noel**¹, Morten Maigaard¹, Dr Peter Lund¹, Dr Ole Højberg¹¹*Aarhus University, Tjele, Denmark*

Methane emissions from agriculture represent a significant portion of anthropogenically derived greenhouse gasses, and reducing methane emissions from ruminants is critical to achieve climate change goals. Ruminants depend on reticulo-rumen microbial fermentation of ingested feed, however methanogenic archaea convert fermentation products (e.g., hydrogen and carbon dioxide) to methane. Individually, the feed additives fat, nitrate and 3-nitrooxypropanol (3-NOP) have proven effective in reducing methane emissions; here we investigate if these additives, alone or in combinations, affect rumen microbiota composition. Holstein dairy cows (n=48) were fed one of 8 treatments per period, arranged in a 2x2x2 factorial design, with two fat levels (30 or 63 g/kg DM), two nitrate levels (0 or 10 g/kg DM) and two 3-NOP levels (0 or 80 mg/kg DM) in a six-period incomplete 8x8 Latin square design. Rumen fluid was collected (stomach tube) from each cow at the end of each three-week period (n=288) and DNA extracted for prokaryote community analysis. For alpha diversity, sampling period affected species richness and Shannon diversity, and 3-NOP increased the species richness and Shannon diversity. For beta diversity, unweighted UniFrac revealed effects of sampling period and 3-NOP, where nitrate, 3-NOP and period affected weighted UniFrac. In conclusion, the rumen microbiota composition was altered by 3-NOP, to a lesser extent nitrate and feed additive supplementation changed composition over time. We observed no effects of fat however an interaction between fat and nitrate was seen on weighted UniFrac. Adding 3-NOP and nitrate in combination caused more differentially abundant genera than when fed alone.

P083 | RUMEN MICROBIOME RESPONSE TO DIFFERENT LEVELS OF BROMOFORM IN SHEEP

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Bromoform is a methane inhibitor and the major active component in the seaweed genus *Asparagopsis*. In order to determine the effect of bromoform on the rumen microbiome a trial with 30 sheep (n=5) was conducted where different doses of bromoform (0, 4.3, 13 and 39 mg/d) were fed for 17 days. In addition the highest dose was also fed once every three days. Rumen gases were quantified in respiration chambers and rumen samples collected after chamber measurements and were analyzed for metabolites and their microbial community composition.

Relative the control, methane emissions were decreased linearly by 10, 30 and 90%, while hydrogen emissions increased. The medium dose and the high dose offered every three days showed similar 30 % methane inhibition. Despite the decreased methane and increased hydrogen emissions no effect on the proportion of the volatile fatty acids, alcohols formate lactate or succinate was observed for the low and medium treatments. Only with the highest daily bromoform dose the proportion of propionate in the rumen was 10% higher compared to the control at the expense of acetate. and low concentrations of formate accumulated. Metabolomic and microbial community analysis is underway to elucidate the changes in rumen function and especially fate of hydrogen during methane inhibition in the rumen

Our data suggest that at 30 % methane inhibition hydrogen removal in sheep does not involve the standard hydrogen removal pathways described in literature.

P084 | SEASONAL CHANGES OF RUMEN AND FECAL ARCHAEAL COMMUNITY OF GRAZING CATTLE

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Canadian cow-calf producers generally place cattle on pasture during summer, therefore, it is important to improve feed efficiency (FE) and lower enteric methane emissions for such cattle populations. As the archaeal community contribute to the enteric methane emission, it is essential to examine their dynamics under grazing practice, so that to better understand their composition and roles in influencing host phenotypes. The current study examined the rumen and fecal archaeal community of the beef cows and heifers raised under common practices in Alberta, who were grazed in spring (tame pasture), summer (native pasture), and fall (deferred native pasture), while fed on drylot diet in winter for two consecutive years. Seasonal changes in total archaeal population were observed, but the shifting pattern was different between the two years. The archaea compositional profiles also showed differences between the two years: in y1 the archaeal profiles did not separate based on the four seasons, while in y2 the season-based clusters were observed. The discrepancies between the two years may be due to the different forage combination of the pastures where the cows grazed on. Regardless the year-to-year variation, Euryarchaeota was always the predominant phyla and Methanobrevibacter was always the predominant genus of the samples. Euryarchaeota was commonly found more abundant and Thermoplasmata was commonly found less abundant in November samples (deferred native pasture). The observed shifts in archaeal populations and composition suggest potential varied methane emissions patterns under grazing system.

P085 | IN VITRO SIMULATION OF ANTIBIOTIC-INDUCED DYSBIOSIS IN A NEW CANINE GUT MICROBIOME MODEL

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The relationship between pets and their owners has evolved towards companionship with an increase aware of animal health. Human-pet interplay led to gut microbes horizontal transfers between the two species, that may be associated with transmission of antibiotic resistance. Nevertheless, up to now, the impact of antibiotics on canine gut microbiota has been poorly described.

The Canine Mucosal Artificial Colon was set-up to reproduce the nutritional, physicochemical and microbial conditions found in the large intestine of medium size dogs. Metronidazole and enrofloxacin were daily administered at in-field doses to reproduce an antibiotic-induced dysbiosis of gut microbiota. Two bioreactors were inoculated with a faecal sample (n=2 donors) and run in parallel for 26 days under control or antibiotic conditions. Luminal and mucus-associated microbiota structure was determined by metabarcoding, and gut metabolites were followed before, during and after antibiotic exposure.

Antibiotics reduced microbial diversity and induced shifts in bacterial populations ($P < 0.05$). In the luminal medium in both donors, an increase in Enterobacteriaceae abundance was observed while Bacteroidaceae, Fusobacteriaceae and Burkholderiaceae decreased. Mucus-associated microbiota was less impacted by antibiotics with a donor-dependent effect. Microbial alterations were associated with decreases in gas production and SCFA concentrations. Microbiota resilience was observed within one week.

This is the first study providing a dynamic in vitro picture of antibiotic impact on canine microbiota structure and function. The model is a relevant tool as alternative to in vivo assays for an in-depth understanding of antibiotic-resistance mechanisms and testing of new restoration strategies in a context of One Health.

P086 | IMPACT OF DAILY ADMINISTRATION OF TWO LACTIC ACID BACTERIA ON THE GUT MICROBIOTA OF ADULT PIGS

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Pigs received two *Lactiseibacillus rhamnosus* strains (HN001 and FNZ129) separately (n=8) from 2 days of age until euthanasia at 19 weeks and their gut microbial communities (caecum, colorectum and faeces) were compared to control pigs. Animals were fed 5×10^{10} CFU/animal/day via sow milk replacer (pre-weaning) or via pellets soaked with bacterial suspensions (post-weaning).

Analysis of gut microbial diversity demonstrated an influence of the *L.rhamnosus* treatments in the caecum, but almost no effect was detected in the colorectum and faeces. Significant decreases in the relative abundances of Prevotella, Lachnospiraceae and Bacteroidales and in acetic acid concentrations were identified in caecal contents of pigs receiving the FNZ129 strain, suggesting a decrease in fibre metabolism. Organisms involved in lactic acid metabolism (Veillonellaceae and Erysipelotrichaceae) were significantly increased in both *L.rhamnosus* treatment groups. Using most probable number enumerations, FNZ129-fed animals showed a significant decrease in numbers of methanogens in the caecum compared to the control animals (6.8×10^6 vs 2.9×10^7 cell/g fresh gut contents), but no significant impact on the relative abundance of methanogens was identified.

Similarly, in piglets, *L.rhamnosus* HN001 influence the bacterial diversity and metabolic activity of the caecal microbial community rather than the colorectal or faecal microbiome (Young et al; 2022). In the current study, we found that *Lactiseibacillus rhamnosus* FNZ129 also had an impact on pigs' methanogen load. Further studies of the hydrogenotrophic caecal community are required to understand this effect.

Young W et.al.(2022) *Lactiseibacillus rhamnosus* HN001 alters the microbiota composition in the cecum but not the feces in a piglet model. *FrontNutr*.9:1002369.doi:0.3389/fnut.2022.1002369

P087 | EFFECTS OF TANNIN SOURCE AND LEVEL ON IN VITRO EQUINE CECAL FERMENTATION

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Strategies are sought to decrease agricultural emissions of greenhouse and volatile nitrogen gases. As dietary inclusion of tannins has been shown to decrease methanogenesis and improve nitrogen retention in ruminants, this study was designed to determine the effects of hydrolysable (chestnut) and condensed tannins (mimosa) on equine cecal fermentation. Equine cecal contents collected the morning of use from a cecal cannulated mare were distributed (10 mL volumes) to culture tubes preloaded with 0.2 g alfalfa and without or with 5, 10, 15, or 20% (wt/vol) chestnut or mimosa tannin (3 tubes/treatment), capped and then incubated anaerobically (100% CO₂) at 39°C. Results revealed both tannins sources decreased ($P < 0.01$) total gas and hydrogen accumulations by as much as 27 and 82%, respectively, when compared to accumulations in controls (11 mL and 0.34 $\mu\text{mol/mL}$ incubation fluid). Mimosa, but not chestnut, treatment decreased ($P < 0.01$) methane accumulations, with decreases ranging from 4 to 83% compared to controls (1.1 $\mu\text{mol/mL}$), and acetate accumulations, by as much as 36% compared to controls (14.8 $\mu\text{mol/mL}$). Neither tannin affected accumulations of butyrate (1.6 $\mu\text{mol/mL}$) and both tannins increased ($P < 0.05$) propionate accumulations by as much as 38% compared to controls (5.7 $\mu\text{mol/mL}$). Ammonia accumulations were decreased ($P < 0.01$) from that in controls (0.24 $\mu\text{mol/mL}$) by as much as 96 and 87% by chestnut and mimosa treatments, respectively. These results indicate tannins may reduce methane and volatile nitrogen emissions in equine cecal fermentations and warrant further research to validate their effects in vivo.

P088 | TOWARDS IMPROVED FUNCTIONAL PREDICTIONS FROM METATAXONOMICS – A STABLE AND TAXONOMY FREE APPROACH TO CONSOLIDATE MICROBIOME GENOMIC DATA

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Microbiomes are studied at genomic levels ranging from single genes/amplicons to community-wide shotgun metagenomes. Individual researchers select the most appropriate level based on the varying advantages and disadvantages of each, including specificity and cost. The range of choice leads to difficulties making direct comparisons between them. The 16S rRNA gene is an economical and routine choice to identify taxa (called metataxonomics) in microbial ecology studies, but different regions of the genes are studied by different researchers. Uncovering the functional potential of individual microbes requires more resource-intensive metagenome-assembled genomes (MAGs). To address these challenges, we have developed a novel identity coding system based on the Life Identification Number (LIN) framework. It allows the consolidation and integration of multiple levels of microbiome genomic datasets.

We applied the approach to link metataxonomic and metagenome-assembled genomic data from ruminant microbiomes. By focusing on sequence similarity we can improve direct comparability between existing studies, that utilise different data generation approaches. Stable classification is robust to future changes in taxonomy. Inaccuracies and inconsistencies in reference databases and published studies are bypassed. The fine-grained resolution of the barcoding approach enhances phylogenetic placement. This provides a rationale for its use in leveraging the advantages of full genome sequences in large studies where it would otherwise not be viable or practical. The development of a distance-based scoring system allows the evaluation of system performance at multiple levels. It has been used to offer new insights on the performance of commonly used variable regions.

P089 | NON-CODING RNAs IN KEY RUMEN METHANOGEN INDICATES NOVEL TARGETS FOR METHANE MITIGATION

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Methane emissions from ruminants contribute to significant portion of the global total greenhouse gas emissions, which causing detrimental effects to the environment and animal production. Tremendous effort has been made to regulate rumen methanogenesis, so that to lower carbon footprint from cattle production chain. However, current mitigation methods either cannot deliver consistent effects or show adverse impact on rumen microbial functions, which hinders the adoption of the methods by the producers. The current study explored the genome of a key rumen methane producing species, *Methanobrevibacter gottschalkii*, using systematic identification method to explore novel targets for manipulating. A total of 97 ncRNA candidates were identified from the genome, 73 of which were reverse complementary to the mRNA-coding genes, whereas the remaining located in the intergenic regions or remote loci where no genes were nearby. Of these candidates, 53 of them were predicted as ncRNAs with unknown functions, while the others were predicted as snoRNAs/miRNAs/RNaseP/telomerase. Five ncRNAs candidates are of specific interests, that they are the asRNAs of the key methanogenesis enzymes (methyl-coenzyme M reductase and N5-methyl-tetrahydromethanopterin-coenzyme M methyltransferase). These asRNAs may regulate the expressions of these genes, and thus further influence the methanogenesis of this methanogen. Such mechanism needs to be further studied.

P090 | PROBIOTIC AND PREBIOTIC SUPPLEMENTATION AMONG SWISS PARA-ATHLETES: A PILOT STUDY ON THE GUT MICROBIOME AND INFLAMMATION STATUS

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Gut-related illnesses are among the causes of morbidity in para-athletes during training, performance and recovery. Therefore, exploring interventions that improve their gut health is important. In this pilot study, we aimed to explore the association of the gut microbiome to the para-athletes' inflammation status at baseline and look into the effect of a probiotic (Bactosan®) and a prebiotic (oat bran) to the gut microbiome and the inflammation status. We conducted a 4-week intervention period, cross-over randomized controlled trial among Swiss para-athletes at the Swiss Paraplegic Center. The gut microbiome was characterized by Oxford Nanopore® sequencing of 16S rRNA amplicons from stool DNA and we measured 31 serum inflammatory markers by bead-based multiplex immunoassays from Biolegend®. There were 14 participants (6 males, 8 females) with mean age of 33.9. At baseline, we found that the gut microbiome alpha diversity is affected by IL1 β , IL2, IL4, IL6, IL8, IL12p70, IL17A, IFN γ , TGF α , erythropoietin, angiopoietin-2, MCSF, G-CSF, GM-CSF, SCF and leptin while beta diversity by angiopoietin-2, IL2p70 and leptin. The gut microbiome diversity remains stable even after probiotic and prebiotic use. Inflammatory status of the para-athletes across the trial was low and was not affected by probiotic or prebiotic use. The trial provided insight on the association of inflammatory markers to the gut microbiome of para-athletes. The lack of change in the gut microbiome and the inflammation status could be explain by the already healthy and good gut health status of the para-athletes at the onset and possibly underexposure to the interventions.

P091 | CASE (CONCATENATED AMPLICON SEQUENCING): A NOVEL WET LAB PROTOCOL FOR STREAMLINED AND AFFORDABLE LIBRARY PREPARATION FOR PACBIO AMPLICON SEQUENCING

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Sequencing technologies are an indispensable tool for investigating microbial communities in microbiome studies. Amplicon sequencing is a cost-effective, sensitive, and scalable technique to gather data on the diversity and composition of microbial populations. Among sequencing platforms, long-reads provide a better resolution of amplicons by providing more base pairs. It also provides a more accurate identification of possible rare species. Further, PacBio HiFi sequences are of exceptional read quality, with less than 1 error in 100,000 bases.

Yet everything comes with a downside, long-read sequencing technologies tend to be more expensive. We developed a novel wet lab protocol for amplicon sequencing that simplifies and reduces the cost of library preparation for PacBio sequencing. We designed universal primer and barcode sets that can be adapted for any target region, such as 16S, 18S or ITS, to use with any type of DNA. We tested our protocol with a range of samples, including complex microbial communities and low-input DNA samples and compared it to existing PacBio amplicon sequencing protocols and Illumina sequencing. Our protocol is catered to reduce costs while getting high-resolution results from PacBio sequencing platforms.

P092 | IMPROVING ISOLATION OF NEXT GENERATION PROBIOTICS STRAINS FROM HUMAN GUT OPTIMIZING CULTURE MEDIA AND ENRICHMENT PROCEDURES

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The term Next-Generation Probiotics (NGPs) refers to microbial strains that can have a positive effect on human health, but do not belong to common probiotic species (e.g., lactic acid bacteria, Bifidobacterium). Several studies highlighted the unexplored source of potentially beneficial microbes in the gut microbiome. For this reason, academic and industrial research is focused on identifying and testing novel microbial strains of gut origin. One of the main issues in NGP culturing was the selection of a suitable culture medium that allowed the growth of these high-demanding species. We tested nine culture media with different formulations in terms of vitamins, minerals and fatty acids to study the culturable fraction of the gut microbiome. We collected bulk microbial cells grown on two plates of each medium and analyzed them by 16S rRNA sequencing of the V3-V4 regions. We sequenced amplicons obtained from the original fecal samples to identify differences among the media and which of them gives a more reliable picture of the gut microbiome. Results obtained allowed us to select four media that supported the growth of the highest number of putative NGP species. Samples were streaked on four media and incubated in aerobic and anaerobic conditions at 37°C to discard facultative anaerobes. To select strict anaerobes, we tested a pre-enrichment step of fecal samples in different broths for 48 h in anaerobic conditions. Fifty-two bacterial colonies were isolated and identified, including the promising NGP candidate *Bacteroides uniformis*.

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P093 | ANALYSING THE ROLE OF GUT MICROBIOTA IN A SOUTH INDIAN COHORT OF HEALTHY AND OBESE INDIVIDUALS

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A stable microbial ecosystem is shaped according to the dietary habits and lifestyle of an individual. Disturbances in the stability such as changes in relative abundance of some key microbial communities has been observed in metabolic disorders like obesity which is an adverse metabolic disease resulting in adipose tissue dysfunction, fat mass gain serving as a risk factor for type 2 diabetes, cardiovascular diseases etc. The gut microbiome abundance data from healthy (BMI<25 kg/m²), overweight (BMI =25-30 kg/m²) and obese (BMI>30kg/m²) South Indian individuals were analysed to understand the changes in microbiota in response to diet, sedentary and non-sedentary lifestyle. The differentially abundant species were determined using statistical methods and decision tree models. In all the groups, Prevotella copri and Faecalibacterium prausnitzii were the most abundant species constituting 35-40% of the total abundance. In healthy individuals, abundance of Bacteroides, Bifidobacterium and Blautia increased with an active lifestyle. In non-sedentary individuals the abundance of Eubacterium and Sutterella significantly increased in overweight and obese individuals while that of Odoribacter decreased compared to healthy. In the sedentary individuals, there was a significant reduction in genera like Alistipes and Barnesiella in overweight and obese individuals while the abundance of Faecalibacterium and Clostridium genera increased in the obese. The physiological effects of the variation in species along with diet can be studied using model generated metabolic fluxes. The combined effect of the species abundance and metabolic fluxes can be used to generate scoring criteria for classifying the healthy and obese individuals.

P094 | DEVELOPMENT OF HIGH THROUGHPUT, MICROTITRE PLATE-BASED GROWTH ASSAYS FOR IMPORTANT GROUPS OF RUMEN METHANOGENS TO ENABLE INHIBITOR DISCOVERY

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Discovery of novel inhibitors of ruminant methane is limited by current methanogen cultivation methods, which require pressurised H₂ + CO₂ headspaces in large volume tubes or serum bottles, taking significant preparation time and resulting in low throughput screens. Assays have been developed for Methanobrevibacter sp. AbM4 and Mbb. boviskoreani JH1 grown on ethanol but these methanogens represent <1% of ruminal methanogens. Here we describe 3 new methanogen growth assays, based on Mbb. ruminantium M1, Mbb. gottschalkii D5, and Methanosphaera sp. which represent over 80% of rumen methanogens. In an anaerobic chamber, BY or BRN10 media were dispensed into 96 well microtitre plates containing potential inhibitors and controls, inoculated with fresh cultures of M1, D5, or WKG6, and sealed in pressure-controlled, stainless steel, cannisters with a CO₂-generating pack. Outside the chamber, cannisters were pressurized to 180 kPa with H₂:CO₂ (80:20) and incubated at 39°C/5 days, allowing sufficient methanogen growth (Spectramax plate reader, OD595nm) to distinguish growth inhibition from uninhibited growth in the negative control. The three assays, along with the JH1 assay, were used to screen lactic acid bacteria extracts (>1500 strains, 43 species) and rank their growth inhibition of one or more of the test methanogens. The assays revealed variation in extract inhibition across the 4 methanogens; extracts from 51 strains inhibited JH1 growth by more than 80%, 17 extracts were most effective against WKG6, while M1 and D5 were less sensitive, showing a maximum of 65% and 25% growth inhibition by extracts, respectively.

P095 | FAECAL MICROBIOTA OF PIGS DURING THE POST-WEANING PERIOD EVALUATED WITH 16S rRNA SEQUENCING

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At weaning, piglets are separated from the sow, causing a sudden diet shift—from sow milk to grains. A disruption of the gut microbiota follows, which may lead to watery diarrhoea, primarily caused by the proliferation of enterotoxin-producing *Escherichia coli*. This post-weaning diarrhoea (PWD) affects newly weaned piglets worldwide. The aim of this study was to follow the development of the gut microbiota during the post-weaning period, to improve PWD-prevention strategies.

Faecal swab samples were collected from 32 healthy piglets from three different Swedish farms with a weaning age of 32 days. They were sampled before weaning, and on days 3, 7, 10, 14 and 21 post-weaning. The microbiota in the 192 samples was characterised by sequencing of the V3-V4 hypervariable regions of the 16S rRNA gene.

At the phylum level, an average of 98% of the operational taxonomic units (OTUs) were classified (range 89-100%). Bacteria from the phyla Firmicutes and Bacteroidetes dominated the faecal microbiota at all time points, with a mean relative abundance of 67% (range 9-100%) and 18% (range 0-75%), respectively. At family level, an average of 70% of OTUs were classified (range 39-100%). The most common families on average were Lachnospiraceae (13%, range 0-57%), Prevotellaceae (11%, range 0-63%), and Oscillospiraceae (10%, range 0-36%).

The composition of the microbiota varied between individuals and across time points. This, and the classification of only 70% of OTUs at the family level, indicates a need for further studies to evaluate the composition of the intestinal microbiota at a more detailed level.

P096 | UTILIZING A METAGENOMICS APPROACH TO DETERMINE MICROBIOMES AND ANTIBIOTIC RESISTANCE GENES ASSOCIATED WITH PERFORATING ABOMASAL ULCERS IN BEEF CALVES

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In western Canada, perforating abomasal ulcers (AU) generally occur in calves 3 to 8 weeks of age, and are invariably diagnosed post-mortem. Some calves may have died peracutely, whereas others may be administered antibiotics and adjunct therapy based on clinical signs consistent with gastrointestinal disease. *Clostridium perfringens* has been associated with AU, but the evidence is circumstantial. Thus, analysing the microbiome may provide some insight into potential pathogens as well as the impact of antibiotic usage on antimicrobial resistance. We used metagenomics (Illumina NovaSeq 6000) to compare the microbial composition of abomasal tissues derived from 12 calves with and 6 calves without AU and to associate antimicrobial use with the presence of antimicrobial resistance genes using AMRFinderPlus. There was a high prevalence of Staphylococcaceae (1.5%), Campylobacteraceae (1.4%) and Enterobacteriaceae (1.4%) across all samples, while *Staphylococcus aureus* (1.4%), *Anaplasma phagocytophilum* (1.3%) and *Microbacterium esteraromaticum* (1.1%) were the most abundant species. Differential abundance (MaAsLin2) only revealed *Streptomyces* sp. REN17 to be less expressed in AU calves. Antimicrobial resistance genes (ARG) encoding for phenicol, macrolide (ribosome methylase), and tetracycline resistance were found in calves with and without a history of antimicrobial therapy. Although there were differences in the predicted activity among groups, they were not significant ($P > 0.05$). We did not identify a microbiota unique to AU, nor could we associate prior antimicrobial therapy to a specific microbiota. A larger sample size is needed to better understand the role of the microbiome in AU etiology, and in the relationship with antimicrobial resistance.

P097 | IMAGE AND GRAPH CONVOLUTION NETWORKS IMPROVE MICROBIOME-BASED MACHINE LEARNING ACCURACY

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The human gut microbiome is associated with a large number of disease etiologies. As such, it is a natural candidate for machine-learning-based biomarker development for multiple diseases and conditions. The microbiome is often analyzed using 16S rRNA gene or shotgun sequencing. Several properties of microbial sequences-based studies hinder machine learning (ML), including non-uniform representation, a small number of samples compared with the dimension of each sample, and sparsity of the data, with the majority of taxa present in a small subset of samples. We suggest combining different taxa to improve microbiome representation for ML using microbial taxonomy, using the image Microbiome (iMic) platform..

iMic translates the microbiome to images and predictions are performed using Convolutional Neural Networks. This drastically improves the performance of static and dynamic ML compared to the state-of-the-art methods and facilitates the interpretation of the classifiers through an explainable artificial intelligence (AI) algorithm.

P098 | MICROBIOME IN THE GUT-SKIN AXIS OF ATOPIC DERMATITIS PATIENTS IN SAUDI ARABIA

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Microbiome is the new organ of the body. Microbiome is very important to be studied and thoroughly investigated to better understand the correlation between Microbiome and human health and Diseases. In this study, 12 children, 6 Atopic Dermatitis (AD) patient and 6 control, were involved to look for a potential explanation of the AD development through differences that may be spotted between their gut microbiome. 12 children, 6 of them AD patients and the other 6 are healthy control participants; age group are between 2 to 7 years old. Fecal samples were collected and reserved for DNA extraction. After DNA extraction, 16S rRNA (V3 – V4 region) were multiplied by PCR and sent to BGI for Sequencing and Bioinformatics. Once the result was received a data analysis were performed. Data statistic showed a slightly fluctuated result for each sample. Operational Taxonomic Unite (OUT) presented 371 Bacteria and the top three most abundant phyla for both groups were Bacteroidetes, Firmicutes, and Proteobacteria. However, on a phyla level AD group has high abundance of Lentisphaerae and TM7 (Saccharibacteria) spp. It was expected to see a typical bacteria composition as shown in other studies for this age group. However, the two group of AD and control presented very minor differences between them in terms of diversity and abundance. We recommend larger size of sampling and more critical cases of AD.

P099 | THE RELATIONSHIP BETWEEN BUCCAL MICROBIOTA AND METHANE EMISSION OF HOLSTEIN FRIESIAN DAMS AND THEIR CALVES

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The objectives of this study were to investigate (1) the parental relationship between dams and calves based on buccal microbiota and (2) whether buccal microbiota is associated with methane emission in dams and calves. Methane emission of 37 Holstein Friesian dams was measured in their dry period. Within 24h of calving, buccal samples were collected from the dams. The calves received colostrum from the dam and were weaned at 10 weeks of age. The calves were housed individually until 3 weeks of age and subsequently group housed until 16 weeks of age. Methane emission of the calves was measured for four weeks (week 13-16) and the buccal samples were collected at the start of methane measurements. Buccal samples of the dams and calves were characterized by metataxonomics. Parental association was assessed by PCA and permutation tests, and association with methane emission by sPLS. There was a clear separation between the buccal microbiota of the dams and the calves, but within calves, the buccal microbiota did not reflect the classification of dams as high or low methane emitters. The correlations between common ASV in true dams-calves pairs were not different from the correlations of random pairs. For both the dams and the calves, no relationship between buccal microbiota and methane emission could be found. Overall, we conclude that (1) dams and calves have a different buccal microbiota, (2) there is no link between the buccal microbiota of dams and their calves, and (3) buccal microbiota is not related to methane emission.

P100 | THE EFFECT OF EXERCISE INTERVENTION AND CARDIORESPIRATORY FITNESS ON THE GUT MICROBIOME: A GENERATION 100 COHORT STUDY

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The composition of gut microbiome can be influenced by multiple factors such as lifestyle, diet, age, geography. We hypothesized that intervention to support healthy aging by promoting exercise might lead to an altered gut microbiome. Here we present unique longitudinal exercise intervention study (G100 study NCT01666340, ClinicalTrials.gov registry) in which we have invited participants (n=73) to a randomized sessions of either high interval intensity trainings (HIIT) or moderate intensity trainings (MICT). Participants were subjected to clinical examinations, physical tests, questionnaires, and gut microbiome sampling after 5 years of continuous exercise intervention. The microbiome of physically active participants exhibits higher diversity and more favourable metabolic capacity compared to sedentary counterparts. Our data supports the hypothesis that exercise, specially the MICT schedule, promotes gut colonization with short-chain-fatty-acid producing bacteria such as Akkermansia spp, Clostridium spp, Prevotella spp. and drives significant increase in species biodiversity. However, unexpectedly we have revealed that more intense exercise routines (HIIT) in elderly population may also foster some of the unhealthy gut biome (e.g. Eggerthellaceae, Escherichia). Here we would like to emphasize that the key for a 'healthy microbiome' is a maintenance of high diversity of those species belonging to beneficial genera, forming symbiotic environment for its host while reducing abundance of dysbiotic species. The participants in this study had unique gut microbiomes supporting the notion that personalized optimization of gut microbiome might be the future direction in this field.

P101 | ANTIMICROBIAL EFFECT OF PINK PEPPER (SCHINUS TEREBINTHIFOLIUS RADDI) ESSENTIAL OIL IN JACKFRUIT STARCH BIODEGRADABLE FILMS

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The incorporation of natural antimicrobial compounds, such as essential oils, in biodegradable films has been explored to reduce the use of artificial preservatives, increasing the shelf life of food products, that could be easily deteriorated by spoilage microorganism, besides the possibility of adding flavour to the end-product. The present study aimed to determine the antimicrobial activity from pink pepper essential oil when it is incorporated to the biodegradable film. A filmogenic solution was made with 3% (w/v) of starch extracted from jackfruit seed and plasticized with 40% (w/w) of glycerol. Pink pepper essential oil 7% (v/v) was emulsified with 1% (w/v) of tween 80 and incorporated into the filmogenic solution after cooling to 35 °C. The filmogenic solution was placed in petri dishes and dried at 30°C. Assays of antimicrobial activity was carried out using strains of *Listeria innocua*, *Escherichia coli* K12 and *Staphylococcus epidermidis*. Film discs were placed on top of the agar plate containing bacteria inoculum (105 CFU.ml⁻¹). Plates were placed in the fridge overnight and then incubated at 37°C for 24h. After this period, the diameter of the halos formed was measured. The films produced in this study showed promising results enhancing the potential of those films as an alternative against synthetic preservatives used to improve the shelf life and sensory characteristics in foods such as hams, cheeses and fresh pasta.

P102 | RESILIENCE OF FAECAL MICROBIOME AFTER A DRUG STRESS IN ADULT AND SENIOR EQUIDS

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The resilience of the large intestine (LI) microbiota may prevent the risk of dysbiosis and limit the associated inflammation state and intestinal mucosa permeability. This property is closely linked to microbiota diversity. Equine LI bacterial diversity decreases with ageing. Thus, senior horses may be more susceptible to challenges they are regularly facing. This study assessed the resilience of the faecal microbiome in senior horses compared to adults after an anthelmintic administration.

Faeces and blood were sampled in 8 senior (26ffl3 years) and 8 adult (7ffl1 years) healthy horses managed under controlled conditions for 2 months. After a 1-month acclimatation, ivermectin was orally administered. Faecal bacteria diversity and composition, cellulolytic bacteria enumeration, short-chain fatty acids (SCFAs) concentration, and blood lipopolysaccharides (LPS) concentration were determined before (D0), two (D2), seven (D7), and 28 days (D28) after administration. Analysis of variance was performed to evaluate the effects of age group, day, and the age group*day interaction.

Ivermectin administration decreased LI cellulolytic bacteria concentration at D2 (p=0.001) and D7 (p=0.001), and SCFAs concentration at D2 (p=0.002) compared to D0. The microbiota structure differed between age groups (p=0.021). There was no difference on bacterial diversity and no interaction was found for all studied variables. This suggested that the microbiota of healthy seniors was as resilient as the adults' one. However, seniors had higher blood LPS concentration than adults (p=0.002) supporting an increased gut permeability. Future investigations are necessary to understand the relation between microbiome diversity, resilience, and increased gut permeability in senior horses.

P103 | IDENTIFICATION AND CHARACTERIZATION CGTase-PRODUCING BACTERIA FROM FRESHLY ISOLATED RUMINAL BACTERIA USING DILUTION-TO-EXTINCTION METHOD FOR BIOTECHNOLOGICAL APPLICATIONS

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The cyclodextrin market has expanded significantly due to their versatility and diverse range of applications, mainly in the food and pharmaceutical industries. However, the enormous cost associated with the synthesis of cyclodextrins, which is exclusively enzymatic by cyclodextrin glycosyltransferase (CGTase) action, is a limiting factor for its use in industrial processes. Bioprospecting new microorganisms producers of CGTase could be a promising strategy to reduce the cost associated with cyclodextrin production. Rumen microorganisms has proven to be good producers of robust industrial enzymes and can be an alternative source for CGTase isoforms. The present study aimed to carry out a screening for ruminal bacteria producers of CGTase using a ruminal bacteria collection recently isolated by dilution to extinction method. A total of 134 ruminal bacteria isolates were anaerobically cultivated in basal liquid medium. Aliquots of cultures were placed to agar plates containing starch and β - and γ - CGTase indicators, phenolphthalein (PHP) and bromocresol green (BCG) dyes respectively, and incubated at 39°C for 48h. Cultures with a surrounding halo were identified as starch degrading and selected for further characterization. Preliminary results confirmed the rumen microbiome potential for CGTase producers. *Streptococcus bovis* isolates demonstrated promising potential for CGTase activity in vitro and corroborated in silico analyses performed with publicly ruminal genomes from the Hungate 1000 collection. Ongoing investigations of these CGTase activity in ruminal bacteria could highlight distinct features of specificity, catalytic capacity and stability compared to the well-known commercial available sources, consequently, broadening the potential of ruminal bacterial for biotechnological applications.

P104 | TRANSCRIPTOMIC AND PROTEOMIC CHANGES ASSOCIATED WITH COBALAMIN-DEPENDENT PROPIONATE PRODUCTION BY THE RUMEN BACTERIUM

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Prevotella ruminicola is an abundant rumen bacterium that can produce propionate in a cobalamin-dependent manner. However, the underlying genes and regulatory mechanisms are poorly understood. To assess whether propionate production in *P. ruminicola* is controlled by a cobalamin-binding riboswitch, we conducted *in silico* analyses and compared transcriptomes and proteomes of cultures grown in the presence and absence of cobalamin. We generated the complete genome sequence of *P. ruminicola* KHP1 and found that it contained four putative cobalamin riboswitches. However, these were not in close proximity to genes putatively involved in propionate production via the succinate pathway, nor were the putative propionate pathway genes co-located in a single operon. Comparative genome analyses of 14 *Prevotella* 1 strains revealed no differences in the presence of candidate propionate pathway genes between propionate-producing and non-producing strains. However, all propionate-producing strains possessed a conserved arrangement of genes encoding a putative transport protein and three subunits encoding a putative methylmalonyl-CoA decarboxylase, upstream of, but in the antisense orientation to co-located genes encoding methylmalonyl-CoA mutase subunits. Cobalamin presence led to the differential expression of 17.5% of the KHP1 genes, including candidate propionate pathway genes. Effects on the KHP1 proteome were less pronounced, and the only differentially expressed enzyme involved in propionate production was the cobalamin-dependent methylmalonyl-CoA mutase, which showed increased abundance in the presence of cobalamin. While our results demonstrate the differential expression of candidate propionate pathway genes in response to cobalamin, these do not appear to be under direct control of a cobalamin riboswitch.

P105 | CONDUCTIVE MATERIALS INCREASE METHANE PRODUCTION AND ENRICH RARE MEMBERS OF THE RUMEN MICROBIOTA

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The dominant electron transfer process in the rumen involves the electron shuttles dihydrogen and formate produced by the microbiota. These shuttles are then used by methanogens to produce methane. But in the rumen the relevance of shuttle-free electron transfers, which are described in other methanogenic environments, is unknown. We hypothesized that conductive materials (CM) would induce microbial enrichment linked to a possible extracellular electron transfer (EET) mechanism in the rumen. We used consecutive batch *in vitro* cultures (ten series, 72 h) to explore the effects of CM on methane production and rumen microbes. Treatments consisted of CM graphene (GPH) or magnetite (MAG) in culture media containing solid or soluble substrates. Triplicate batch cultures (three donor sheep) contained 36 mL of culture media and 4 mL of rumen fluid or microbial suspension from the previous culture. Incubations without CM were the Control. Compared to the Control, methane production increased by >40% (P<0.001) from incubation #3 in GPH and MAG, regardless of substrate. The rumen microbial community shifted throughout the incubation series and was influenced by substrate and CM (P<0.001). The final CM incubation #10 in soluble substrate showed enrichment of genera that were poorly or not detected in the Control and the initial inoculum. Methanococcus methanogens increased 7-fold and *Disulfobrevibacter* by 22% in MAG; while *Methanococcus* increased 10-fold in GPH. Both CM increased *Treponema* and *Syntrophococcus* bacteria by >3-fold. Our results suggest the presence of an EET mechanism in the rumen ecosystem that should be validated using complementary approaches.

P106 | STRUCTURE AND NETWORK ANALYSIS OF THE RUMEN AND HINDGUT MICROBIOTA OF COWS REVEALS POTENTIAL ADAPTATION TO ADVANCED DURATION OF HIGH-GRAIN FEEDING

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The microbial communities in cattle gastrointestinal tract are diverse in their composition and response to external perturbations. A change in feeding regime represent a challenge for the microbiota, which need to adapt to the new substrates. The aim of this study was to evaluate how the rumen and fecal microbiota react to a switch from forage to high-grain feeding and to the duration on the new diet. Nine non-lactating, rumen-cannulated cows were used in this experiment (1 week on forage and 4 weeks of high-grain feeding). Microbial composition was analyzed through 16S rRNA gene sequencing and correlation networks were used to study the variations in the microbial communities within each week. Abundance analysis showed effects of diet on microbial composition in both rumen and feces, and different response to the duration on high-grain diet. In the rumen, diet change reduced species diversity and richness, which in turn corresponded to higher density, transitivity and assortativity in the network for the first week on high-grain compared to the other weeks on high-grain. In feces, the transitivity and assortativity increased towards the end of the experiment, while the density decreased, which was accompanied by a tendency for an increase in alpha-diversity indices in those samples. Despite an initial decline, *Succinivibrionaceae* and *Roseburia* in the rumen and *Ruminococcaceae* in feces recovered by week 4 on high-grain. Overall, the dietary shift and duration of feeding caused major changes to the structure of the microbial communities in the gut, with differential responses, depending on sample origin.

P107 | DISTINCT EFFECTS OF ANAEROBE GUT FUNGI ON FEED LIGNOCELLULOSE COMPOSITION AND STRUCTURE DURING ITS DEGRADATION

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Manipulation of the rumen microbiome is a promising avenue to address major challenges in ruminant farming, but requires better understanding of the microbiome function. Rumen microbiome-derived anaerobic gut fungi (AGF) are powerful degraders of feed lignocellulose, whose activity increase animal performance in vivo, and strongly enhances digestibility of feed in rumen fluid in vitro cultures. Despite their importance, these organisms remain less well studied as their bacterial counterparts. Consequently, we have very limited understanding of how their unique biochemical and physical degradative mechanisms affect lignocellulose substrates, and thereby feed digestibility.

Here, we assessed the effects that AGF and their enzymes had on the composition and structure of complex lignocellulose feeds. We hypothesized that AGF with different enzymatic degradative potential and distinct morphological features for penetration of feed particles, would have distinct roles or niches in feed digestion. We therefore isolated and selected representatives of three fungal genera and assessed how these AGF affected lignocellulose composition in situ, for common ruminant feed components wheat straw and timothy grass hay. Feed fibre analysis of solids recovered from in vitro cultures demonstrated that *Neocallimastix* degrades hemicellulose and cellulose in the feed at similar rates, while *Piromyces* and *Caecomyces* preferentially degrade hemicelluloses. Growth of the AGF on cellulose further demonstrated strongly distinct abilities to swell and disintegrate this model substrate.

The activity of the three AGF isolates thus resulted in very different effects on lignocellulose composition and structure, and strongly suggest that these fungi have distinct roles in the microbiome during feed digestion.

P108 | DEVELOPMENT OF A NOVEL ENSEMBLE BINNING TOOL AND ITS EVALUATION ON AN EARLY LIFE RUMEN MICROBIAL DATASET

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Metagenome-assembled genomes (MAGs) are critical for understanding microbial communities, but binning assembled contigs can be a challenging process, involving a variety of tools and approaches. Binning tools can be split into two types: individual and ensemble, where individual is independent binning, and ensemble takes the output of multiple binning tools and refines the result. A core aspect of most ensemble bidders typically rely on an approach first utilized by DAS tool, which first score bins based on completeness and contamination using universal single copy genes (USCGs), choose the 'best bin', and remove the best bins contigs from the remaining pool of bins, and rescore until there is a non-redundant bin set.

There are two important limitations to this strategy, the first is the inherent issue with USCGs, which is not necessarily a good approach for genomes with reduced sizes or for less well represented genomes. The second is the scoring approach does not necessarily maximize utilization of read data or generation of high-quality MAGs.

We assess the importance of these by producing a standalone tool utilizing a new graph-based approach which is non reliant on USCGs. We then evaluated our tool on a large early life rumen microbial dataset, as well as the synthetic CAMI challenge datasets, finding promising improvements. Our approach offers a new alternative for ensemble binning improving MAG attainment as well as avoiding the disadvantages of current tools.

P109 | CONSERVED GUT MICROBIAL GUILDS ACROSS THE HUMAN LIFE STAGES**Dr Ezgi Özkurt**^{1,2}, Dr Rebecca Ansorge^{1,2}, Dr. Clémence Frioux³, Dr Falk Hildebrand^{1,2}¹Quadram Institute Bioscience, Norwich, United Kingdom, ²Earlham Institute, Norwich, United Kingdom, ³Inria, INRAE, CNRS, Univ. Bordeaux, Bordeaux, France

The gut microbiome plays a crucial role in human health, but its significance during different life stages remains uncertain. Nevertheless, research has shown that the gut microbiota follows a predictable transition from infancy to adulthood. As the gut microbiome ages, it becomes less diverse, has a more unique composition, and a lower abundance of bacteria that produce short-chain fatty acids (SCFAs). Although certain bacteria are commonly associated with early or late life stages, our knowledge of associated bacterial guilds and their interactions is still limited.

Our study examined age-type gut microbiomes and their evolution over time by analyzing nearly 9,700 publicly available faecal metagenomes from 36 studies worldwide. We found that individuals in the same life stage tend to have consistent microbial guilds coexisting in their gut despite differences in genetic background and lifestyle. We hypothesize that these microbial guilds form the basis of a healthy gut flora. Analyzing microbial composition and intricate interaction networks among bacteria revealed crucial colonization patterns that decline in the mature adult microbiome but reappear in the elderly. Finally, we investigated the evolutionary forces shaping microbial communities in different age groups, contextualizing our findings within an evolutionary framework. Our study underscores the importance of microbial guilds in the gut and their evolution during different phases of human life.

P110 | BACILLUS AMYLOLIQUEFACIENS -516 HARBORS INTERESTING PROPERTIES FOR BEING USED AS A FUTURE BENEFICIAL DIRECT-FED MICROBIAL IN RUMINANTS**Dr Raphaelle Gresse**¹, Dr Giuseppe Copani², Dr Bruno Ieda Cappellozza², Ms Jeanne Danon³, Dr Vincent Niderkorn¹, Dr Evelyne Forano³¹Université Clermont Auvergne, INRAE, VetAgro Sup, UMR Herbivores, Saint Genes Champanelle, France,²Chr. Hansen, Animal and Plant Health & Nutrition, Hørsholm, Denmark, ³Université Clermont Auvergne, INRAE, UMR 454 MEDIS, Saint Genes Champanelle, France

In the last decades, using direct-fed microbial has improved livestock animals' production and overall health status. The ability of *Bacillus* spp. to form endospores confers to this species the benefit of surviving harsh conditions (e.g. high temperature), representing an advantage in terms of long-term storage and possible feed application compared to non-spore-forming bacteria. In this study, the potential of *Bacillus amyloliquefaciens* -516 to be used as a direct-fed microbial product in ruminants was assessed using various *in vitro* techniques. Cultures started from spore bulks showed that the 516 strain was able to germinate, survive and grow in several media containing rumen juice. Gas composition analysis of *in vitro* culture in a medium containing 40% rumen juice using gas chromatography revealed that germination of *B. amyloliquefaciens* -516 could reduce oxygen level thus favoring an anaerobic environment for rumen microbes. At last, the enzymatic capacity of the *B. amyloliquefaciens* 516 strain in pure and diluted rumen fluid was assessed on purified polysaccharides and complex substrates. Results revealed that the strain produces both xylanase and amylase in rumen content and possesses the ability to release sugars from complex substrates such as corn, barley, wheat, and soy flour. Taken together these data suggest that *Bacillus amyloliquefaciens* -516 could be a good candidate for being used as a direct-fed microbial in ruminants. Further *in vivo* testings of this strain in dairy cows are needed to confirm the *in vitro* findings.

P111 | StORF-REPORTER: PSEUDOGENE DETECTION REVEALS INSIGHTS INTO HISTORICAL GENE SHARING DYNAMICS IN THE RUMEN MICROBIOME

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In recent years, it has become apparent that prokaryotic genomes contain large numbers of pseudogenised genes which may provide valuable insights into the recent functional history of their host environment. Accurate detection of pseudogenes will therefore enable the expansion of our knowledge of an organism's historic pangenome and the functional capabilities it previously possessed. However, pseudogenes are difficult to detect ab initio and are not routinely reported by gene prediction tools.

We present StORF-Reporter (Stop-ORF-Reporter), a tool that processes an annotated genome and returns missed genes (functional and/or pseudogenised) from unannotated regions. We applied StORF-Reporter to the unannotated regions of the Hungate collection of genomes and found on average ~100 pseudogenised genes (with in-frame stop codons) per genome. Many of these had high scoring similarity to known Swiss-Prot proteins and form widespread gene families across the different genera of the Hungate collection.

To investigate whether or not the pseudogenised genes are present across other collections, we further applied the methodology to high-quality MAGs from the rumen microbiome. This revealed gene families spanning multiple genera with copies of both intact and pseudogenised versions.

These pseudogenised genes represent a pangenomic 'graveyard' which may alter our understanding of the definition of core and accessory genes for many rumen microbiome species.

P112 | BENCHMARKING SECOND AND THIRD-GENERATION SEQUENCING PLATFORMS FOR MICROBIAL METAGENOMICS

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Shotgun metagenomic sequencing is a frequent method to explore the taxonomic diversity and metabolic potentials of complex microbial communities. Current investigations mostly employ second generation short read sequencing, yet advances in third generation long read technologies provide opportunities to overcome some of the limitations of short read sequencing. Here, we compared seven platforms, comprising second-generation sequencers (Illumina HiSeq3000, MGI DNBSEQ-G400 and DNBSEQ-T7, ThermoFisher Ion GeneStudio S5 and Ion Proton P1) and third generation sequencers (Oxford Nanopore Technologies MinION R9 and Pacific Biosciences Sequel II). We created three uneven synthetic microbial communities composed of up to 87 genomic microbial strains DNAs per mock, spanning 29 bacterial and archaeal phyla, and representing the most complex and diverse synthetic communities used for sequencing technology comparisons. Our results show that third generation sequencing have advantages over second generation platforms in analysing complex microbial communities, but require careful sequencing library preparation for optimal quantitative metagenomic analysis. Our sequencing data also provides a valuable resource for testing and benchmarking bioinformatics software for microbial metagenomics.

P113 | INCREASING RESOLUTION OF MYCOBIOME PROFILING IN THE HUMAN GUT**Mr David Schneider**¹, Dr Ezgi Özkurt¹, Dr Falk Hildenbrand¹¹Quadram, Norwich, United Kingdom

The gut microbiome has major roles in human health, as it regulates the uptake of nutrients, helps in the education of the immune system and protects against pathogens. It is a complex environment consisting of more than one thousand different species of bacteria, archaea, fungi and viruses, containing nearly 10,000,000 unique genes. The bacterial part of the gut microbiome has been the focus of research for the last decade, whereas research investigating the mycobiome, which represents approximately 0.1% of the microbiome diversity is still in its infancy. This is mainly because current sequencing technologies have limited resolution to reach very low abundant microbes. Using density gradient, size-based filtering and protoplast-forming enzymes, we were able to enrich fungi more than 10-fold in a mixed community of bacteria and fungi as well as in human faeces samples. Metagenomic sequencing showed a much more diverse eukaryotic space than without enrichment in *in vivo* human faeces. The present results show that enrichment of fungi in complex microbiomes is possible, but that there is much space for improvement to gain an unbiased, robust and repeatable method.

P114 | FECAL MICROBIOTA DEVELOPMENT FOLLOWING EARLY-LIFE RUMEN MODULATION: A ONE-YEAR STUDY**Mrs Hanna Huuki**^{1,2}, Mrs Johanna Vilkkil¹, Mrs Aila Vanhatalo², Mrs Ilma Tapio¹¹Natural Resources Institute Finland, Production systems, Genomics and breeding, Jokioinen, Finland,²University of Helsinki, Faculty of Agriculture and Forestry, Department of Agricultural Sciences, Helsinki, Finland

Early-life rumen modulation's long-term impact on fecal microbiota in ruminants remains understudied. In this study, treated calves (T-group, n=4) received 5-10 ml of fresh rumen liquid 3x/week between 2-8 weeks, while their control siblings (C-group, n=4) remained untreated. The animals were kept in individual pens until weaning, and fecal samples were collected weekly from 1-8 weeks and monthly from 2-12 months age. The community composition of bacteria and anaerobic fungi (AF) was determined using 16S rRNA and ITS1 metataxonomic sequencing. The quantity of bacteria increased after the first week but temporarily declined around weaning (P=0.02). Before month 4, the quantity of AF was extremely low but increased thereafter, reaching the highest counts at 12 months (P=0.02). The Shannon diversity of bacteria increased until month 6, while AF reached the highest diversity at month 12. The Bray–Curtis distances showed that the communities were strictly divided into pre- and post-weaning communities. Bacteria developed until month 6 (P < 0.001), and AF until month 8 (P < 0.001), but the treatment didn't affect either kingdom. Within bacteria, Actinobacteriota and Proteobacteria reduced by month 4 and were replaced by Bacteroidota, Verrucomicrobiota, Patescibacteria, and Desulfobacterota phyla. In AF, Caecomyces 1, SK3, and Piromyces spp. were more abundant at the early stage, while from month 6 onwards, BlackRhino became dominant, and Orpinomyces spp. and Cyllamyces spp. increased in abundance. The results suggest that early-life rumen modulation had little effect on the long-term fecal microbiota development, which was driven by age and diet.

P115 | EXPLORING THE SMALL INTESTINAL MICROBIOTA: OPTIMIZATION OF AN IN VITRO GUT MODEL

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Research concerning the human gut microbiome expanded extensively but the small intestinal microbiota remained mainly unexplored, despite its importance to human health and immune system development. The characterization of the small intestinal microbiota is challenging, due to the need of invasive sampling techniques and ethical restrictions. These limitations and lack of knowledge could be resolved by use of in vitro models, mimicking both physiological and microbial environment, such as the Simulator of the Human Intestinal Ecosystem (SHIME). This is a dynamic semi-continuous model which simulates the gastrointestinal tract, optimized to study the colon microbiota. Yet, expanding the model by including also the small intestinal microbiota, would boost the knowledge on the functionality, dynamics and composition of this poorly accessible community. Furthermore, this would allow a more representative in vitro gastrointestinal model and serve as possible platform to study small intestinal diseases linked to microbial alterations, such as Crohn's ileitis.

To reach this goal, the mouth, small intestinal and colon microbial communities were established in a SHIME by use of an antegrade colonization with saliva microbiota and a retrograde colonization with fecal microbiota. The microbial community composition and metabolic activity were regularly monitored.

The results show unique communities per gastrointestinal compartment (mouth, proximal small intestine, terminal ileum and proximal colon), including both saliva (*Veillonella*, *Streptococcus*, *Fusobacterium*) and proximal colon (*Bacteroides*) genera residing in the proximal small intestine and/or terminal ileum in line with in vivo literature. As next step, the model will be validated through use of human in vivo samples.

P116 | ANTIBIOTIC RESISTANCE IN GUT BACTERIA ISOLATED FROM HEALTHY HUMANS

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^{*} This work was sponsored by Probi AB.

The occurrence of multidrug-resistant pathogens is an immense threat to human health with a global dimension. The routine application of antibiotics in clinical practice exerts a constant pressure on the gut microbiome causing an increased spread of antibiotic resistance as a bacterial survival mechanism. Consequently, we now face a reduced efficiency in combating infectious diseases, measured by increasing numbers of mortality cases attributable to previously treatable infections. Knowledge of the scale of human gut bacteria antibiotic resistance, the underlying resistance mechanisms, and patterns of resistance acquisition at intra- and inter-species levels are essential to create strategies to limit the spread of resistance.

We report the extent of antibiotic resistance in a total of 428 isolated gut bacteria from six healthy volunteers. This diverse bacterial collection represents the culturable fraction derived under favourable conditions supporting the growth of a maximum number of diverse human gut commensal bacteria. Strains were tested for antibiotic resistance to a set of clinically significant antibiotics representing the main classes – tetracyclines, penicillins, glycopeptides, aminoglycosides, macrolide, lincomycin, chloramphenicol, fluoroquinolones, nitroimidazole - using the disc-diffusion method. Coupled phenotypic and genotypic data show certain resistance distribution patterns and resistance expression mechanisms. An accumulative resistance effect was observed for the vancomycin operon. Resistance to vancomycin and tetracycline was prevalent among cultured bacteria despite volunteers not having been recently exposed to antibiotics. These results show the importance of commensal gut bacteria as reservoirs of resistance genes, potentially transferable to incoming pathogenic bacteria.

P117 | ENGINEERING BUTYROGENIC TROPHIC CHAINS IN THE HUMAN COLON – DRIED CHICORY ROOT AS INTACT PLANT-CELL FIBRE PRODUCT TO BOOST HUMAN HEALTH

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Short-chain fatty acids (SCFA) produced by the human gut microbiota modulate human health, notably when absorbed in the distal colon. Engineering trophic chains that delay SCFA production to the distal colon offer unexplored health benefits. Dried chicory root is an intrinsic fibre with a pectin and (hemi)cellulose plant-cell matrix naturally shielding intracellular inulin from rapid fermentation. We hence hypothesized that dried chicory root can promote trophic chains that generate SCFA and are beneficial for human health. Exploratory in vitro faecal batch-fermentation experiments implied delayed butyrate production from dried chicory root. In an in vitro synthetic community, we demonstrated that *Anaerostipes rhamnosivorans* in the presence of *Bifidobacterium animalis* and *Bacteroides xylanisolvens* (cell-wall degrader) formed a butyrogenic trophic chain from dried chicory root. Replicating these results in vivo in subjects at risk for type 2 diabetes, we observed that faecal acetate, propionate and butyrate all increased by more than a quarter of their original levels following dried chicory root intake. Especially *Bifidobacterium* and *Anaerostipes* spp. increased up to four-fold, while *Blautia* spp. and other *Lachnospiraceae* spp. decreased. We attributed these changes to a delayed colonic fermentation caused by the plant-cell matrix. Paralleling the large microbiota changes, also bowel function was strongly modulated. Further systemic effects were manifested by increased fasting plasma SCFA levels and reduced diabetes biomarkers, especially in subjects with low baseline *Blautia* spp.. In conclusion, dried chicory root with an intact plant-cell matrix can generate butyrogenic trophic chains promoting SCFA production in the human distal colon and their health-related effects.

P118 | HOST-GUT MICROBIOTA CROSS-TALK VIA NCRNAS IN AUTISM SPECTRUM DISORDERS

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Autism spectrum disorders (ASD) is a neurodevelopmental condition often accompanied by gastrointestinal disorders and dysbiosis. Autism aetiology is attributed to gene-environmental interaction and gut pathogens represent interesting environmental factors.

Host-microbial cross-talk, via small ncRNAs, has been proposed. We investigated the microbial and non-coding RNAs (ncRNAs) profile in the stools of children with autism and neurotypical controls.

Small RNA-seq and customized bioinformatics analysis were applied to identify and annotate ncRNAs, while metatranscriptomic for microbiome profiling.

Dysbiosis was found in all children with ASD. The ncRNAs classes distribution did not differ between autism and control groups. We identified: miRNAs, piRNAs (for the first time in feces of children with ASD) and lncRNAs. Functional analysis of miRNA and piRNA targets returns genes involved in cell-cell junctions, bacterial invasion, inflammation and metabolite signalling, and all these processes are associated to autism.

The short reads from small-RNA-seq were aligned against a lncRNA database. We found 14 lncRNAs significantly differentially expressed. This is an unusual method to study lncRNAs, so we checked the gene coverage and the exclusive mapping of identified sequences against the human transcriptome obtaining a homogenous gene coverage for all lncRNAs, while only two univocally mapped on lncRNA genes (LINC02249 and PRDX6-AS1).

The small RNAseq allowed the profiling of dysregulated miRNAs and piRNAs. Aligning small reads against lnc genes we propose a pioneering approach to investigate lncRNAs, supplying the degradation of RNA in stools. These allow the study of possible host and gut microbiota cross-talk via sncRNAs and lncRNAs.

Acknowledgment: GEMMA-euProject www.gemma-project.eu.

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A

A. Draper, Lorraine	OT03
Abbott, Wade	P028
Abdalla, Adibe	P068
ABDALLA, ADIBE LUIZ	P033
Abecia, Leticia	P008
Adegbeye, Moyosore	P029
Aden, Konrad	P081
Ahn-Jarvis, Jennifer	P036
Albers, Ruud	OT07
Albertsen, Mads	OT12
Alfano, Vincenzo	P016
Alfieri, Roberta	P118
Al-hindi, Rashad	P098
Almeida, Mathieu	P004, P112
Al-odat, Ma'en	P073
Alric, Monique	P035
Altshuler, Ianina	P104
Amadio, Ariel	P037, P057
AMAR, Mohamed	P030
Amos, Gregory	P012
Ampe, Bart	P066
Anderson, Robin	P087
Anderson, Thea	P028
Andersson-Engels, Stefan	OT03
Andres-Lasheras, Sara	P006
Andrews, Catherine	P086, P094
Andrews, Simon	P061
Anguita, Juan	P008
Ansorge, Rebecca	OT18, P109
Apper, Emmanuelle	P085
Aransay, Ana Maria	P008
Ariza-Nieto, CJ	P044
Ariza-Nieto, Claudia Janeth	P056
Arora, Trisha	OT02
Attwood, Graeme	P021, P024, P078, P086, P094
Attwood, Graeme T.	P104
Aubrey, Wayne	P111
Aubry, Aurelie	P046
Auria, Guiseppe	P054

B

Babu, Ambrin Farizah	P054
Bakshani, Cassie	OT12
Bannink, Andre	P058
Baraille, Marylou	P055, P102
Barbera, Giorgia L.	OT10
Bargiela, Rafael	P072
Barriaes, Diego	P008
Barron, Luis Javier	P027

B

Bassett, Shalome	P086, P094
Baudot, Aurélien	OT07
Bednarska, Natalia	P100
BÉGUET-CRESPEL, Fabienne	P030
Beisvåg, Vidar	P100
Belanche, Alejandro	P020, P031, P052
Benavides-Cruz, Juan Carlos	P029
Benninga, Marc A.	P015
BÉRA-MAILLET, Christel	P030
Bereswill, Stefan	OT02
Bermingham, Emma	P067
Bernard, Maria	P040
Berry, David	P077
Bertelli, Claire	P002
Berthelot, Valerie	P050
Berthiller, Franz	P062
Bertolo, Alessandro	P090
Bhatta, Raghavendra	P087
Biagini, Lucia	P062
Biggs, Patrick J.	P104
Bird, Leanne	P094
Biscarrat, Paul	P041
Biswas, Ambarish	P086
Blanquet-Diot, Stéphanie	OT01, P004, P009, P013, P035, P053, P080, P085
Boirie, Yves	P035
Bonneau, Mathis	P005
Bootsma, Erik	P059
Boraston, Alisdair	P028
Bosser, Alex	P099
Nikoleta Boteva	P116
BOUCHON, Matthieu	P018
Boumpoutou, Rufin	P005
Brans, Johanna	P046
Bresser, Lucas	P015
BRESSUIRE, Christelle	P030
Brezočnik, Lucija	P079
Bron, Auriane	P053
Broudiscou, Laurent-Philippe	P050
Brown, Aaron	OT13
Brul, Stanley	P015
Brun, Morgane	P009, P013, P035, P085
Bruneau, Nicolas	P040
Buckland, Maria	P001
Bui, Thi P. N.	P117
Busche, Svenja	P081
Butowski, Nina	P067
Buttet, Marjorie	P055, P102

C

Cabello, Alfonso	P072
Caderni, Giovanna	P045
Cadiou, Julie	P051
Calenge, Fanny	P040
Campossela, Simona	P090
Canarruto, Giulia	P016
Cann, Isaac	P076
Caporaso, Greg	OT11
Carbone, Vincenzo	P078
Caron De Fromentel, Claude	P016
Carvell-Miller, Laura	P012
Castelo, Janire	P008
Castillo-Lopez, Ezequias	P062, P106
Casuscelli di Tocco, Francesca	P016
Cave, Nick	P067
Cazzaniga, Monica	OT02
Centelles Lodeiro, Javi	P059
Cerón-Cucchi, María Esperanza	P037, P057
Chakoory, Oshma	OT14
Chatterley, Laura	P012
Chaudemanche, Cyril	P035
Chavarri, Maria	P027
Chavarría, Nicolás	P037, P057
Cheng, Yanfen	P076
Cherbuy, Claire	P041
Chiappori, Federica	P118
Chomistek, Nora	P006
Claesson, Marcus	OT02
Clare, Amanda	P111
Clavel, Thomas	OT06, P026
Collins, Stephanie	P048
Conlon, Michael	P038
Consiglio, Arianna	P118
Copani, Giuseppe	P110
Cornu, Agnes	OT08, P043
Cotter, Paul	P034
Coville, Jean-Luc	P040
Cox, Eric	P017
Cravero, Silvio	P057
Creevey, Chris	OT13, P046, P108
Creevey, Christopher	P111
Cressard, Crystèle	P005
Cressard, Pierre	P005
Cristina Ferreira Bonomo, Renata	P101
Cristobal-Carballo, Omar	P046
Crompton, Les A.	P065
Crost, Emmanuelle	P036
Crouzet, Laureen	P021, P024, P086, P094
Cupaioli, Francesca Anna	P118

D

D' Souza, Tanya	OT03
Da Silva, Kévin	P112
Danon, Jeanne	P110
Daud, Noshin	P069
Dauros Singorenko, Priscila	P023
David, Aymeric	P004
De Filippis, Francesca	OT16, P047, P071, P092
De Filippo, Carlotta	P045
De Nicola, Francesca	P016
De Paepe, Kim	P115
Dekker, James	P086
Delbaere, Karen	P115
Della Croce, Clara	P045
DellaRosa, Maria	P083
Demay, Romain	P005
Denis, Sylvain	P004, P053, P080, P085
Derbré, Frédéric	P005
Deschamps, Charlotte	P004, P035, P085
Devlin, Edward	P022
Deyaert, Stef	OT07
Di Cagno, Raffaella	P071
Diederer, Kay	P015
Diez Gutierrez, Lucía	P027
Dillon, Lucy	OT13
Dimonaco, Nicholas	OT13, P111
Diwedi, Bidhi	P014
Domingo-Almenara, Xavier	OT02
Doré, Joël	P051
Dragsted, Lars O.	OT10
Duca, Frank	OT11
Dufour, Claire	OT08, P043
Sylvia Duncan	P116
Duong, Rich	P039
Duplessis, Melissa	OT15
Durif, Claude	OT01, P009, P013, P085

d

de Benito, Amparo	P072
de Carvalho Batista Muniz, Izabella	P101, P103
de Jonge, Wouter J.	P015
de la Torre-Hernández, María Eugenia	P064
del Campo, Rosa	P072
de Vos, Willem M.	P117

E

Ehrlich, S. Dusko	P004
Eikanger, Katrine S.	OT12
EL FAHIME, Elmostafa	P030
El-Nezami, Hani	P054
Engel, Erwan	P080
Ercolini, Danilo	OT16, P047, P071, P092
Esposito, Alessia	P071, P092
Etienne, Camille	P045
Etienne-Mesmin, Lucie	OT01, P004, P009, P013, P035, P080

F

Falà, A. Kate	P034
Falony, Gwen	P059
Farfan, Mauricio	P013
Farquharson, Freda	P060
Fernández-López, Laura	P072
Ferreira Bonomo, Renata Cristina	P103
Ferreira Santana, Renata	P101, P103
Ferrer, Manuel	P072
Ferrillo, Alessandra	OT12
Ferris, Conrad	P001
Ferrocino, Ilario	P071
Feskens Edith J. M.	P117
Flueck, Joelle	P090
Flynn, Cassandra	P042
Forano, Evelyne	P110
Forbes, Ken	P073
Fournier, Elora	P004, P080
Frankel, Gad	OT02
Friel, Ruairi	P074
Frioux, Clemence	OT18
Frioux, Clémence	P109
Frost, Gary	P036

G

Gagic, Dragana	P104
Gahan, Cormac	P009
Gahan, Cormac G.M.	OT02
Galosi, Livio	P062
Garcia-Gutierrez, Enriqueta	P034
GATTI, BRUNO SCATENA	P033
Geiger, Hartmut	OT04
Gerard, Christine	P050
Gérard, Philippe	OT07, P045
Gere, José	P037

G

Giovanelli, Lisa	P045
Glisic, Marija	P090
Gobbetti, Marco	P071
Godoy Santos, Fernanda	OT13, P001, P101, P103
Godoy-Santos, Fernanda	P046
Gomes, Pierre	P050
Gómez, Aharón	P052
Gomez-Gallego, Carlos	P054
Goossens, Evy	P017
Goossens, Karen	P049, P066
Gratz, Silvia	OT19, P022, P116
Graviou, Dominique	P018, P105
GREGO, ELOISA	P033
Grenier, Bertrand	P070
Gresse, Raphaelae	P110
Gruninger, Rob	P006
Guan, Leluo	P006, P084, P089
Guéraud, Françoise	P045
Guerrieri, Francesca	P016
Guery, Benoît	P002
GUIBOUT, Héloïse	P030
Gundogdu, Ozan	P010
Gutierrez Torrejon, Martin	OT19
Gutierrez-Merino, Jorge	OT05

H

Haberg, Asta	P100
Hagen, Live	OT12, P028
Halifa, Ruben	OT08, P043
Hangelsbroek Roland W. J.	P117
Hanhineva, Kati	P054
Hanrahan, Naomi	OT03
Hardt, Wolf-Dietrich	OT02
Harris, Danielle	P081
Harris, Hannah	P032
Haslam, David B.	OT04
Hassan, Mahmoud	P052
Hay, Susan	P069
Hay M., Susan	P063
Hayes, Maria	P038
Hazon, Mathilde	P005
Heavens, Darren	P091
Heimesaat, Markus M.	OT02
Herman, Chloe	OT11
Hernández-García, Pedro Abel	P064
Hertig-Godeschalk, Anneke	P090
Herzog, Mathias Klaus-Maria	OT02
Hess, Matthias	P039

H

Hildebrand, Falk	OT18, P091, P109
Hildenbrand, Falk	P113
Hill, Colin	OT03
Hill, Jeremy	P086, P094
Hilton, Anthony	P012
Hitch, Thomas	OT06, P026
Hofacre, Charles	P070
Højberg, Ole	P082
Holck, Jesper	P060
Holden, Nicola	OT17
Hong, Jiwon	P023
Howard, Lynsey	IT05
Hov, Johannes R.	P072
Hoyles, Lesley	OT09
Huang, Rongcai	P031
Humbert, Delphine	P085
Hund, Alexandra	P096
Huuki, Hanna	P114
Huws, Sharon	OT13, P001, P046, P101, P103
Huynh, Vy A.	P014

I

Iannone, Valeria	P054
Iebba, Valerio	P016
Ieda Cappelozza, Bruno	P110
Irazoqui, Matías	P037, P057
Izquierdo, Mariana	P01

J

Jang, Jiyeon	P061
Jardet, Déborah	P040
Jaspers, Yerrick	P015
Jegou, Sarah	P004
Jelinski, Murray	P096
Jennings, Susan	P026
Jimenez, Carolina	P068
JIMENEZ, CAROLINA RODRIGUEZ	P033
Jiménez, Elisabeth	P020, P031
Jokela, Rosa	P117
Jordan, Jack	P001
Jørgensen, Malte S.	OT10
Juge, Nathalie	P036
Jujihara, Nicolas	P028
Julliand, Samy	P055, P102
Julliand, Véronique	P055, P102
JUSTE, Catherine	P030

K

Kaka Bra, Kardokh	OT02
Kaminska, Dorota	P054
Kandari, Anwar	P019
Kanti Kar, Soumya	P099
Kaper, Fred	P117
Kathagen, Gunther	P059
Kelly, William	P021, P024, P078, P086, P094
Kemp, Stephan	P015
Kettunen, Mikko	P054
Kidane, Alemayehu	OT12
Kiltie, Anne	P069
Kiltie E., Anne	P063
Kindermann, Angelika	P015
King, Marissa	P028
Kirwan, Stuart	P074
Kissner, Maya	P007
Klahr Fritz, Mikaela	P095
Klávus, Anton	P054
Koehnke, Jesko	P073
Kolehmainen, Marjukka	P054
Koopman, Nienke	P015
Koren, Omry	OT20, P003, P097
Kreuzer-Redmer, Susanne	P062
Kurz, Alina	P072

L

Lagarde, Fabienne	P080
Lagarrigue, Sandrine	P040
Lamas, Bruno	P080
Lamérand, Maryline	P049, P066
Langella, Philippe	P041
Laursen, Martin F.	OT10
Lavín, Jose Luis	P008
Lawther, Katie	OT13, P001
Le Bourvellec, Carine	OT08, P043
Le Chatelier, Emmanuelle	P004
Le Joubioux, Florian	P053
Leahy, Sinead	P021, P024
Leanti La Rosa, Sabina	IT02
Lecoeur, Alexandre	P040
Lecommandeur, Emmanuelle	P005
Leijon, Mikael	P095
Lemoine, Lisa	P007
Léveque, Mathilde	P080
Levin, Evgeni	P015
Levrero, Massimo	P016
Lewis, Eva	P046

L

Li, Xuan	P011, P075
Li, Yang	P024, P094
Li, Yuqi	P076
Licciulli, Flavio	P118
Licht, Tine R.	IT03, OT10
Llanes, Nuria	P052
Lok, Johnson	P054
Longo, Vincenzo	P045
Lonsdale, Zoe	P012
Louis, Petra	P060
Louvandini, Helder	P068
LOUVANDINI, HELDER	P033
LOUVANDINI, PATRICIA	P033, P068
Louzoun, Yoram	OT20, P003, P097
Low, Kristin	P028
Lowe, Katherine	P086
Lu, Jing	P048
Lund, Peter	P082
Lyons, Laura	P056

M

Ma, David	P036
Ma, Jing	P076
MacCallum, Donna	P022
Mackie, Roderick	P025
Mahoney-Kurpe, Sam C.	P104
Maigaard, Morten	P082
Maldonado, Elaina	OT05
Maluquer de Motes, Carlos	OT05
Marinelli, Ludovica	P115
Marotta, Roberto	P047
Marre, Sophie	OT14
Marroquí, Laura	P034
Martin, David	P005
Martin, Jennifer	OT19, P116
MARTIN, Bruno	P018
Martín García, Ignacio	P020
Martineau, Christine	OT15
Martín-García, Ignacio	P031
Martín-Ruiz, Itziar	P008
Mathis, Greg	P070
Matrin, Jenny	P019
Maudet, Claire	P045
Mayengbam, Shyam	P042
Mayorga, O	P044
Mayorga, Olga	P056
Mayorga-Mogollón, Olga Lucia	P029

M

Maziar, Aida	P042
McAllister, Tim	P006, P089
McArthur, Simon	OT09
McCann, Joshua	P025
McKinley, Aileen	OT19
Melgar, Silvia	OT03
Mendoza, Germán David	P064
Mercenier, Annick	OT07
Mercier-Bonin, Muriel	P080
Meslier, Victoria	P004, P112
Mezzelani, Alessandra	P118
Mihalynuk, Liam	P028
Mikkonen, Santtu	P054
Monbet, Valérie	P005
Mondot, Stanislas	P051
Montenegro-Borbolla, Elena	P002
Montoya, Alejandra Carolina Villa	P074
Moomin, Aliu	P063, P069
Moon, Christina D.	P104
Morabito, Christian	P004
Moreno, Elena	P072
Moreno, Santiago	P072
Morgavi, Diego	P018, P031, P105
Morrison, Steven	OT13, P046
Mosca, Ettore	P118
Mosoni, Pascale	OT08, P043
Mu, Chunlong	P011, P075
Muetzel, Stefan	P083
Mukhopadhyaya, Indrani	OT19, P116
Mukhopadhyay, Subhankar	OT05
Munro, Carol	P022
Muriel, Alfonso	P072
Murros, Kari E.	P01

N

Na, Sang Weon	P084
Nale, Janet	OT17
Nally, Ken	OT03
Newbold, C. Jamie	P065
Newbold, Charles James	P056
Newbold, CJ	P044
Ngom, Serigne Inssa	P030
Niderkorn, Vincent	P110
Noel, Samantha	P082

O

O Sullivan, Deborah	P009
O'Brien, Leah	P108
O'Donnell, Caroline	P074
O'Flaherty, Vincent	P074
O'Hara, Eoin	P006
Opsomer, Geert	P066
Ortiz Chura, Abimael	P105
Ortiz-Chura, Abimael	P037, P057
Ortiz-Cuadros, Ronnal	P029
O'Sullivan, Déborah	P013
Otero, Yolanda	P053
Oyama, Linda	OT13, P001, P101, P103
Ozkurt, Ezgi	OT18, P091

Ö

Özkurt, Ezgi	P109, P113
--------------	------------

P

Pacífico, Cátia	P062, P106
Pakalapati, Jnana Sree	P001
Palacios, Ainhoa	P008
Palevich, Nikola	P078, P104
Palm, Noah W	P008
Palma Hidalgo, Juan Manuel	P020, P065
Palma-Hidalgo, JM	P044
Pangga, Gladys Maria	P010
Pascal, Géraldine	P040
Patel, Trushar	P028
Paturel, Alexia	P016
Peña-Cearra, Ainize	P008
Pepke, Frédéric	P041
Perez Molina, Jose A.	P072
Peters, Audrey	OT02
Peters, Jason	P086
Petri, Renee	P048
Petri, Renée	OT15, P096
Petri, Renee M.	P062
Peyret, Pierre	OT08, OT14, P043
Phillips, Anthony	P023
Piazza, Silvano	P016
Piche, Jessie	P042
Pickup, James	P001
Pierre, Fabrice	P045
Pihlajamaki, Jussi	P054

P

Plaza Oñate, Florian	P112
Plaza-Oñate, Florian	P004
Podar, Mircea	P112
Podgorelec, Vili	P079
POMIES, Dominique	P018
Pons, Nicolas	P112
Pope, Phil	P028
Pope, Phillip	OT12
Pope, Phillip B.	P104
Popova, Milka	P018, P031, P105
Potter, Adam	P069
Pouget, Mélanie	P035
Poulin-Laprade, Dominic	OT15
PRESUTO, LIVIA	P033
Procházková, Nicola	OT10
Proost, Sebastian	P059
Psifidi, Androniki	P010
Puhlmann, Marie-Luise	P117

Q

Quinquis, Benoit	P004, P112
------------------	------------

R

R. Stockdale, Stephen	OT03
RACHED, Bahia	P030
Raes, Jeroen	OT10, P059
Raghuvanshi, Ruma	P051
Raikos, Vassillios	P019
Rajasekaran, Krsana	P086, P094
Raju, Sajan C.	P072
Ramírez Saad, Hugo César	P064
Ramos, Nadia	P037, P057
Ramos-Morales, Eva	P044, P052, P056
Rasmussen, Morten A.	OT10
Ratel, Jérémy	P080
Redmer-Kreuzer, Susanne	P106
Reid, Peter	P024, P078
Reid, Peter M.	P104
Reilly, Kerri	P078
Reinhardt, Camila	P042
Reisinger, Nicole	P062, P106
Reitlo, Line	P100
Reynolds, Chris K.	P065
Ribeiro, Gabriel	P006
Ricci, Sara	OT15, P062, P096, P106

R

Richmond, Anne	P010
Rivera-Chacon, Raul	P062, P106
Roager, Henrik M.	OT10
Rocha, Miguel	P068
Rodríguez, Hector	P008
Romera-Recio, Eva	P052
Romero, Pedro	P031, P052
Ronimus, Ron	P078
Roques, Simon	P099
Rosendal, Thomas	P095
Rosin, Aline	P007
Roskam, Emily	P074
Rossi, Giacomo	P062
Rossini, Valerio	OT03
Roth, Nataliya	P070
Roulston, Corey	P001
Roume, Hugo	P112
Rous, Colombe	P051
Roussel, Charlène	OT01
Royer, Isabelle	OT15
Rubino, Francesco	OT13, P046
Ruettimann, Belinda	P090
Rupnik, Maja	P079

S

Saccaro, Angelo	P068
Saenz, Jessica	P027
Sáenz, Johan S.	P072
Saiz-Gonzalo, Gonzalo	OT03
San vicente, Leire	P027
Sang, Carrie	P078
Saris, Per E. J.	P014
Sauvaitre, Thomas	OT01
Schneider, David	P113
Schnorr, Stephanie	P077
Schofield, Linley	P078
Schwartz-Zimmermann, Heidi E.	P062
Scoley, Gillian	OT13
Scott, Karen	OT19, P019, P073, P116
Sebek, Léon	P099
Segura-Wang, Maia	P070
Sehgal, Ratika	P054
Seifert, Jana	P072
Sendagorta, Elena	P072
Sener-Aydemir, Arife	P062, P106
Seoane, Iratxe	P008
Seppen, Jurgen	P015

S

Sequino, Giuseppina	OT16, P092
Sereika, Mantas	OT12
Serin, Spencer	P028
Serrano-Villar, Sergio	P072
Shanahan, Fergus	OT03
Shannon, Emer	P038
Shaw, Sophie	OT19
Shayya, Nizar	OT02
Shen, Shuqi	P107
Shtosel, Oshrit	P097
Shtossel, Oshrit	OT20
Shtusel, Oshrit	P003
Sierra-Alarcón, AM	P044
Sierra-Alarcón, Andrea Milena	P029
SILAN, Ece	P091
Silvio, Cravero	P037
Singh, Raminder	OT03
Sivignon, Adeline	OT01
Sjölund, Marie	P095
Slattery, Helen	P034
Smidt Hauke	P117
Smith, Paul E.	P031
Solis, Selvin	OT05
Solvang, Michael	P060
Sommer, Morton	P054
Song, Deguang	P008
Soni, Priya	P094
Soni, Pryia	P086
Sørensen, Emil	OT12
Spacova, Irina	IT06
Stahl, Selina	OT04
Stecher, Bärbel	IT01
Stensvold, Dorte	P100
Stoikidou, Theano	P001
Stoyanov, Jivko	P090
Stucki, Gerold	P090
Swift, Simon	P023

T

Takahashi, Lumena	P037, P057
Takala, Timo M.	P014
Talbot, Guylaine	OT15
Tancredi, Alessandra	P017
Tapio, Ilma	P114
Teh, Koon Hong	P021
Telatin, Andrea	P036
Then K., Chee	P063
Thirion, Florence	P004, P030
Thomas, David	P067
Thorn, Camilla	P074
Tikkanen, Ida	P054
Tincati, Camilla	P072
Tingley, Jeff	P028
Tito-Tadeo, Raul	P059
Tondini, Sara	P025
Tong, Brian	P086, P094
Tongue, Sue	OT17
Torra, Josep	P052
Torraville, Sarah	P042
Tralau, Tewes	P007
Troseid, Marius	P072
Tsekitsidi, Eirini	OT10

t

te Velde, Anje A.	P015
-------------------	------

U

Ungerfeld, Emilio M.	P031
Uriot, Ophélie	OT01, P004, P009, P035

V

Valentino, Vincenzo	OT16, P071
Valido, Ezra	P090
Vallier, Marie	P053
Van de Wiele, Tom	OT01, P053, P115
Van den Abbeele, Pieter	OT07
Van Herreweghen, Florence	OT01
Van Hese, Ilke	P049, P066
Van Immerseel, Filip	P017
Van Landuyt, Josefien	OT01
Vandaele, Leen	P049, P066
Vanhatalo, Aila	P114
Vanholsbeeck, Frederique	P023
Vargas, Juan	P056
Vázquez Castellanos, Jorge	P059
Vazquez-Urbe, Ruben	P054
Vera-Ponce De León, Arturo	OT12
Ver-Loren-Van Themaat, Emiel	P058
Vermeulen, Astrid	P059
Vieira-Silva, Sara	P059
Vilkkki, Johanna	P114
Villalobos-Arias, Karla	P064
Vilte, Daniel	P037
Vitaglione, Paola	P047
Vitali, Francesco	P045
VOLAND, Laurianne	P018

V

van de Guchte, Maarten	P051
van Dongen, Katja C. W.	P117
van Gastelen, Sanne	P058, P099
van Laar, Harmen	P099
van Schaik, Willem	IT04
van Leeuwen, Pim	P015
van Munster, Jolanda	P107

V

van de Guchte, Maarten	P051
van Gastelen, Sanne	P058, P099
van Laar, Harmen	P099
van Schaik, Willem	IT04
van Leeuwen, Pim	P015
van Munster, Jolanda	P107

R

Walker, Nicola	P058
Wallgren, Per	P095
Wang, Dongyang	P076
Warren, Fred	P032
Warren, Frederick	P036
Waschina, Silvio	P081
Waters, Sinéad	P074
Wellekens, Jozefien	P115
Weninger, Savanna	OT11
White, Joni	P023
WILKINS, JOHN-PAUL	P088
Willats, William	OT12
Wisløff, Urlik	P100
Witteck, Thomas	P096
Wittlinger, Jan-Philipp	P077
Wottlin, Lauren	P087
Wu, Haiqin	P011, P075
Wu, Ziming	P001

X

Xia, Dong	P010
Xing, Xiaohui	P028

Y

Yan, Tianhai	P046
Yáñez Ruiz, David R.	P020
Yanez-Ruiz, David R.	P052
Yáñez-Ruiz, David	P031
Yazbek, Hiba	P051
Young, Wayne	P067
Yuan, Qi	P042

Z

Zaheer, Rahat	P006
Zandberg, Wesley	P028
Zebeli, Qendrim	P062, P106
Zerjal, Tatiana	P040
Zeuner, Birgitte	P060
Zhong, Pei	P076
Zhou, Mi	P084, P089
Zhu, Weiyun	P011, P075, P076

Ž

Žlender, Tanja	P079
----------------	------

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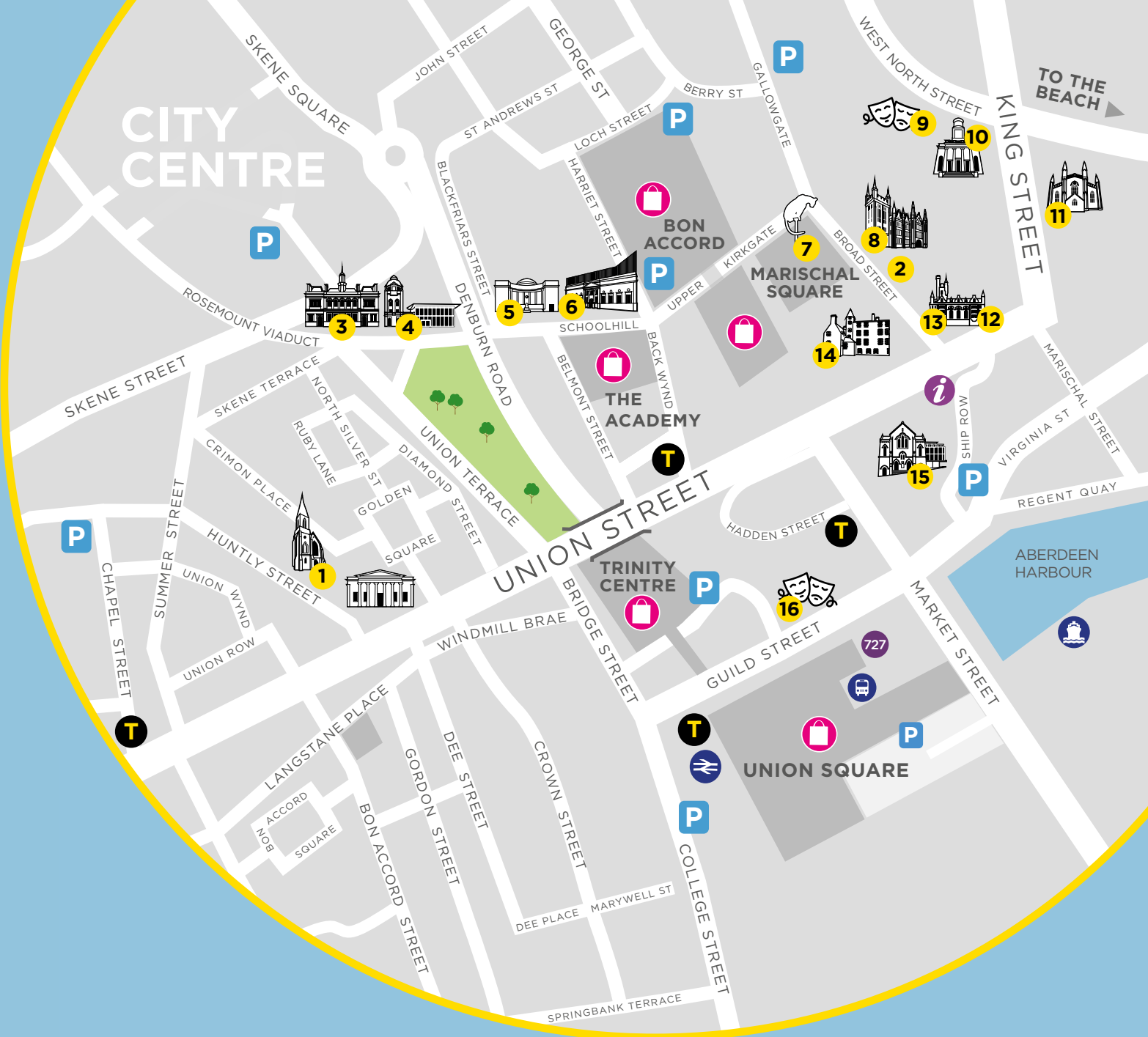
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- 10** ARTS CENTRE
- 11** ST ANDREW'S CATHEDRAL
- 12** THE TOLBOOTH MUSEUM
- 13** TOWN HOUSE (Civic Welcome Reception Venue)
- 14** PROVOST SKENE'S HOUSE
- 15** MARITIME MUSEUM
- 16** TIVOLI THEATRE
- 17** P&J LIVE AT TECCA (Conference Venue)
- 18** ARDOE HOUSE HOTEL (Conference Dinner Venue)
- 19** BEACH BALLROOM (Ceilidh Venue)



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For general event enquiries, please contact:

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